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Potential use of ozone in SARS-CoV-2 / COVID-19.

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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.
O₃SS: 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/m³). 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 (O₃SS), 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 (\pm 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 than 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/m³).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 water⁴⁵ 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 H₂O₂ 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 Paulesu⁵² 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 severe 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 fibrosis⁶⁴ 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} lung⁷⁰ 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, O₃SS 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 saline solution as used during cardiopulmonary bypass, the cells of the patient's organism use more glucose than when it is oxygenated. Therefore, it is concluded that dissolved O₂/O₃ mixture, free radicals, hydrogen peroxide and hexagonal aqueous structures formed during the bubbling of aqueous NaCl solutions with a mixture of O₂/O₃ 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 (O₃SS), 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 O₃SS

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 O₃SS

Saturation of the physiologic saline solution 0.9% at 3 μg / kg b.w. for 10 min. Administer to the patient under bubbling with the same parameters at 80/120 drops/min. Twice a week for 6 treatments. After administering the O₃SS, administer i.v. Glutathione (GSH) 600 mg + Vit. C 1 g dissolved in 100 mL of physiologic solution. Twice a week for 6 treatments.

Interventional protocol with O₃SS

Saturation of the physiologic saline solution 0.9 % at 5 μg / kg b.w. for 10 min. Administer to the patient under bubbling with the same parameters at 80/120 drops/min. Every day during 5 days. The following 5 days lower the concentration to 3 μg / kg b.w. 10 treatments in total. After each O₃SS, administer i.v. GSH 1,2 g + Vit. C 2 g. dissolved in 100 mL of physiologic solution. Administer 10 treatments, twice a week.

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/IIb 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 $\mu\text{g}/\text{NmL}$ - 80 $\mu\text{g}/\text{NmL}$) 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 $\mu\text{g}/\text{mL}$).

Disposables to administer the therapy should be free of phthalates and resistant to ozone. For more detail about device 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/m³). 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 (O₃SS), 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.

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