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Potential use of ozone in SARS-CoV-2 / COVID-19.
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Original drafters of the paper: Adriana Schwartz, Scientific Secretary of ISCO3, Gregorio Martínez-Sánchez, President of ISCO3.

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Note: The paper "Potential use of ozone in SARS-CoV-2 / COVID-19" has been drafted, discussed and approved by ISCO3 having into account three key points:

1. The World Health Organization (WHO), specialized agency of the United Nations, whose mandate is public health, has officially recognized that "Currently, there are no vaccines or specific pharmaceutical treatments available for COVID-19." [1]
2. To fight this pandemic the WHO have called "countries to take urgent and aggressive action"; and stating that "this is not just a public health crisis, it is a crisis that will touch every sector – so every sector and every individual must be involved in the fight."[2] So ISCO3 as part of the world health sector wants to be involved in the fight against this pandemic.
3. As there are not "vaccines or specific pharmaceutical treatments available" this paper offers a contribution to fight the coronavirus proposing **the potential use of ozone therapy**, as a complementary therapy, exclusively based on scientific available data as is explained in detail in this paper.

Abbreviation / Acronyms

ACE2: Angiotensin-converting enzyme 2.
CDC: Centres for Disease Control and Prevention (USA).
COVID-19: Coronavirus disease 19.
CT: Computed tomography.
EBOO: Extracorporeal Blood Oxygenation-Ozonation.
EPA: Environmental Protection Agency (USA).
FDA: Food and Drug Administration (USA).
GSH: Glutathione
HSP: Heat Shock Proteins.
MAH: Major Autohemotherapy.
MiAH: A variant of the Minor Autohemotherapy.
MSCs: Mesenchymal Stem Cells.
O_3SS: Ozonized Saline Solution.
OSHA: Occupational Safety and Health Administration (USA).
SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2.
UC: Umbilical Cord.
WHO: World Health Organization.

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Summary

The emergence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2; provisionally named 2019 novel coronavirus or 2019-nCoV) disease (COVID-19) in China at the end of 2019 has caused a large global outbreak and is a major public health issue. The COVID-19 has been characterized as a "pandemic" by the World Health Organization (WHO). The most official recent and available data on March 12th, 2020 has shown that almost 125,000 cases have now been reported to WHO, from 118 countries and territories. In the past two weeks, the number of cases reported outside China has increased almost 13-fold, and the number of affected countries has almost tripled.[3] "4,291 people have lost their lives (…) in the days and weeks ahead, we expect to see the number of cases, the number of deaths, and the number of affected countries climb even higher."[2] The scope of this paper is to review **"the potential ozone utilization that serves as a complementary therapy"** in the management of COVID-19. Evidence acquisition terms (ozone, SARS-CoV-2 and COVID-19) was searched in the scientific data bases.

Ozone can be used in the disinfection of viral contaminated environments. Its maximum anti-viral efficacy requires a short period of high humidity (>90% relative humidity) after the attainment of peak ozone gas concentration (20 – 25 ppm, 39-49 mg/m^3). As a gas it can penetrate all areas within a room, including crevices, fixtures, fabrics, hospital room, public transport, hotel room, cruise liner cabin, office, etc. and under surfaces of furniture, much more efficiently than manually applied liquid sprays and aerosols. **The environment to be treated must be free of people and animals due to the relative toxicity of ozone via inhalation**.

Systemic ozone therapy can be "**potentially**" useful in SARS-CoV-2. The rationale and mechanism of action has already been proven clinically in other viral infections and has been shown to be highly effective in research studies. The mechanism of action will be by 1) The induction of adaptation to oxidative stress, hence a re-equilibration of the cellular redox state. 2) The induction of IFN-gamma and proinflammatory cytokines. 3) The increase of blood flow and tissue oxygenation to vital organs. 4) It has the potential actions to act as an auto-vaccine when administered in form of minor autohemotherapy.

The recommended routes of administration are: Major Autohemotherapy (MAH), Ozonized Saline Solution (O_3SS), Extracorporeal Blood Oxygenation-Ozonation (EBOO), and a variant of the Minor Autohemotherapy (MiAH). Clinical protocol should be adhered to with the standard doses and procedures as defined in the Madrid Declaration of Ozone Therapy. It is a complementary therapy because while the infected patient will continue to be treated with allopathic medicine, at the same time the patient will receive the treatment that this paper is proposing.

At least three clinical trials using major autohemotherapy are currently being undertaken in China and more clinical trials and data are needed to confirm the efficacy of ozone therapy as a complementary therapy in COVID-19 diseases.

Keywords

Ozone, Ozone therapy, COVID-19, SARS-CoV-2, Ozonized Saline Solution, Major autohemotherapy.

Introduction

Coronaviruses are important human and animal pathogens. At the end of 2019, a novel coronavirus was identified as the cause of a cluster of pneumonia cases in Wuhan (Hubei Province of China) and caused a large global outbreak representing a major public health issue.[4] It rapidly spread, resulting in an epidemic throughout China, with sporadic cases reported globally. In February 2020, the World Health Organization designated the disease COVID-19, which stands for coronavirus disease 2019.[5] The virus that causes COVID-19 is designated severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2); previously, it was referred to as 2019-nCoV. SARS-CoV-2 is closely related to two bat-derived severe acute respiratory syndrome-like coronaviruses, bat-SL-CoVZC45 and bat-SL-CoVZXC21, in particular BetaCoV/bat/Yunnan/RaTG13/2013 are similar to the human SARS-CoV-2.[6] It is shown to have large genetic diversity and rapid evolution.[7]

SARS-CoV-2 is spread by human-to-human transmission via respiratory droplets or direct contact, and infection has been estimated to have a mean incubation period of 6.4 days and a basic reproduction number of (2.24 - 3.58) days.[4] Among patients with pneumonia caused by SARS-CoV-2, fever was the most common symptom, followed by cough, malaise and dry cough at the prodromal phase.[8] Bilateral lung involvement with ground-glass opacity was the most common finding from computed tomography (CT) images of the chest. CT images demonstrated progression during the early stage from illness onset.[9]

There are currently no antiviral drugs licensed by the U.S. Food and Drug Administration (FDA), by the Spanish Drug Agency and Health Products (AEMPS) or by the Italian Drug Agency to treat patients with COVID-19. To our knowledge, no antiviral drugs to treat patients with COVID-19 have been licensed in any country in the world so far. **This point has been officially confirmed by WHO: "Currently, there are no vaccines or specific pharmaceutical treatments available for COVID-19."**[1] Some *in-vitro* or *in-vivo* studies suggest potential therapeutic activity of compounds against related coronaviruses, but there are no available data from observational studies or randomized controlled trials in humans to support recommending any investigational therapeutics for patients with confirmed or suspected COVID-19 at this time.

Remdesivir, an investigational antiviral drug, was reported to have *in-vitro* activity against SARS-CoV-2.[10] A small number of patients with COVID-19 have received intravenous remdesivir for compassionate use outside of a clinical trial setting. A randomized placebo-controlled clinical trial of remdesivir for treatment of hospitalized patients with pneumonia and COVID-19 has been implemented in China. A randomized open label trial of combination lopinavir-ritonavir, duranavir, danoprevir, cobisistat, Anti-CD147 Humanized Meplazumab, Eculizumab, Bevacizumab, Recombinant Human Angiotensin-converting Enzyme 2 (rhACE2), NK cells, Umbilical Cord (UC)-Derived Mesenchymal Stem Cells (MSCs),

immunoglobulins, sphingosine-1-phosphate receptor regulators Fingolimod, hydroxy-chloroquine, intravenous vitamin C, Vitamin D, INF beta, glucocorticoids, **ozonated autohemotherapy** (This is one of the many other compounds tried without successful available data yet), traditional Chinese medicine remedies and others treatment has been also been conducted in hospitalized patients with pneumonia and COVID-19 in China, but no results are available to date. Clinical trials of other potential therapeutics for COVID-19 are being planned.[11,12]

In addition to viral spread through a respiratory route, SARS-CoV in the intestinal tract, kidney and sweat glands may be excreted via feces, urine and sweat, thereby leading to virus transmission.[13] The angiotensin-converting enzyme 2 (ACE2) very likely serves as the binding site for SARS-CoV-2, the strain implicated in the current COVID-19 epidemic, similarly to strain SARS-CoV implicated in the 2002-2003 SARS epidemic.[14] The major comorbidities of the fatality cases include hypertension, diabetes, coronary heart disease, cerebral infarction, and chronic bronchitis. The source of the virus and the pathogenesis of this disease are still unconfirmed. No specific therapeutic drug has been found.[15]

Ozone therapy could be used in the treatment of COVID-19 in two therapeutic categories:
1) Disinfection (count with high scientific background):
   a) Contaminated environments (hospitals, transport, vehicles, all surfaces where the virus may have been deposited etc.);
   b) In aqueous solutions such as disinfections of drinking water, waste water treatment, laundry facilities, and food processing.[16]
2) Potential systemic application as a complementary medicine in order to:
   a) Improve the health status of the patients and reduce the viral load,[17-19]
   b) In the form of ozonated water mouthwash to reduce the incidence of ventilator-associated pneumonia in patients connected to mechanical ventilation.[20]

The scope of this paper is to review the potential mechanisms of action of ozone utilization that serve as complementary therapy in the management of COVID-19.

Evidence Acquisition Terms included in the information search

COVID-19, SARS-CoV-2, SARS, ozone, ozone therapy, viral pneumonia.

Bibliographic databases consulted: MEDLINE/PubMed, SciELO, LILACS, PAHO, EMBASE, ZOTERO ISCO3, WHO International Clinical Trials Registry Platform, NIH. U.S. National Library of Medicine and informational databases with search engines such as Google and Google Scholar.

Types of documents: original articles, published thesis, clinical reports, ongoing clinical trials and bibliographic reviews.

Languages: English, Russian, and Spanish. Dates of publication: 1980 to 2020.

Exclusion criteria: lack of free access to complete text due to financial constraints and/or, studies presenting inadequate scientific evidence.

Environmental disinfection

To reduce the spread of COVID-19 virus, environmental infection control procedures should be implemented.[21-25] In United States health care settings, the CDC states routine cleaning and disinfection procedures are appropriate for COVID-19 virus.[24] Products approved in USA by the Environmental Protection Agency (EPA) for emerging viral pathogens contain as active components: hydrogen peroxide, sodium hypochlorite, peroxyacetic acid, ethanol, isopropyl alcohol, alkyl dimethyl benzyl ammonium chlorides, didecyl dimethyl ammonium chloride, octyl decyl dimethyl ammonium chloride, sodium carbonate peroxyhydrate, sodium dichloro-s-triazinetrione and others.[26]

The importance of environmental disinfection was illustrated in a study from Singapore, in which viral RNA was detected on nearly all surfaces tested (handles, light switches, bed and handrails, interior doors and windows, toilet bowl, sink basin) in the airborne infection isolation room of a patient with symptomatic mild COVID-19 prior to routine cleaning.[25] Viral RNA was not detected on similar surfaces in the rooms of two other symptomatic patients following routine cleaning (with sodium dichloroisocyanurate). Of note, viral RNA detection does not necessarily indicate the presence of infectious virus. Factors influencing the survival of these viruses on surfaces include: strain variation, titre, surface type, suspending medium, mode of deposition, temperature and relative humidity, and the method used to determine the viability of the virus. Environmental sampling has identified contamination in field-settings with SARS-CoV and influenza virus, although the frequent use of molecular detection methods may not necessarily represent the presence of viable virus.

Once contaminated from the environment, hands can then initiate self-inoculation of mucous membranes of the nose, eyes or mouth. Mathematical and animal models, and intervention studies suggest that contact transmission is the most important route in some scenarios. Infection prevention and control implications include the need for hand hygiene and personal protective equipment to minimize self-contamination and to protect against inoculation of mucosal surfaces and the respiratory tract, and enhanced surface cleaning and disinfection in healthcare settings.[27]

Viruses have been studied during their interaction with ozone.[28-31] **After 30 s of exposure to ozone, 99 % of the viruses were inactivated** and demonstrated damage to their envelope proteins, which could result in failure of attachment to normal cells and breakage of the single-stranded RNA.[28] Ozone gas however has a number of potential advantages over other decontaminating gases and liquid chemical applications.[32] Thus ozone is a natural compound, is easily generated *in situ* from oxygen or air, and breaks down to oxygen with a half-life of about 20 min (± 10 min depending on the environment).[16] As a gas it can penetrate all areas within a room, including crevices, fixtures, fabrics, and the under surfaces of furniture, much more efficiently tan manually applied liquid sprays and aerosols.[33] The only significant disadvantages are its ability to corrode certain materials, such as natural rubber, on prolonged exposure, and its potential toxicity to humans.

The Occupational Safety and Health Administration (OSHA) in USA, has set Public Health Air Standards of 0.1 ppm for 8 h or 0.3 ppm for 15 min as the limit of the amount of ozone to which people can be safely exposed.[34] Air cleaners which utilize ozone must not generate ozone levels above the Public Health Standards, which are far below any antimicrobial activity or effective odour control. Low ozone concentrations, below the EPA-acceptable indoor limit, have been used as air cleaners, but their effectiveness has been questioned by many studies.[35,36] At high ozone concentration, ozone has been used to decontaminate **unoccupied spaces** of some chemical and biological contaminants and odours such as smoke.

Maximum anti-viral efficacy of ozone requires a short period of high humidity (>90% relative humidity) after the attainment of peak ozone gas concentration (20 – 25 ppm, 39-49 mg/m^3).[16] A study showed that under the treatment with ozone virus-containing samples dried onto hard surfaces (plastic, steel and glass), and soft surfaces such as fabric, cotton and carpet, were equally vulnerable to the treatment.[33] Using appropriate generators at appropriate ozone concentrations, ozone will help to decontaminate rooms, hospital room,[37] public transport, hotel room, cruise liner cabins, offices, etc. **The environment that is to be decontaminated must be free of peoples and animals due to the toxic nature of ozone by inhalation**.[38] In a case of accidental inhalation it is recommended to follow the first aids measures recommended by ISCO3.[39] Ozone gas has been also used in the disinfection of hospital laundry.[40] In addition, it may be used in the treatment of waste water residues.[41] Conventional sewage treatment reduce the amount of all viruses but, further ozonation reduced the amounts of several viruses to undetectable levels, indicating that this is a promising technique for reducing the transmission of many pathogenic human viruses.[42]

Aqueous solutions of ozone are in use as disinfectants in many commercial situations, including waste water treatment,[43] laundries,[44] drinking water[45] and food processing.[46,47] Ozone is considering a highly effective disinfectant for virus control.[48] Ozone exposure reduced viral infectivity by lipid peroxidation and subsequent lipid envelope and protein shell damage.[29]

Therapeutic actions of ozone in viral diseases

Ozone can inactivate viruses via direct oxidation of its components.[29] However the virucidal activity *in vivo* becomes uncertain when viruses are in biological fluids or, even worse, when they are intracellular (pneumocytes, hepatocytes, epithelia, CD4+ lymphocytes, monocytes, glial and neuronal cells) because, the potent antioxidant system protects viral integrity.[49] That is why it is irrational to use direct IV injection of gas or other non-recommended methods of application of ozone.[50] Ozone therapy represents a useful adjunctive and complementary therapy but neither ozone, nor H_2O_2 reach sufficient concentrations in tissues because free pathogens are protected by plasma antioxidants and intracellular viruses are inaccessible.[51] However, in order to explore the efficacy of ozone therapy in viral diseases, Bocci and Paulesu[52] explain the possibility that ozone may act *in vivo*. The following mechanisms may have some relevance:

a) *A prolonged ozone therapeutic treatment appears able to induce an adaptation to oxidative stress,* hence a re-equilibration of the cellular redox state, which is a fundamental process for inhibiting viral replication that will be blocked. The paradoxical mechanism by which ozone (a potent oxidant) can induce an antioxidant response, is currently demonstrated not only at a proteomic level, but also at a genomic one. Ozone at therapeutic dose modulates the nuclear factor Nrf2 and NfκB and induces the re-equilibrium of the antioxidant environment.[53-58] Oxidative stress and innate immunity have a key role in lung injury pathways that control the severity of acute lung injury during viral infections like SARS.[59]

b) *The induction of cytokine synthesis, such as IFN and IL,* in ozonated blood has been shown to be possible. Although ozone is a weak inducer, the reinfused lymphocytes and monocytes, by migrating through the lymphoid system, can activate other cells that, in time, will lead to a stimulation of the immune system. This may represent an important process because it is known that an acute viral disease becomes chronic either because the virus is particularly virulent, or because the heterogenous viral population evolves rapidly and escapes immune control, or because the immune system becomes tolerant to viral antigens and becomes unable to counteract the infection. Moreover, besides the induction of HO-1,[58] a protective enzyme, the release of some heat shock proteins (HSP) such as HSP60, HSP70 and HSP90 are also influential in viricidal activity. These proteins are potent activators of the innate immune system, able to induce the synthesis of proinflammatory cytokines by the monocyte-macrophage system and the activation of antigen-presenting cells.[49,60]

c) *Oxygen-ozone therapy certainly improves oxygenation.*[61,62] The patients with SARS are prone to have mild non-specific hepatitis,[63] lung fibrosis[64] and renal failure may be present.[65] Ozone therapy stabilizes hepatic metabolism and fibrinogen and prothrombin plasma levels tend to normalize in infected patients, suggesting an improvement of the hepatic protein synthesis.[49] There is a lot of research demonstrating the protective effect of ozone to prevent oxidative damage to heart,[66,67] liver,[68,69] lung[70] and renal tissue.[71]

d) During blood ozonation *ex vivo* for the minor AHT, using ozone concentrations near 90 *μg*/mL per mL of blood, it may be feasible to induce the oxidation of free viral components, which could represent an inactivated and immunogenic vaccine.[49,72,73]

e) *Ozonized Saline Solution*. This method was formalized by the Ministry of Health of the Russian Federation in the early 1980´s and has been officially implemented in public health hospitals, specifically for the specialties of orthopedics, dermatology, gynecology and obstetrics.[74,75] In 2004, it was also officially recognized in Ukraine.[76] The method is supported by a large amount of scientific papers and a strong clinical experience about the benefits of this therapy.[77]

The method consists of bubbling and saturating a physiological solution (0.9%) with ozone-oxygen mixture at concentrations that are calculated depending on the patient's

weight. Its administration takes about 20-30 min. Unlike Major Autohemotherapy, the Ozonized Saline Solution has proven to be especially effective in viral diseases such as Epstein Barr, Cytomegalovirus, Papillomavirus, HIV, Herpes Zoster, Herpes Simplex, etc. Since the Saline Solution is a plasma expander, O_3SS represents a greater amount of blood being treated than MAH and therefore, the number of sessions may need to be reduced.

An analysis of bibliographic data on the interaction of ozone with NaCl in aqueous solutions, allows us to conclude that the decomposition of ozone in aqueous solutions of NaCl is not accompanied by formation of products other than oxygen. In particular, no noticeable amounts of hypochlorites and chlorates are observed. This is particularly significant for medicinal application of ozonized isotonic solutions.[78,79]

When ozone dissolves in water, free radicals, hydrogen peroxide (in an insignificant amount!), hexagonal water structures and small molecules are formed. Hexagonal water molecules formed during ozonation of aqueous solutions improves transport across the cell membrane not only of electrolytes, but possibly also of other substances.[80]

Boyarinov G.A. and Sokolov V.V.[81,82] showed that when an ozonized cardiopulmonary bypass (cardiopulmonary cardiopulmonary bypass) is performed, the cells of the patient's organism use more glucose than when it is oxygenated. Therefore, it is concluded that dissolved O_2/O_3 mixture, free radicals, hydrogen peroxide and hexagonal aqueous structures formed during the bubbling of aqueous NaCl solutions with a mixture of O_2/O_3 gas, determine the therapeutic effect of ozonated physiological solution.

The procedure is not only effective and safe, but it is much more economical and easier to implement.

Recommended administration route

Recommended administration routes are the systemic and in this order: Ozonized Saline Solution (O_3SS), Major Autohemotherapy (MAH), Extracorporeal Blood Oxygenation-Ozonation (EBOO) and a variant of the Minor Autohemotherapy (MiAH) (using 90 μg/mL). The summary of the administration of each procedure is described in the Madrid Declaration on Ozone Therapy.[74] In addition, a step by step procedure is available in writing according to the Good Clinical Practice to conduct each procedure and can be downloaded from the ISCO3 web site (www.isoc3.org).[83-85]

Recommended clinical protocol with O_3SS

These recommendations are based on the clinical experience of the ozone therapist and should be submitted to further clinical trials. Note that currently there are three clinical trials in China using the MAH, but preliminary results are not available yet.[17-19]

Preventive protocol with O_3SS

Saturation of the physiologic saline solution 0.9% at 3 μg/NmL for 10 min. Once the solution has been saturated, set up the same parameters and proceed to administer to the patient under continuous bubbling at 80/120 drops/min. Administer twice a week for 6 treatments.
After administering the O_3SS, administer i.v. Glutathione (GSH) 600 mg + Vit. C 1 g dissolved in 100 mL of physiologic solution. Once a week for 3 treatments.

Interventional protocol with O_3SS

Saturation of the physiological saline solution 0.9% at 5 µg / NmL for 10 min. Administer to the patient under continuous bubbling with the same parameters described at a rate of 80/120 drops / min. Apply daily, for 5 days. In the following 5 days the concentration is reduced to 3 µg / NmL and administered daily. 10 treatments in total. Immediately after O_3SS, administer i.v. GSH 1.2 g + Vit. C 2 g. dissolved in 100 mL of physiological solution. Administer twice a week. Four (4) treatments in total.

Since the disease concurs with an acute oxidative stress, we include GSH due to its capacity to donate electrons and stabilize the free radicals generated by the virus. GSH is a non-enzymatic antioxidant, and is one of the first lines of defence against oxidative damage. During aging, GSH content declines and the immune system undergoes a deficiency in the induction of Th1 response. Reduced secretion of Th1 cytokines, which is associated with GSH depletion, could weaken the host defences against viral infections.[86]

Devices (Ozone Generators and Disposables)

The ozone must be produced by a medically reliable and certified generator. Ozone generators are medical devices classified within the European Union as medical device class IIa and to have the CE seal accompanied by four numbers (art. 9, Council Directive 93/42/EEC, in accordance with Annex IX of the same directive). The generator must allow the measurements of precise ozone concentrations (from 1 μg/NmL - 80 μg/NmL) and produce ozone exclusively from medicinal oxygen grade coming from a medical quality certified container.

The equipment should have the facility to regulate the output flow between 200-500 mL / min and be able to administer continuous flow at very low concentrations (2-5 μg/mL).

Disposables to administer the therapy should be free of phthalates and resistant to ozone. For more detail about devise and disposable consult ISCO3/DEV/01/01.[87]

Concluding Remarks

Ozone can be useful for disinfection, its maximum anti-viral efficacy requires a short period of high humidity (>90% relative humidity) after the attainment of peak ozone gas concentration (20 – 25 ppm, 39-49 mg/m^3). In any case, spaces have to be free of people because of the toxicity of ozone by inhalation. The environment to be treated must be free of people and animals due to the relative toxicity of ozone via inhalation.

Systemic ozone therapy can be potentially useful in SARS-CoV-2. The rationale and mechanism of action have already been proven clinically with other viral infections and have been shown to be highly effective in research studies. The mechanism of action is as follows: 1) The induction of adaptation to oxidative stress, hence a re-equilibration of the cellular redox state. 2) The induction of IFN-gamma and proinflammatory cytokines. 3) The increase of blood flow and tissue oxygenation to vital organs (i.e. renal, pulmonary and cardiac circulation). 4) It has the potential to act as an auto-vaccine when administered in the form of minor autohemotherapy.

The recommended systemic administration is: Ozonized Saline Solution (O_3SS), Major Autohemotherapy (MAH), and Extracorporeal Blood Oxygenation-Ozonation (EBOO). Clinical protocols should comply with the standard doses and procedures defined in the Madrid Declaration of Ozone Therapy. [71] At least three clinical trials using major autohemotherapy are in progress in China and more clinical trials are needed to confirm the efficacy of ozone therapy as complementary therapy in the treatment of COVID-19 diseases. It is a complementary therapy because while the infected patient is treated with allopathic medicine, at the same time the patient is also receiving the complementary proposed treatment.

References

1. WHO. Responding to community spread of COVID-19 Interim guidance 7 March 2020. WHO file:///C:/Users/USER-PC/Downloads/WHO-COVID-19-Community_Transmission-2020.1-eng.pdf) (Acessed on 12/03/2020)2020.
2. Ghebreyesus T. WHO Director-General's opening remarks at the media briefing on COVID-19 - 11 March 2020. https://www.who.int/dg/speeches/detail/who-director-general-s-opening-remarks-at-the-media-briefing-on-covid-19---11-march-2020 (Acessed on 12/03/2020)2020.
3. Ghebreyesus T. WHO Director-General's opening remarks at the media briefing on COVID-19 - 12 March 2020. https://www.who.int/dg/speeches/detail/who-director-general-s-opening-remarks-at-the-mission-briefing-on-covid-19--12-march-2020) (Acesses on 12/03/2020)2020.
4. Lai CC, Shih TP, Ko WC, Tang HJ, Hsueh PR. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and coronavirus disease-2019 (COVID-19): The epidemic and the challenges. *Int J Antimicrob Agents.* Feb 17 2020:105924.
5. WHO. World Health Organization. Director-General's remarks at the media briefing on 2019-nCoV on 11 February 2020. https://www.who.int/dg/speeches/detail/who-director-general-s-remarks-at-the-media-briefing-on-2019-ncov-on-11-february-2020 (Accessed on March 3, 2020). 2020.
6. Li X, Zai J, Zhao Q, et al. Evolutionary history, potential intermediate animal host, and cross-species analyses of SARS-CoV-2. *J Med Virol.* Feb 27 2020.
7. Phan T. Genetic diversity and evolution of SARS-CoV-2. *Infect Genet Evol.* Feb 21 2020;81:104260.
8. Habibzadeh P, Stoneman EK. The Novel Coronavirus: A Bird's Eye View. *Int J Occup Environ Med.* Feb 5 2020;11(2):65-71.
9. Xiong Y, Sun D, Liu Y, et al. Clinical and High-Resolution CT Features of the COVID-19 Infection: Comparison of the Initial and Follow-up Changes. *Invest Radiol.* Mar 3 2020.

10. Wang M, Cao R, Zhang L, et al. Remdesivir and chloroquine effectively inhibit the recently emerged novel coronavirus (2019-nCoV) in vitro. *Cell Res.* Mar 2020;30(3):269-271.
11. WHO. World Health Organization. International Clinical Trials Registry Platform. http://apps.who.int/trialsearch/ [Accessed on 07/03/2020]. 2020.
12. NIH. U.S. National Library of Medicine. Clinical Trials.gov https://www.clinicaltrials.gov/ [Accessed on 07/03/2020]. 2020.
13. Ding Y, He L, Zhang Q, et al. Organ distribution of severe acute respiratory syndrome (SARS) associated coronavirus (SARS-CoV) in SARS patients: implications for pathogenesis and virus transmission pathways. *J Pathol.* Jun 2004;203(2):622-630.
14. Gurwitz D. Angiotensin receptor blockers as tentative SARS-CoV-2 therapeutics. *Drug Dev Res.* Mar 4 2020.
15. Deng SQ, Peng HJ. Characteristics of and Public Health Responses to the Coronavirus Disease 2019 Outbreak in China. *J Clin Med.* Feb 20 2020;9(2).
16. Hudson JB, Sharma M, Vimalanathan S. Development of a Practical Method for Using Ozone Gas as a Virus Decontaminating Agent. *Ozone: Science & Engineering.* 2009;31:216-223.
17. Guangjian N, Hongzhi Y. Clinical study for ozonated autohemotherapy in the treatment of Novel Coronavirus Pneumonia (COVID-19). ChiCTR2000030165. Academy of Medical Engineering and Translational Medicine, Tianjin University. 2020-02-24. http://www.chictr.org.cn/showproj.aspx?proj=49947 (Accessed 8/03/2020). . 2020.
18. Linlin H, Xiangdong C. A randomized controlled trial for the efficacy of ozonated autohemotherapy in the treatment of Novel Coronavirus Pneumonia (COVID-19). ChiCTR2000030006. Union Hospital, Tongji Medical College, Huazhong University of Science and Technology. 2020-02-19. http://www.chictr.org.cn/showproj.aspx?proj=49737 (Accessed 8/03/2020). . 2020.
19. Huiling H, Tong X. A multicenter randomized controlled trial for ozone autohemotherapy in the treatment of novel coronavirus pneumonia (COVID-19). ChiCTR2000030102. Tianjin Huanhu Hospita. 2020-02-23. http://www.chictr.org.cn/showproj.aspx?proj=49747 (Accessed 8/03/2020). 2020.
20. Soodmand M. A survey of the effect of ozonated water mouthwash on oral health and incidence of ventilator-associated pneumonia in patients connected to mechanical ventilation in intensive care units - A randomized clinical trial. IRCT20180213038720N2. Registration date: 2019-12-18, 1398/09/27 https://en.irct.ir/trial/41951 (Accessed 8/03/2020). . 2019.
21. WHO. World Health Organization. Home care for patients with suspected novel coronavirus (nCoV) infection presenting with mild symptoms and management of contacts. Updated February 4, 2020. https://www.who.int/publications-detail/home-care-for-patients-with-suspected-novel-coronavirus-(ncov)-infection-presenting-with-mild-symptoms-and-management-of-contacts (Accessed on March 8, 2020). 2020.
22. CDC. Centers for Disease Control and Prevention. Interim guidance for persons who may have 2019 Novel Coronavirus (2019-nCoV) to prevent spread in homes and residential communities. https://www.cdc.gov/coronavirus/2019-ncov/hcp/guidance-prevent-spread.html#First_heading (Accessed on March 08, 2020). 2020.
23. WHO. World Health Organization. Infection prevention and control during health care when novel coronavirus (nCoV) infection is suspected. January 25, 2020. https://www.who.int/publications-detail/infection-prevention-and-control-during-health-care-when-novel-coronavirus-(ncov)-infection-is-suspected-20200125 (Accessed on March 08, 2020). 2020.
24. CDC. Centers for Disease Control and Prevention. Interim Infection Prevention and Control Recommendations for Patients with Confirmed 2019 Novel Coronavirus (2019-nCoV) or Patients Under Investigation for 2019-nCoV in Healthcare Settings. February 3, 2020. https://www.cdc.gov/coronavirus/2019-nCoV/hcp/infection-control.html (Accessed on March 08, 2020). 2020.
25. Ong SWX, Tan YK, Chia PY, et al. Air, Surface Environmental, and Personal Protective Equipment Contamination by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) From a Symptomatic Patient. *JAMA.* Mar 4 2020.
26. EPA. United States Environmental Protection Agency. EPA's Registered Antimicrobial Products for Use Against Novel Coronavirus SARS-CoV-2, the Cause of COVID-19. 03/03/2020. https://www.epa.gov/pesticide-registration/list-n-disinfectants-use-against-sars-cov-2 (Accessed 8/03/2020). 2020.
27. Otter JA, Donskey C, Yezli S, Douthwaite S, Goldenberg SD, Weber DJ. Transmission of SARS and MERS coronaviruses and influenza virus in healthcare settings: the possible role of dry surface contamination. *J Hosp Infect.* Mar 2016;92(3):235-250.
28. Roy D, Wong PK, Engelbrecht RS, Chian ES. Mechanism of enteroviral inactivation by ozone. *Appl Environ Microbiol.* Mar 1981;41(3):718-723.
29. Murray BK, Ohmine S, Tomer DP, et al. Virion disruption by ozone-mediated reactive oxygen species. *J Virol Methods.* Oct 2008;153(1):74-77.
30. Lin YC, Wu SC. Effects of ozone exposure on inactivation of intra- and extracellular enterovirus 71. *Antiviral Res.* Jul 2006;70(3):147-153.

31. Kekez MM, Sattar SA. A new ozone-based method for virus inactivation: preliminary study. *Phys Med Biol.* Nov 1997;42(11):2027-2039.
32. Barker J, Vipond IB, Bloomfield SF. Effects of cleaning and disinfection in reducing the spread of Norovirus contamination via environmental surfaces. *J Hosp Infect.* Sep 2004;58(1):42-49.
33. Hudson JB, Sharma M, Petric M. Inactivation of Norovirus by ozone gas in conditions relevant to healthcare. *J Hosp Infect.* May 2007;66(1):40-45.
34. OSAHA. Occupational Safety and Health Administration. Occupational Safety and Health Standards. Toxic and Hazardous Substances. 1910.1000 TABLE Z-1 Limits for Air Contaminants. https://www.osha.gov/laws-regs/regulations/standardnumber/1910/1910.1000TABLEZ1 (Accessed 8/03/2020). 2020.
35. Dyas A, Boughton BJ, Das BC. Ozone killing action against bacterial and fungal species; microbiological testing of a domestic ozone generator. *J Clin Pathol.* Oct 1983;36(10):1102-1104.
36. Foarde KK, VanOsdell DW, Steiber RS. Investigation of Gas-Phase Ozone as a Potential Biocide. *Applied Occupational and Environmental Hygiene* 1997;12(8):535-542.
37. Lemon SM. SARS: Clearing the air. In: Knobler S, Mahmoud A, Lemon S, Mack A, Sivitz L, Oberholtzer K, eds. *Learning from SARS: Preparing for the Next Disease Outbreak: Workshop Summary*. Washington (DC): National Academies Press; 2004:376.
38. Bocci V, Borrelli E, Travagli V, Zanardi I. The ozone paradox: ozone is a strong oxidant as well as a medical drug. *Med Res Rev.* Jul 2009;29(4):646-682.
39. ISCO3. First Aids in Ozone Therapy. (Inhalatory exposition and accidental over dose). 2015; 16. Available at: www.isco3.org. Accessed March 8, 2020.
40. Cardoso CC, Fiorini JE, Ferriera LR, Gurjao JW, Amaral LA. Disinfection of hospital laundry using ozone: microbiological evaluation. *Infect Control Hosp Epidemiol.* Apr 2000;21(4):248.
41. Wang J, Shih Y, Wang PY, Yu YH, Su JF, Huang CP. Hazardous waste treatment technologies. *Water Environ Res.* Oct 2019;91(10):1177-1198.
42. Wang H, Sikora P, Rutgersson C, et al. Differential removal of human pathogenic viruses from sewage by conventional and ozone treatments. *Int J Hyg Environ Health.* Apr 2018;221(3):479-488.
43. Gottschalk C, Libra JA, Saupe A. *Ozonation of Water and Waste Water: A Practical Guide to Understanding Ozone and its Application*: ohn Wiley & Sons; 2008.
44. Cardis D, Tapp C, DeBrum M, Rice RG. Ozone in the Laundry Industry-Practical Experiences in the United Kingdom. *Ozone: Sci. Eng.* 2007;29:85-89.
45. Shin GA, Sobsey MD. Reduction of Norwalk virus, poliovirus 1, and bacteriophage MS2 by ozone disinfection of water. *Appl Environ Microbiol.* Jul 2003;69(7):3975-3978.
46. Kim JG, Yousef AE, Dave S. Application of ozone for enhancing the microbiological safety and quality of foods: a review. *J Food Prot.* Sep 1999;62(9):1071-1087.
47. Naito S, Takahara H. Ozone Contribution in Food Industry in Japan. *Ozone Sci. Eng.* 2006;28:425–429.
48. Wolf C, von Gunten U, Kohn T. Kinetics of Inactivation of Waterborne Enteric Viruses by Ozone. *Environ Sci Technol.* Feb 20 2018;52(4):2170-2177.
49. Bocci V. *Ozone: A new medical drug*. Netherlands: Springer; 2011.
50. ISCO3. Non-recommended routes of application in ozone therapy *ISCO3/LEG/00/10.* 2017:13. www.isco3.org.
51. Burgassi S, Zanardi I, Travagli V, Montomoli E, Bocci V. How much ozone bactericidal activity is compromised by plasma components? *J Appl Microbiol.* May 2009;106(5):1715-1721.
52. Bocci V, Paulesu L. Studies on the biological effects of ozone 1. Induction of interferon gamma on human leucocytes. *Haematologica.* Nov-Dec 1990;75(6):510-515.
53. Martinez-Sanchez G. Mechanisms of action of O3. Genomic pathways. *Ozone Therapy Global Journal.* 2019 2019;9(1):21-22.
54. Delgado-Roche L, Riera-Romo M, Mesta F, Hernández-Matos Y, Barrios JM, Martínez-Sánchez G. Medical Ozone Promotes Nrf2 Phosphorylation Reducing Oxidative Stress And Proinflammatory Cytokines In Multiple Sclerosis Patients. *Rev Esp Ozonoterapia.* 2018 2018;8(2 Supp 1):48-49.
55. Martinez-Sanchez G, Delgado-Roche L. Up-date on the mechanisms of action of ozone through the modification of cellular signaling pathways. Role of Nrf2 and NFkb. *Rev Esp Ozonoterapia.* 2017 2017;7(2):17-18.
56. Bocci V, Valacchi G. Nrf2 activation as target to implement therapeutic treatments. *Front Chem.* 2015;3:4.
57. Re L, Martinez-Sanchez G, Bordicchia M, et al. Is ozone pre-conditioning effect linked to Nrf2/EpRE activation pathway in vivo? A preliminary result. *Eur J Pharmacol.* Nov 5 2014;742:158-162.
58. Pecorelli A, Bocci V, Acquaviva A, et al. NRF2 activation is involved in ozonated human serum upregulation of HO-1 in endothelial cells. *Toxicol Appl Pharmacol.* Feb 15 2013;267(1):30-40.
59. Imai Y, Kuba K, Neely GG, et al. Identification of oxidative stress and Toll-like receptor 4 signaling as a key pathway of acute lung injury. *Cell.* Apr 18 2008;133(2):235-249.

60. Larini A, Bocci V. Effects of ozone on isolated peripheral blood mononuclear cells. *Toxicol In Vitro.* Feb 2005;19(1):55-61.
61. Lintas G, Liboni W, Simonetti V, et al. Long-term cerebrovascular reactivity mediated by ozone autohemotherapy: a NIRS study. Paper presented at: Terzo Congresso del Gruppo Nazionale di Bioingegneria; 2012, 2012.
62. Zaky S, Fouad EA, Kotb HIM. The effect of rectal ozone on the portal vein oxygenation and pharmacokinetics of propranolol in liver cirrhosis (a preliminary human study). *British Journal of Clinical Pharmacology.* Mar 2011 2011;71(3):411-415.
63. Guan YJ, Tang XP, Yin CB, Yi ZQ. [Study on the damage of liver in patients with SARS]. *Zhongguo Wei Zhong Bing Ji Jiu Yi Xue.* May 2004;16(5):267-270.
64. Venkataraman T, Frieman MB. The role of epidermal growth factor receptor (EGFR) signaling in SARS coronavirus-induced pulmonary fibrosis. *Antiviral Res.* Jul 2017;143:142-150.
65. Khan G. A novel coronavirus capable of lethal human infections: an emerging picture. *Virol J.* Feb 28 2013;10:66.
66. Simonetti V, Quagliariello V, Franzini M, Iaffaioli RV, Maurea N, Valdenassi L. Ozone Exerts Cytoprotective and Anti-Inflammatory Effects in Cardiomyocytes and Skin Fibroblasts after Incubation with Doxorubicin. *Evid Based Complement Alternat Med.* 2019;2019:2169103.
67. Delgado-Roche L, Hernandez-Matos Y, Medina EA, Morejon DA, Gonzalez MR, Martinez-Sanchez G. Ozone-Oxidative Preconditioning Prevents Doxorubicin-induced Cardiotoxicity in Sprague-Dawley Rats. *Sultan Qaboos Univ Med J.* Aug 2014;14(3):e342-348.
68. Adali Y, Eroglu HA, Makav M, Guvendi GF. Efficacy of Ozone and Selenium Therapy for Alcoholic Liver Injury: An Experimental Model. *In Vivo.* May-Jun 2019;33(3):763-769.
69. Tezcan AH, Ozturk O, Ustebay S, Adali Y, Yagmurdur H. The beneficial effects of ozone therapy in acetaminophen-induced hepatotoxicity in mice. *Pharmacol Rep.* Apr 2018;70(2):340-345.
70. Kaldirim U, Uysal B, Yuksel R, et al. Ozone therapy ameliorates paraquat-induced lung injury in rats. *Exp Biol Med (Maywood).* Dec 2014;239(12):1699-1704.
71. Wang L, Chen H, Liu XH, et al. Ozone oxidative preconditioning inhibits renal fibrosis induced by ischemia and reperfusion injury in rats. *Exp Ther Med.* Dec 2014;8(6):1764-1768.
72. Bocci V, Zanardi I, Travagli V. Ozonation of human HIV-infected plasmas for producing a global vaccine: How HIV-patients may help fight the HIV pandemia. *Virulence.* May-Jun 2010;1(3):215-217.
73. Bocci V, Travagli V, Zanardi I. The failure of HIV vaccines: a new autovaccine may overcome some problems. *Medical Hypotheses.* Jun 2009 2009;72(6):662-664.
74. Schwartz-Tapia A, Martínez-Sánchez G, Sabah F, et al. Madrid Declaration on Ozone Therapy. . *ISCO3.* 2015:50.
75. Peretiagyn SP, Struchkov AA, eretiagyn NC, Kulechina NB, Inventors; 2289413, assignee. Ozonization Method of Saline Solution2006.
76. Shmakova IP, Nazarov EI. Methods of application of ozone in medicine (guidelines). 2004.
77. Maslennikov OV, Kontorshikova CN, Gribkova IA. *Ozone therapy in Practice. Health Manual, Ministry Health Service of The Russian Federation The State Medical Academy Of Nizhny Novgorod, Russia.* http://www.absoluteozone.com/assets/ozone_therapy_in_practice.pdf. 1 ed2008.
78. Razumovskii SD, Konstantinova ML, Grinevich TV, Korovina GV, Zaitsev VY. Mechanism and kinetics of the reaction of ozone with sodium chloride in aqueous solutions. *Kinetics and Catalysis.* 2010;51(4):492-496.
79. Boyarinov GA, Gordetsov AS, Peretyagin SP, Matusyak KS, Ovchinnikov YV, Boyarinova LV. The analysis of interaction of ozone and sodium chloride in Aqueous solution. *Rev Esp de Ozonoterapia.* 2016;6(Supp 1):77.
80. Gorbunov SN, Korhouknov AE, Mozhaev MV, et al. [Structural-molecular transformations of water solutions of electrolytes under the influence of medical ozone]. *Meditsinskii almanakh.* 2013 2013(3):38-40.
81. Boyarinov GA, Sokolov VV. *Ozonized cardiopulmonary bypass (experimental justification and clinical results).* Nizhny Novgorod,1999.
82. Boyarinov GA, Monakhov AN, Medvedev AP, Chiginev VA, Beaver VM, Gamzaev AB. *The effect of the ozonized cardioplegic solution on cardiodynamics during cardiac valve prostheses // In the book: Ozone in biology and medicine: Abstract. Doc. II Vseros. Scientific and practical confer. with international participation Nizhny Novgorod.* Nizhny Novgorod1995.
83. ISCO3 I. ISCO3/MET/00/01 Major Autohemotherapy (AHTmayor).
84. ISCO3. ISCO3/MET/00/02 Minor Autohemotherapy. www.isco3.org2016.
85. ISCO3. Extracorporeal blood oxygenation-ozonation (EBOO) *ISCO3/MET/00/22.* 2016;1:9.
86. Amatore D, Celestino I, Brundu S, et al. Glutathione increase by the n-butanoyl glutathione derivative (GSH-C4) inhibits viral replication and induces a predominant Th1 immune profile in old mice infected with influenza virus. *FASEB Bioadv.* May 2019;1(5):296-305.
87. ISCO3. Guidelines and Recommendations for Medical Professionals Planning to Acquire a Medical Ozone Generator. *International Scientific Committee of Ozone Therapy* www.isco3.org 2019. Accessed 11/03, 2020.