Ozonoterapija u liječenju bolesti COVID-19: analiza literature o mehanizmu djelovanja, učinkovitosti i sigurnosti

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UNDERGRADUATE PROGRAMME OF STUDY IN NURSING

OXYGEN OZONE THERAPY IN THE TREATMENT OF COVID-19: CURRENT STATE OF EVIDENCE ON THE MECHANISMS OF ACTION, EFFECTIVENESS AND SAFETY

Final thesis no. 46/SES/2022

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Bjelovar, December 2022

obrazac ZR - 001

Veleučilište u Bjelovaru

Trg E. Kvaternika 4, Bjelovar

1. DEFINIRANJE TEME ZAVRŠNOG RADA I POVJERENSTVA

Student: **Milović Andreja** JMBAG: **0314021959**

Naslov rada (tema): **Ozonoterapija u liječenju bolesti COVID-19: analiza literature o mehanizmu djelovanja, učinkovitosti i sigurnosti**

Područje: **Biomedicina i zdravstvo** Polje: **Kliničke medicinske znanosti**

Grana: **Infektologija**

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3. **Đurđica Grabovac, dipl.med.techn., član**

2. ZADATAK ZAVRŠNOG RADA BROJ: 46/SES/2022

This requirements for this thesis are:

1. To explain the course and public health significance of coronavirus disease 2019 (COVID-19) pandemic.
2. To discuss the most important and recognized therapeutic effects of systemic oxygen ozone treatment.
3. To elaborate on the mechanism and effectiveness of oxygen ozone therapy for various applications, as well as it purported role as monotherapy or an effective adjuvant treatment in COVID-19.
4. To review relevant clinical trials that used systemic intravenous therapy by ex vivo ozonization, known internationally as major-autohemotherapy (M-AHT), for COVID-19 treatment.
5. To critically appraise the available literature and provide recommendations for further practice.
6. To highlight the role of nursing professional in safe preparation and use of oxygen ozone therapy techniques, education and preparation of the patients, assistance in administration of the prescribed therapy and in the monitoring of the effects.

Datum: 17.05.2022. godine

Mentor: **dr. sc. Tomislav Meštrović**

Acknowledgments

I would like to thank my mentor, dr. sc. Tomisav Meštrović, MD, PhD, for his skillful guidance and support in writing this thesis, as well as my co-mentor, prof. Gerardo Tricarico, MD, DMD, for his clinical mentorship, sharing his expertise in ozonetherapy and experience in its use in COVID-19. My heartfelt gratitude goes out to my family for their support and understanding: to my late father, who was a pioneering patient from the beginning of my clinical experience with ozonetherapy; to my dear husband for his steady patience; to my loving mother for her care. Warm thanks to my colleagues and friends for their encouragement. A special thanks goes to all the faculty members of the Undergraduate Professional Program of Study in Nursing and the Bjelovar University of Applied Sciences staff. With deep gratitude, I am extending my thanks to the predecessors of our teachers for developing, preserving, and generously sharing their knowledge so that we can continue to help those in need. Lastly, I would like to thank all our patients for their willingness to explore the roads less traveled and make indispensable contributions to our body of knowledge.

Table of Content

1. INTRODUCTION

The Coronavirus Disease 2019 (COVID-19) epidemic, caused by the novel *betacoronavirus* named Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV2), has presented many challenges to humankind in different arenas since its onset in November 2019 and declaration of pandemic in March 2020. The world faced several pandemic waves, with consecutive lockdown measures affecting every aspect of daily life. On 26th November 2022, the Johns Hopkins University and Medicine COVID-19 tracker showed 641,233,928 confirmed cases and 6,630,179 deaths globally, estimating an observed case-fatality ratio of 1.03% (1). Croatia reported 1,252,886 confirmed cases, 17,292 deaths, and a 1.38% observed case-fatality ratio (2).

The rapid spread of SARS-CoV-2, together with a lack of adequate treatment, created an overwhelming burden on the health care systems across the world. Research and exploration of old and new therapeutic modalities was urged and facilitated by the open access to published results, which allowed for the timely sharing of valuable information.

Oxygen ozone therapy refers to the use of an oxygen-ozone gas mixture in medicine, and it has been in use for more than a hundred years. The first application in medicine was as a wound disinfectant during the pre-antibiotic era of World War I. Since modern medical ozone generators and ozone-resistant materials have been made, ozone therapy have been used successfully in almost all clinical branches of medicine, as well as in dentistry and veterinary medicine (3).

The World Federation of Ozone Therapy (WFOT) states that ozone therapy is currently practiced in over 50 countries by more than 26,000 health professionals, with an increasing trend (4).

In Croatia, there are currently no registered professional associations for ozone therapy as part of the national professional societies of physicians, dentists, nurses, and veterinarians, nor are there any other associations related to ozone therapy. So far, ozone therapy is practiced only in private clinics in the areas of dentistry, dermatology, orthopedics, sports medicine, regenerative medicine, and integrative medicine. An ongoing project in the application of ozone therapy in vascular diseases at the University Hospital Center Zagreb represents the beginning of the use of ozone therapy in public hospitals. In

2021, the Croatian Nursing Council offered a continuing education lecture titled *Perioperative care for the patient for ozone therapy of the spine* (5). The number of scientific publications about ozone therapy in Croatia is very modest. Search results for published articles showed 4 articles in veterinary medicine, 3 in dentistry, and 2 review articles on ozone therapy in general (6). A search for academic sources showed 8 theses in dentistry, 1 in medicine, 1 in biotechnology, and 1 in veterinary medicine (7). As more research demonstrates the efficacy and safety of ozone therapy in a wide range of diseases, health care professionals in Croatia are becoming more interested in education and up-to-date training.

To illustrate the scientific interest in publications of articles containing the words "ozone therapy," a full-text search on the PubMed Central database on 26th November 2022, resulted in 13,310 records (8). Calculating the average daily article appearance since the beginning of the COVID-19 pandemic shows a significant increase. 2.7 articles per day in 2019, 4.3 articles per day in 2020, peaking at 5.3 articles per day in 2021, and continuing with 4.8 articles per day in 2022 so far.

The well-proven antimicrobial, anti-inflammatory, and immunomodulatory effects of ozone therapy in the treatment of infectious diseases, as well as the effects of cytokine modulation, oxidative stress modulation, improvement of circulation and tissue oxygenation, reparation of vascular endothelium, and hypo-coagulation effects, were key leaders in the exploration of the application of ozone therapy in COVID-19 treatment (9-11).

Studies have demonstrated the effectiveness and safety of ozone therapy as a complementary therapy in the treatment of COVID-19 patients. Depending on the routes of administration, various clinical effects were achieved in various degrees of disease severity, resulting in a reduction of symptoms, a reduced time period for returning a PCR negative test, reduced hospitalization time, and a reduced mortality rate. The most frequent systemic routes of oxygen-ozone application were: major autohemotherapy (M-AHT or MAH), intravenous infusion of ozonated saline solution (O3SS), and rectal insufflation (RIO3). There were no adverse reactions reported in the use of M-AHT, with only minor transient discomfort reported in RIO3 and O3SS, attesting to the safety of ozone therapy when applied properly (12). COVID-19 prophylaxis with ozone therapy studies showed a

decreased incidence rate. Post-COVID-19 studies showed significant clinical improvements (13).

This is the first study in Croatia reviewing the use of oxygen ozone therapy in all three aspects of COVID-19 treatment: prophylaxis, acute treatment, and post-COVID-19 conditions treatment, as well as the first thesis on the subject of oxygen ozone therapy from the nursing perspective (14). The thesis is composed of four parts. In the first part, fundamental knowledge about COVID-19 and its public health significance is presented. In the second part, an overview of oxygen ozone therapy with a short history, an explanation of the mechanism of action, therapeutic effects, routes of administration, and clinical applications are presented. The third part extends to the use of oxygen ozone therapy in the treatment of COVID-19, prophylaxis, and post-COVID-19 treatment, systematically reviewing recently published clinical trials and case studies and eliciting the most important mechanisms of action, therapeutic effects, and safety. The fourth part is dedicated to the role of the nursing professional in oxygen ozone therapy, with the explanation and critical comparison of three procedures of the major autohemotherapy method, one of the most frequent and complex application routes.

2. AIM

The aim of this narrative review thesis is to present the current state of evidence on the use of oxygen ozone therapy in the treatment of COVID-19.

The following are the objectives:

- to present the fundamental knowledge about COVID-19
- to present the fundamental knowledge about oxygen ozone therapy
- to describe the mechanisms of action of oxygen ozone therapy in the treatment of COVID-19
- to demonstrate the effectiveness and safety of oxygen ozone therapy in the prophylaxis and treatment of acute and post-COVID-19 condition
- to explain the role of the nursing professional in oxygen ozone therapy and describe the major autohemotherapy (M-AHT) technique

3. METHODS

Different literature sources and search strategies were used to write this narrative literature review thesis. Relevant textbooks in the fields of oxygen ozone therapy, medical microbiology, clinical infectology, epidemiology, and nursing in the English, Croatian, and Italian languages were consulted. Online sources were searched using Google search engine and Google Scholar search database.

Medical bibliographic databases: MEDLINE through PubMed and PubMed Central search engines; Cochrane library; ScienceDirect, ProQuest ,Springer, Ebsco essentials, and ISCO3 library; clinical trials registries: U.S. National Library of Medicine (ClinicalTrials.gov), The European Union Clinical Trials Register (clinicaltrialsregister.eu) and Chinese Clinical Trial Registry (chictr.org.cn); and preprints at medrxiv.org.

Key words used in english were "ozone therapy", "oxygen ozone therapy", "COVID-19", "Sars-CoV-2". Croatian literature was searched using the central portal of Croatian scientific journals Hrčak (hrcak.srce.hr) for published articles and Croatian digital academic archives and repositories Dabar (dabar.srce.hr) for academic theses. Key words in Croatian were "ozonoterapija", "ozon terapija", "koronavirus", "COVID-19". Additional key words were searched according to different relevant subjects throughout the thesis.

Systematic review methodology according to PRISMA recommendations was used in order to facilitate effective literature research strategy, reduce bias in article selection process, and increase the quality of the descriptive presentation of the study results in this narrative review thesis format. The inclusion criteria were: all primary sources of information: clinical trials, case-control studies, case series, and case reports with clear outcomes and adverse events reports, without limitation in number of participants. The exclusion criteria were: review articles, other secondary sources articles and gray literature, articles without available full text; environmental and animal studies related to ozone. On 17th October 2022, 127 articles were screened using the key words "ozone therapy COVID-19". Mendeley Reference Manager was used to eliminate duplicates. Manual selection of 47 relevant articles was performed first by screening the titles and the abstracts, then reviewing the full text. 27 articles were selected for the narrative review: 22 related to ozone therapy treatment of COVID-19, 3 related to ozone therapy as prophylaxis for COVID-19, and 2 related to treatment of post-COVID-19 conditions.

4. DISCUSSION

4.1. Coronavirus disease (COVID-19)

The coronavirus disease (COVID-19) is a viral infectious disease caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) that originated in Wuhan, China, in December 2019. It is characterized by flu-like symptoms, atypical pneumonia, severe acute respiratory syndrome (SARS), and multi-organ system damage (15).

4.1.1. Etiology

Coronaviruses (CoV) are pleomorphic viruses with an envelope, a helical symmetry nucleocapside, and a single-strand, positive-sense RNA genome (+ssRNA). SARS-CoV-2 has four structural proteins (nucleocapsid (N), envelope (E), membrane (M), spike (S)), non-structural proteins, and accessory proteins. Coronaviruses are ubiquitous, infecting a wide range of wild and domestic animals and humans causing common cold (16).

The subfamily Coronavirinae has four genera named according to the Greek alphabet: α, β, γ and δ-CoV. Human coronaviruses (HCoVs) are classified into the two first genera: alfacoronavirus (HCoV-229E, HCoV-NL63) and betacoronavirus (HCoV-HKU1, HcoV-OC43, SARS-CoV, SARS-CoV-2, MERS-CoV) (16,17). SARS-CoV-2 genomic sequencing showed 79% similarity to SARS-CoV, 50% similarity to MERS-CoV, and 85% homology with bat coronavirus (15).

4.1.2. Epidemiology

SARS-CoV-2 is transmitted directly by the release of respiratory droplets and aerosol particles when they are inhaled, ingested, or come into contact with oral, nasal, and ocular mucosa. Indirect transmission occurs through contact with contaminated surfaces, materials, stools, urine, and blood serum (18). Research showed that viral RNA was detected simultaneously in different clinical specimens during the infection (19). In some cases, viruses may persist in the stool for two months after the beginning of the infection (15). Different variants of SARS-CoV-2 are showing different virulence, transmission dynamics, and clinical manifestations. Tracking the basic reproduction number and prevalence of asymptomatic cases is used in assessing the transmission risk, epidemiological burden, and public health significance.

Table 4.1.2.1. shows COVID-19 cases and mortality by countries in the region in comparison with world and European figures on 26th November 2022. It is presented using the observed case-fatality ratio per 100 confirmed cases and the fatality rate per 100,000 persons in the general population (1, 20).

Table 4.1.2.1. Comparison of the COVID-19 cases and mortality on 26.11.2022.

Country	**Confirmed cases**	**Deaths**	**Observed case-fatality% ratio**	**Fatality per 100,000 population**
World	641,233,928	6,630,179	1.1	N/A
Europe	264,065,011	2,133,199	0.8	N/A
Croatia	1,252,608	17,288	1.4	421.12
Slovenia	1,252,901	6,928	0.6	333.25
Bosnia and Herzegovina	400,466	16,200	4.0	493.78
Serbia	2,420,801	17,357	0.7	198.65
North Macedonia	344,710	9,568	2.8	459.25
Montenegro	283,607	2,789	1.0	444.06
Kosovo	272,206	3,202	1.2	176.87

4.1.3. Pathogenesis

The SARS-CoV-2 replication cycle, molecular and cellular pathogenesis have been extensively studied in order to understand the disease development, find a specific treatment, and implement effective prevention. Viral cell invasion starts with the attachement of the S1 component of the spike glycoprotein (S) to the angiotensin-converting enzyme 2 (ACE-2) receptor on the host cell membrane (21). ACE-2 receptors are present in respiratory epithelial and intestinal enterocytes, and endothelial cells of other organ systems making them possible targets of SARS-CoV-2 offering the initial explanation of multiple organ system involvement. The degree of dysregulation of the renin–angiotensin system (RAS) and ACE/ACE2 expression has been studied in relation to the degree of disease severity in different stages of COVID-19 (22).

Current understanding of COVID-19 being an endothelial disease explains the correspondence between all five functions of the vascular endothelium and their exaggerated physiological reactions contributing to the disease's development and severity, as illustrated in Figure 4.1.3.1. (23-25).

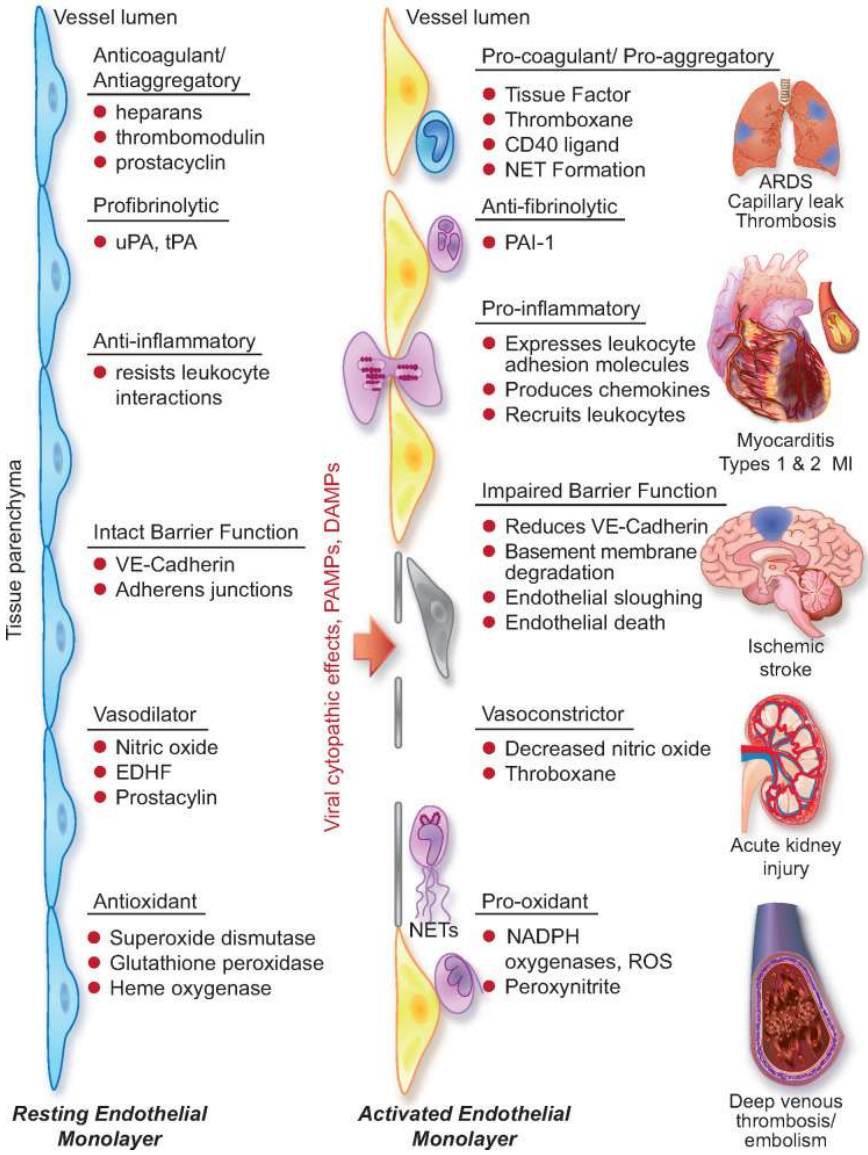

Figure 4.1.3.1. Vascular endothelium in COVID-19 pathogenesis (23)

The vascular endothelium consists of a monolayer of cells of mesenchymal origin with complex multifunctionality as a structural barrier with anticoagulant, anti-inflammatory, antioxidant, and vasodilatating properties (23). Endothelial cells can be activated directly by the cytotoxic effects of viral invasion or indirectly by proinflammatory cytokines (IL-1, TNF) derived from pathogens and dying cells. Enothelial cells produce a special polysaccharide layer protecting the luminal side called glycocalyx (from Greek *glyks*, meaning "sweet," and *kalux*, meaning "shell"), which is crucial in maintaining vascular hemostasis, as seen in Figure 4.1.3.2. Glycocalyx gets damaged by chemical stress from metabolic causes (hyperglycemia), oxidative stress, hyper-inflammatory stimuli, or pharmacological causes (corticosteroids, chemoterapy), as well as

mechanical damage and senescence, all of which are related to risk factors for the infection and COVID-19 severity (24,25).

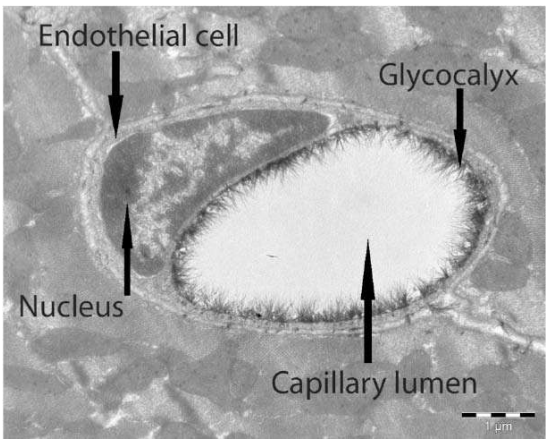

Figure 4.1.3.2. Glycocalyx in a left ventricular myocardial capillary, electron microscopy (26)

The main therapeutic effect of the M-AHT is the reparation of vascular endothelium, making ozone therapy especially valuable in the prevention and early treatment of symptoms in order to prevent the development of severe symptoms, complications, and long COVID-19.

4.1.4. Immunity

The innate and adaptive immune response to SARS-CoV-2 has been extensively researched. As in other viral infections, an increase of IgM in the acute phase and a consequtive increase of IgG in the convalescent phase is noticed in COVID-19 and post vaccination response. More population studies are required to evaluate the cross-reactivity and the duration of B and T cell responses. Assessment of the protection against emerging variants in the context of previous HCoV infections, primary SARS-CoV-2 infection and vaccine-induced immune response remains under investigation (27).

4.1.5. Clinical manifestations

The incubation period for SARS-CoV-2 ranges from 0 to 14 days and varies in different variants, averaging 4–6 days. The highest viral load detected in the nasopharynx is within the first 5 days after symptom onset, while contact studies are confirming the highest transmission risk is a few days before and within the first 5 days after onset. The WHO stated that the proportion of asymptomatic infections ranged from 1.4% to 78.3%

throughout the pandemic. From symptomatic cases, 40% develop a mild disease, 40% a moderate disease, 15% a severe disease, and 5% a critical disease (28).

COVID-19 presents with a wide range of symptoms of varying severity, classified by the WHO into the following three categories: non-severe (mild, moderate), severe, and critical, as shown in Table 4.1.5.1. (28). NIH Clinical classification of COVID-19 offers six categories: asymptomatic infection; mild, moderate, severe, and critical illness; persistent symptoms or organ dysfunction after acute COVID-19 (29).

Table 4.1.5.1. WHO COVID-19 disease severity classification (28)

COVID-19 Severity	**Symptoms**
Non severe - **Mild**	Fever, cough, sore throat, malasia, muscle pain, fatigue, altered or loss of sense of taste and smell, headache, vomiting, diarrhea, no evidence of pneumonia, shortness of breath, dyspnea or hypoxia.
Non severe - **Moderate**	- adults - clinical features of pneumonia (fever, cough, shortness of breath and tachypnoea) without hypoxia; SpO2≥90% breathing room air - children - clinical signs of non-severe pneumonia with no signs of severe disease
Severe	- adults - clinical pneumonia lung infiltrates >50% plus a respiratory rate > 30 breaths/min, severe respiratory distress, or SpO2<90% breathing room air, or ratio of arterial partial pressure of oxygen to fraction of inspired oxygen (PaO2 /FiO2) <300 mm Hg - children - signs of pneumonia plus central cyanosis, SpO2< 90%, inability to feed, very rapid breathing or chest in-drawing
Critical	acute respiratory distress syndrome (ARDS), sepsis, septic shock, acute thrombosis, disseminated intravascular coagulation (DIC), multi organ dysfunction and multisystem inflammatory syndrome in children (MIS-C) and adolescents.

Based upon autopsy findings, the most frequent immediate cause of death in COVID-19 has been respiratory failure, with pneumonia as the main condition leading to it. Septic shock, multi-organ failure, pulmonary thromboembolism, and heart decompensation were among other findings (30).

4.1.6. Diagnosis

The diagnosis of COVID-19 consists of different components: medical history, clinical presentation, physical examination, microbiological testing, and clinical criteria.

The two most commonly used diagnostic tests for SARS-CoV-2 infection are a nucleic acid amplification test (NAAT) that detects viral RNA directly, called a reverse transcription polymerase chain reaction (RT-PCR) assay, and an antigen test that detects viral protein. The serology test detects the presence of antibodies in the blood, confirming a prior or recent infection. The RT-PCR test is considered the gold standard for detecting current SARS-CoV-2 infections (15).

A typical chest X-ray demonstrates bilateral peripheral ground-glass opacity, with areas of consolidation developing later in COVID-19's clinical course. Typical laboratory findings in COVID-19 include leukopenia and lymphopenia, high acute-phase reactants, cell free hemoglobin, elevated D-dimers and prothrombin time (PT), activated partial thromboplastin time (APTT), and fibrinogen (FIB) (29).

4.1.7. Treatment

Currently, there is no specific treatment for the Sars-Cov-2 virus and COVID-19 disease. COVID-19 is a complex systemic disease, requiring different treatment strategies in different phases of the disease and in different treatment settings.

Treatment recommendations and guidelines have been continuously reviewed and updated by expert panels, committees, and work groups in different leading organizations (29,31). According to NIH guidelines, therapies are divided into six categories: antiviral therapy; anti-SARS-CoV-2 antibody products; immunomodulators; antithrombotic therapy; concomitant medications; and supplements (29). Anti-inflammatory medications, antibiotics, and oxygen therapy have been widely used. Remdesivir is currently the only U.S. Food and Drug Administration (FDA) approved antiviral drug (32). Eight medications have been approved by the European Medicines Agency (EMA) (33).

4.1.8. Prognosis

Different factors such as preexisting medical conditions, age, race, lifestyle, and socioeconomic status have been investigated in order to assess the risk of infection, severity of the disease, and prognosis. Data showed that the hospitalization rate was six times higher and the mortality rate was twelve times higher in patients with co-morbid conditions than among those without (28). Factors associated with the incidence and severity of COVID-19 are heart disease, hypertension, chronic respiratory diseases,

diabetes, obesity, chronic kidney disease, cerebrovascular disease, cancer, pregnancy, smoking, HIV infection, transplant recipients, patients aged >70 years, and other conditions and treatments effecting endothelial integrity (28,29,31).

4.1.9. Prevention and control

Public health measures are aimed at reducing viral transmission. The development of the vaccines was aimed at stimulating specific immunity and controlling the waves of epidemics. The promotion of salutogenesis and immune support is aimed at keeping the natural immunity and overall health of the population robust. Four vaccines for COVID-19 prevention have been authorized by the FDA and six by the EMA. Both agencies recently approved two new, adapted, bivalent booster vaccines (32,33). Only one drug is approved for SARS-CoV-2 pre-exposure prophylaxis (PrEP) (29).

4.1.10. Post-COVID-19 Condition (PCC)

WHO states that 10–20% of people experience a range of post-COVID-19 effects, the most common of which are fatigue, weakness, anxiety, shortness of breath, palpitations, memory loss, concentration difficulties, and insomnia (31). The first outcomes of an ongoing, general population cohort study in Scotland found that 6% of symptomatic cases had not recovered and 42% only partially recovered (34). Systematic review and meta analysis of the two most common post-COVID-19 symptoms lasting for 12 or more weeks following COVID-19 diagnosis were fatigue experienced by one in every three individuals and congitive impairment affecting one in every five indivudials (35).

To standardize terminology, the Center for Disease Control (CDC) assigned the ICD-10-CM diagnosis code "U09.9 Post-COVID-19 condition, unspecified" in October 2021 (36). PCC is also recognized as a disability condition (37). The current WHO definition specifies a three-month time frame from the onset of symptoms, with a minimum of two months in individuals with a probable or confirmed viral infection (38).

Clinical manifestations differ considerably depending on individual susceptibilities and comorbidities, posing challenges in the treatment and management of the post-COVID-19 condition, commonly called long COVID-19 (15, 29). Different countries are addressing the emerging long COVID-19 healthcare and socioeconomic burden in various

ways, including the establishment of specialized clinics and rehabilitation centers, as well as the additional education of health care professionals.

4.2. Oxygen ozone therapy

Oxygen-ozone therapy (O2/O3) is the use of a gaseous mixture of oxygen and ozone for therapeutic purposes in medicine. Ozone therapy (OT or O3) is a shortened synonym used interchangebly in practice and literature as it will be in this thesis. It is important to emphasize that ozone is always used mixed with medical grade oxygen in the proportion range of 0.05% - 5% of ozone with 95% - 99.95% of oxygen.

Ozone therapy is considered a medical intervention and is therefore practiced by trained medical professionals. A physician who practices ozone therapy is called an ozone therapist. Ozone therapy is typically used as a complementary therapy, an adjunct to standard allopathic treatment (39). The use as an alternative therapy and monotherapy in clinical practice has also been investigated.

The ozone oxygen mixture is a unique and versatile therapeutic agent that has many simultaneous therapeutic effects. Versatility is also seen in the numerous routes and methods of administration. Ten parenteral and nine topical or loco-regional routes of application provide both systemic and local therapeutic effects (40).

Ozone is instantaneously very reactive and short-lived; therefore, it has to be prepared in-situ for immediate use in the gaseous form. Its nature presents challenges in keeping it stable in fluids, oils, and creams for topical applications over an extended shelf life; therefore, appropriate production techniques and storage requirements need to be respected.

4.2.1. A brief historical overview of oxygen ozone therapy

Ozone was discovered in 1785, forteen years after oxygen, by the Dutch physicist M.Van Marum. Fifty-five years later, it was synthesized by a German chemist, C. F. Schönbein, and named ozone using the Greek word *ozein,* meaning "strong smell" (41). The discovery of antimicrobial properties and the use of ozone as a disinfecting agent happened more than seventy years prior to the discovery of penicillin. Water treatment plants were the first massive users of ozone, which nowadays is used in farming and other

industrial applications. In 1896, Nikola Tesla patented the first ozone generator in the USA; four years later, he founded the *Tesla Ozone Company* and was the first to ozonize olive oil and produce it for medical use (42).

The use of ozone in medicine was initially mostly external and topical for disinfection, wound healing, and the healing of gangrenes and ulcers. Internal routes of application evolved from these early applications as technology advanced: rectal insufflation (1936), autohemotherapy (1961), and ozonized saline solution (1977). The following decades led to the expansion of clinical applications, research, the spread of knowledge, and popularity of ozone therapy across the world (3).

Professional medical ozone therapy associations have been established around the world on both national and international levels. The World Federation of Ozone Therapy (WFOT) was founded in 2005 to promote the use of ozone therapy and increase international cooperation. Currently, it has members representing 31 countries (4). The International Scientific Committee of Ozone Therapy (ISCO3), founded in 2010, is an independent international scientific medical body with the aim of scientifically determining and standardizing safe protocols to maximize the effects of ozone therapy by improving and updating *The Madrid Declaration on Ozone Therapy* every five years (43).

4.2.2. Physical and chemical properties of ozone

Ozone is a triatomic allotrope of oxygen, a gaseous molecule consisting of theree atoms of oxygen, with the molecular formula O3. It is very reactive and unstable, 1.5 times as dense as its parent molecule oxygen, and it has a characteristic fresh smell detectable at very low concentrations (44). The chemical structure of ozone is shown in Figure 4.2.2.1. as a planar, polar molecule with three interchangable, resonating molecular forms (45). The molecule has 18 valence electrons; two unpaired electrons are formed in reaction with an oxygen free radical. Electrochemical potential is + 2.076. Ozone is considered to be part of the reactive oxygen species (ROS) group. Ozone is a coroless to pale blue gas at room temperature, a dark blue liquid at -112 °C, and a violet-blue black crystal at -193.2 °C When concentrated, it becomes explosive in the gaseous and liquid states, while at low concentrations it decomposes slowly. It has a very high electron affinity, making it unstable and spontaneously transforming into oxygen with a release of energy in the form of heat

(46). The solubility of ozone in water is ten times greater than that of oxygen, which is important in its reaction with biological tissues where it also changes the electrochemical potential of the tissues, and in the preparation and use of ozonated water (47).

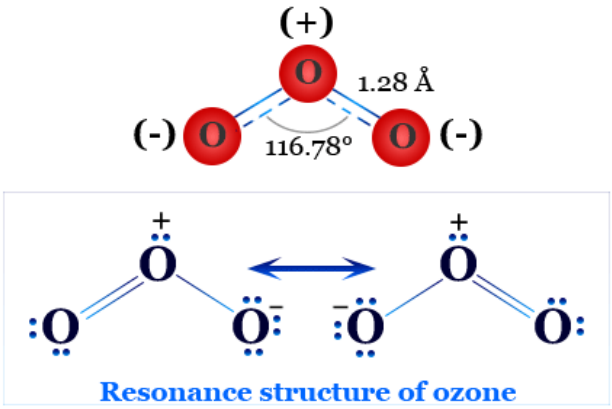

Figure 4.2.2.1. Chemical structure of ozone molecule (45)

4.2.3. Methods of generating ozone

The formation of ozone in nature is a complex process that varies depending on the starting ingredients in different parts of the atmosphere and is kept in balance. Endogenous ozone is naturally present in different tissues. It was first noticed in the immune system, where activated neutrophils generate ozone via the antibody- and amino acid-catalyzed water oxidation pathway (48).

The process of artificially generating ozone from oxygen has been based on one of nature's three ways: using electric current as in lightning storms, ultraviolet rays, and ionizing radiation. Figure 4.2.3.1. shows a schematic presentation of ozone formation from oxygen molecules through the exposure to a powerful electric charge (49).

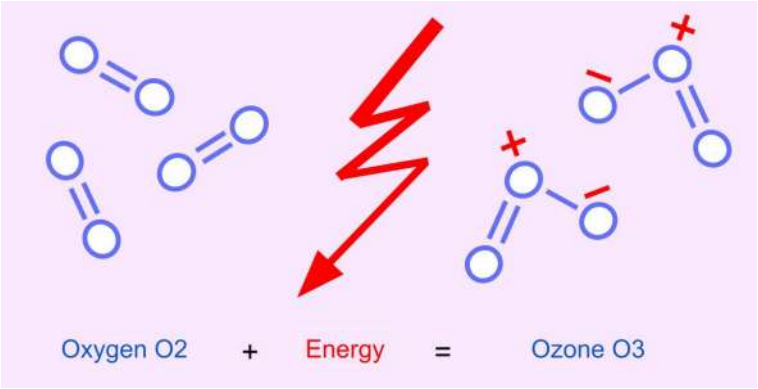

Figure 4.2.3.1. Schematic presentation of ozone formation using electrical energy (49)

It is important to make a distinction between ozone generator devices used for non-medical and medical purposes. Ozone generators for use in industry use air, oxygen, or water as the starting ingredients. Medical ozone generators (MOG) are certified as medical devices and only use medical-grade oxygen with a purity of at least 99.5% O2 that is stored in certified high-pressure cylinders. Air cannot be used because of all the other elements in it; the main concern is the presence of 78% nitrogen, which would become highly toxic nitrogen compounds (40). Most medical ozone generators use a strong electrical field generated between 2–4 high-voltage tubes connected in series to achieve a voltage difference between 4 000 and 13 000 volts. Pure oxygen molecules are passing through the tubes as the energy from the electric discharge breaks them into oxygen atoms, which, in the presence of an excess of oxygen molecules, form the triatomic ozone molecule. Depending on the voltage, the distance between the electrodes, and the speed of oxygen flow, different concentrations of ozone are produced (40). Within the European Union, certification of medical devices is carried out under Directive 93/42/EEC MDD with Class IIB. Materials used in the production of MOGs and disposables for the administration of ozone therapy have to be ozone resistant and certified (39).

4.2.4. Concentration and hormetic dose-response effect

The ozone concentration is inversely proportional to the oxygen flow. Historically, the concentration of ozone has been expressed in µg/mL, which is still widely used in the literature, the "normalized ozone concentration" unit "µg/Nml" is recommended (39).

The total ozone dose is equivalent to the gas volume (mL) multiplied by the ozone concentration (µg/NmL). *Dose O3 (µg) = Volume O3 (mL) x Concentration O3 (µg/NmL).*

The hormetic dose response relationship is very important to the understanding of ozone therapy, as different doses have different effects in different routes of application. Ozone, as a pro-oxidant molecule, is a hormetine that causes harmful oxidative stress in high doses while stimulating a beneficial antioxidant response in low doses. Therefore, it is important that the recommended dosage guidelines are followed and treatment is optimized for each individual patient by carefully applying the up-dosing system (50).

Based upon their effects doses can be devided in three broad categories; non-effective, therapeutic and toxic. Therapeutic doses are divided into three main groups: low, medium and high as shown in Table 4.2.8.1. Research shows that a safe therapeutic effect

ranges from ozone concentrations smaller than 10 μg/NmL to 50 μg/NmL. A total ozone dose of up to 6.0 mg per day is considered safe and effective in applications with systemic effects, but it must be individually calibrated depending on the current condition of the patient. Concentrations greater than 80 μg/NmL are not recommended due to possibility of low hemolysis (1%-2%) (40).

Low doses stimulate protective cellular reorganization, mitochondrial activity, and nuclear transcription pathways, having immunomodulatory and antioxidative modulatory effects. They are used in diseases where the immune system is strongly compromised (cancer, HIV, etc.) and in patients with a heavy oxidative stress burden. Medium doses also have an immunomodulatory and antioxidant stimulating effect for use in the treatment of chronic degenerative diseases (chromic obstructive pulmonary disease (COPD), atherosclerosis, diabetes, Parkinson's disease, Alzheimer's disease, senile dementia). High doses have inhibitory effects and are often used in autoimmune diseases, ulcers, infections, and chronic wounds (39).

4.2.5. Toxicity and safety

The toxicity of ozone lies in its strong oxidative nature, reacting with organic and inorganic substances in different environments. Different tissues react differently depending on their antioxidant capacity, route of application, and dose (50).

The respiratory system in humans is the most vulnerable to even low levels of ozone exposure because of its anatomical and physiological properties. The protective surfactant of the respiratory tract lining fluids (RTLFs) cannot provide sufficient buffering and antioxidant protection because it is physiologically present in a minimal amount to allow the alveolar surface to perform an effective exchange of gases. Chronic exposure to ozone over a large alveolar surface can lead to chronic inflammation (51). An initial physiologic protection is built into the olfactory apparatus which detects the smell of ozone at low concentrations of 0.02 mg/m3, which is ten times lower than the permissible limit in the work place. Permissible Exposure Limit (PEL) of 8 hours, established by the U.S. Occupational Safety and Health Administration (OSHA) is 0.1 ppmv or 0.2 mg/m3 and can cause lachrymation and irritation of upper respiratory airways whereas 50 ppmv is categorized as Immediately Dangerous to Life and Health (IDLH) (52). To prevent air pollution and toxicity all unused ozone has to be converted back to oxygen using an ozone

catalyst, sometime called destructor. Most modern MOGs have built-in ozone catalysts, otherwise a separate device is used (40).

4.2.6. Mechanisms of ozone action and therapeutic effects

The powerful oxidizing effect on inorganic and organic matter makes ozone a powerful desinfectant. In reaction with organic compounds in biological tissues and fluids, it forms peroxide compounds and ozonides. The products of the reaction of ozone with biomolecules are called secondary messengers or signaling molecules, and they conduct the biological effect of ozone because the ozone molecule is no longer present. Detailed studies of ozone reactions with unsaturated, saturated, nitrogen-, and sulfur-containing biological molecules have been conducted (47).

Most of the complex reactions and mechanisms that ozone engenders were studied in the reaction of ozone with human blood, leading to an understanding of the diverse therapeutic effects of M-AHT. Figure 4.2.6.1. shows a schematic representation of the key molecular mechanisms of action of the oxygen-ozone gas mixture on the blood and blood vessels during M-AHT (53). The timing of the reactions is important, as these form the foundation for the development of the therapeutic methods and protocols. Oxygen is ten times less water-soluble than ozone and slowly saturates all hemoglobin. At the same time, ozone gets instantaneously dissolved in the water part of plasma, reacts with the antioxidants and other components in the blood, creating signaling molecules, also called "ozone messengers," and then disappears. Two main signaling molecules are: reactive oxygen species (ROS) and lipid oxidation products (LOPs) (54). ROS are formed in the early reaction of ozone with the water part of plasma and are considered to be early effector molecules. ROS are normally produced by mitochondria in response to cellular stress, acting on mitochondria-associated endoplasmic reticulum membranes (MAMs) (55). ROS as hormetin molecules in the process called mitohormesis have bioregulatory functions. Low levels of ROS signal an increase in mitochondiral activity and the initiation of a nuclear response in order to protect the cell from harmful effects, while high levels of ROS signal a reduction in mitochondiral response (55). Hydrogen peroxide, being the main representative of the ROS group, acts on all components of the blood, triggering complex biochemical reactions. The erythrocytes release more oxygen, increasing tissue oxygenation. Platelets with increased growth factors aid tissue healing. Mononuclear

leucocytes modulate immune responses via the nuclear transcription factor kappa B (NfkB) pathway (56). Lipid oxidation products (LOPs) are considered late effector molecules and form 5-7 minutes after exposure to ozone through the oxidation of polyunsaturated fatty acids (PUFA). As a main LOPs, 4-hydroxynonenal (4-HNE) is transported all over the body when treated blood i s renifused. This turns on the molecular switch Nrf2/Keap1 and the Antioxidant Response Element (ARE) in the DNA for gene expression, which increases the production of antioxidants. Ozone derivatives have a beneficial effect on the lumen of the blood vessels, acting to regenerate the endothelial glycocalix and protect the vascular matrisome (53,55).

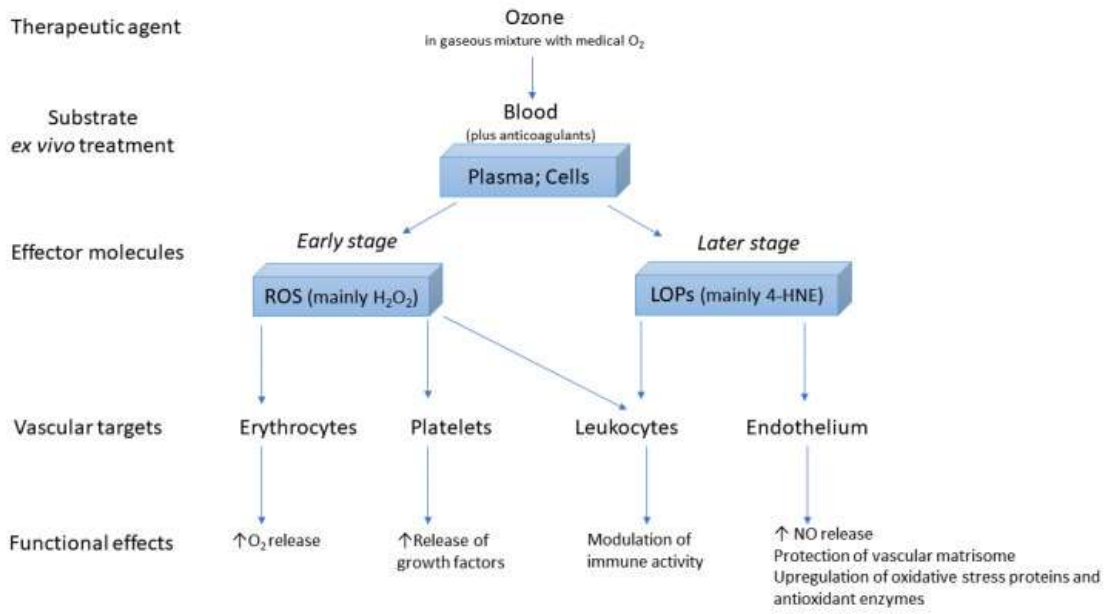

Figure 4.2.6.1. Molecular mechanisms of action of the oxygen ozone mixture on the blood (53)

The therapeutic effects of ozone therapy applied in clinical practice are the following: oxidative stress modulator, immune modulator, metabolic activator, metabolic regulator, anti-inflammatory, antimicrobial, analgesic, detoxifying, and increasing tissue oxygenation and circulation (47).

In summary, ozone is an oxidant molecule with an antioxidant effect (55). Ozone therapy presents deliberate, controlled, transient, light oxidative stress, resulting in strong upregulation of the antioxidative response, counteracting the depleting effects of chronic oxidative stress caused by chronic degenerative diseases, infections, etc (53).

4.2.7. Methods of ozone therapy administration with clinical indications

The Madrid Declaration on Ozone Therapy categorizes ozone therapy administration routes based upon the therapeutic effect into two groups: local and systemic (39). The following parenteral and loco-regional routes of application are listed as the most common routes with systemic effects: major autohemotherapy (M-AHT or MAH), minor autohemotherapy (Mi-AHT or MiAH), ozonized saline solution (O3SS), rectal insufflation (RIO3), vaginal insufflation (VIO3), extra-corporeal blood oxygenation (EBBO), application of ozone injections into trigger, acupuncture, and reflexology points, sauna, or quasi-total-body exposure to ozone. The most common routes of application in the category with local effects are the following: auricular flushing; gassifications in a plastic bag or the gloves technique for skin treatment; topical application of water, oil, and ozonated creams; subcutaneous, intramuscular, intradiscal, paravertebral, and intra-articular injections (39).

Ozone can be used as a gas or dissolved in water, saline solutions, oils, and creams. In the gas form, it is always used as an oxygen-ozone mixture and can be applied using injections, catheters, and gassification bags. Direct injection of the gas mixture can be: subcutaneous, intradermal, intramuscular, intra-articular, intradiscal, intraforaminal, and intralesional. Insufflations of the gas into cavities are performed using catheters. Direct treatment of the skin with ozone gas is done using gassification plastic bags or gloves. Ozone can be used dissolved in water in the form of ozonated water for enteral use and in different types of rinses (oral, vaginal, skin). Ozone can be dissolved in oils and creams for topical use. Direct intravascular application of ozone is not recommended because of the bubble formation and risk of gas embolism. A special equipment is used when ozone is applied in dentistry (40).

The four most commonly used methods of ozone therapy in COVID-19 treatment are: major autohemotherapy (M-AHT or MAH), minor autohemotherapy (Mi-AHT or MiAH), ozonized saline solution (O3SS) and rectal insufflation (RIO3).

Table 4.2.7.1. is showing dose comparison chart of these four systemic routes of administration (39,40,57).

The ozone dosage will depend on the general condition of the patient, age, clinical indications, and the route of ozone therapy application.

Table 4.2.7.1. Dose comparison chart for four most common systemic routes of application

Systemic route of application	**Oxygen ozone gas mixture**	**Low level**	**Medium level**	**High level**
Major Autohemotherapy (M-AHT or MAH)	Concentration - C (µg/NmL) Volume - V(mL) Dose - D (mg)	10-20 50-100 0.5-2.0	20-30 100-150 2.0-6.0	35-40 150-200 5.25-8.0
Minor Autohemotherapy (Mi-AHT or MiAH)	Concentration - C (µg/NmL) Volume - V (mL) Dose - D (mg)	5-10 5 0.025-0.050	15-20 5-10 0.075-0.100	30-40 5-10 0.150-0.400
Ozonated Saline Solution (O3SS)	Concentration - C (µg/NmL) Volume - V (mL) Dose - D (mg/kg)	0.4 200 0.001	0.8 200 0.002	2 200 0.005
Rectal Insufflation (RIO3)	Concentration - C (µg/NmL) Volume - V (mL) Dose - D (mg)	10-15 100 1.0-1.5	20-25 150 3.0-3.75	25-40 200 5.0-8.0

4.2.7.1. Major autohemotherapy (M-AHT or MAH)

Major autohemotherapy is the most frequently used methods of administration of oxygen-ozone mixture intravenous therapy in practice and is often considered a "true systemic therapy." It was also one of the most frequently used application in COVID-19 treatment and convalescence. The biological tissue treated is venous blood, collected outside of the body using specially designed M-AHT sets and kits. After the initial reaction with the gaseous mixture is complete (5-7 minutes), the blood is immediately reinfused as the patient remains connected to the M-AHT infusion system throughout the entire procedure. It is important to underline that because a small amount of blood (under 300 ml) is treated for a short time and immediately reinfused, autohemotherapy is not considered to be a transfusion medicine. Figure 4.2.7.1.1. shows a patient in the reclined position during the reinfusion phase using an M-AHT kit with a plastic bag with three lines in the reinfusion phase. The medical ozone generator is visible on the right side, toward the wall.

The volume of extracted blood ranges from 50 to 200 ml. The recommended safe range is 1.5%–2% of the total circulating blood volume (CBV), transposed to 1.2–1.3 mL/kg.

Figure 4.2.7.1.1. M-AHT plastic bag kit with three lines during reinfusion phase

For example, if a person weighs 85 kg, 1.2 x 85 = 102 ml of blood can be extracted. Ozone concentrations for systemic use range from 10 µg/NmL to 40 µg/NmL (39). The recommended anticoagulants are: Anticoagulant Citrate Dextrose Solution A (ACD-A), USP (2.13% free citrate ion) in a proportion of 7 mL -10 mL per 100 mL of blood, or citrate of sodium 3.8% in a proportion of 10 mL per 90-100 mL of blood to be ozonized. Heparin is not advisable because it can induce thrombocytopenia and platelet aggregation (39,40). The number of treatment sessions and their frequency in a single treatment cycle, as well as the time interval between cycles, depend on the type of disease treated, the phase of the treatment, and the condition and reaction of the patient.

One treatment cycle may vary between 5-20 sessions. Cycles can be repeated every 5–6 months. Clinical data shows that an improvement occurs between the fifth and tenth session. It is considered that after the 12th session, the antioxidant defense mechanism has already been activated. M-AHT or O3SS can be administered two to three times a week, and RIO3 can be administered daily depending on the patient's response (39). Treatments in COVID-19 varied in different studies.

Most common clinical indications for M-AHT are: chronic degenerative pathologies, periferal vascular disease, problems with microcirculation, diabetes, diabetic foot, decubitus ulcers, dry macular degeneration, ulcerative colitis, immune system disorders, psoriasis, allergies, acne, chronic bacterial infections, herpes simplex and zoster, HBV, HCV, HIV, asthma, COPD, metabolic syndrome, liver damage, vascular headaches, depression, neurovascular disorders and neurodegenerative diseases (Amyotrophic lateral sclerosis, multiple sclerosis, Parkinson's, and Alzheimer's disease), fibromialgia, and adjuvant therapy in the treatment of tumors (57).

4.2.7.2. Minor autohemotherapy (Mi-AHT or MiAH)

Minor autohemotherapy is the treatment of a small amount of venous blood (5–10 ml) outside of the body in a syringe with an oxygen–ozone mixture. The blood is then reinjected into the muscle. The concentration range of ozone (10 μg/NmL to μ40 g/NmL) and the blood gas ratio of 1:1 are the same as in M-AHT. The Mi-AHT procedure is simpler than M-AHT as no special infusion kits or anticoagulants are used. A 20 ml syringe is first filled with the amount of gas mixture at the prescribed concentration. The same volume of venous blood is extracted into the syringe without anticoagulant. The blood and gas is manually mixed for 30 seconds and slowly injected into the muscle, most commonly in the gluteal region (40). Dosages are shown in Table 4.2.7.1.

The main therapeutic effect of Mi-AHT is considered to be stimulation of the immune system, and it is used as an "autovaccine" in different disorders. It is used in dermatological diseases (allergies, acne, dermatitis, eczema, psoriasis, and furunculosis), autoimmune diseases, and adjuvant therapy in chronic diseases and cancer (39).

Mi-AHT is easily tolerated and can be administered with single or multiple injections and repeated 2-3 times a week. The treatment cycle usually involves 5–10 weekly sessions (39,47). Clinical results showed a marked improvement in the therapeutic

response when both M-AHT and Mi-AHT were used at the same time, suggesting a synergistic effect with no adverse effects (40).

4.2.7.3. Ozonized Saline Solution (O3SS)

The ozonized saline solution method of systemic ozone therapy is an intravenous infusion of ozonized saline solution prepared outside of the body prior to and during the infusion. The reaction of sodium chloride and ozone is complex and depends on the temperature, pH, and starting concentration of the reactants. Initial concern for the use of O3SS in medicine was the formation of toxic hypochloric acid, which was later on proved to be very minimal. The challenge is to achieve a stable concentration of ozone due to its reactivity and short half-life in solutions (47). The presaturation of the saline solution is done in four ways, each of which uses continuous bubbling of ozone until saturation concentration is achieved.

The main concern with this method is intravascular microbubbles, which can lead to gas embolism and pain at the venepunture site.

The recommended ozone dosages in O3SS are low in comparison to other systemic routes of application and are calculated according to body weight. Low dosage is 1 μg/kg, medium or average is 2 μg/kg, and high is 5 μg/kg body weight. Dissolved ozone concentration in saline solution is between 22%-25% of ozone gas concentration. Therefore, when calculating the concentration that needs to be set at the ozone generator, it has to be multiplied by four (39).

The ozonated saline solution is as effective as the other systemic applications, when properly applied. The mechanism of action of ozone on the activation of the nuclear transcription factor Nrf2 was first discovered in experimental models that used O3SS (40). Two to three sessions per week may be administered within a cycle of ten sessions.

Clinical evidence of O3SS has been proven in use in the following pathologies: acute appendicitis, brain trauma, diabetic foot, obstructive jaundice, vulvar leukopathy, delayed fetal growth and lymphovenous stasis of lower limbs (39). O3SS provides a valuable alternative for patients who refuse blood treatment due to religious or personal beliefs.

4.2.7.4. Rectal insufflation (RIO3)

Rectal insufflation is the local-regional administration of an oxygen-ozone gas mixture via catheter into the rectum, producing local and systemic effects. Historically, rectal applications of medicine have been effectively used due to the anatomical and physiological features allowing absorption of medicine through rectal mucosa, reaching venous and lymphatic circulation. The superior hemorrhoidal vein communicates with the hepatic portal system; therefore, medications absorbed in the lower part of the rectum directly reach the systemic circulation (40).

The mechanisms of action of the ozone previously described above are also proposed in RIO3. Ozone dissolves rapidly in luminal water, but its availability, absorption speed and amount are reduced and variable because of the luminal content.

RIO3 is often used in pediatric and elderly patients, as well as in cases with fragile venous access. RIO3 has been gaining popularity as a non-invasive systemic therapy and has been used as an alternative to M-AHT. It is simpler to perform and more economical, with long-term clinical results similar to M-AHT (40). Nowadays, there are commercially available kits and equipment for home use, where a person may self-administer the treatment prescribed by their physician (58).

Treatment has to be performed when the rectal ampoule is empty. The gas is most often administered manually with a 50 ml syringe clamping the catheter after each syringe. The gas is slowly administered in steps of 50 ml every 2-5 minutes. Recommended starting volume is 100 ml and gradually increased with every session till 400-500 ml depending on the patient's tolerance. The patient is advised to rest for 15 minutes after RIO3 to avoid rapid gas expulsion and allow the completion of the ozone reaction and absorption (40).

Ozone concentration, gas volume, and the dose recommendations are shown in Table 4.2.7.1. Dose calculation based upon the patient's weight is 75 μg for 1 kg; it is recommended to start the treatment with half the dose or adjusting it according to the pathology (39). As in M-AHT, ozone concentration should not exceed 40 μg/ml to avoid local damage to enterocytes (40).

No adverse effects were observed during rectal ozone application, apart from a transient bloating feeling that passes within 5 minutes as gas is absorbed. Therefore, it is important to slowly administer the gas and instruct the patient to breathe deeply, relax, and retain the gas to maximize the therapeutic effect.

Treatment cycles of 15-20 sessions once a day, or every 2-3 days, repeated at a 3-4 month interval in the first year are recommended (39).

The therapeutic effects of RIO3 on local, regional, and systemic levels, covering different acute and chronic pathologies, are clinically proven. In gut-associated diseases, RIO3 has biochemical, bactericidal, and modulatory effects on microbiota and gut-associated lymphoid tissue (GALT), strengthening the immune system (40). Therapeutic effects on type II diabetes, chronic hepatitis, ulcerative colitis, hemerhoids, infectious diseases, brain disorders, fibromyalgia, and cardio-vascular and pulmonary diseases have also been well documented (40,47,57, 59-61).

4.2.8. Clinical applications of ozone therapy in various medical specialties

The list of medical specialties that have clinical and research-based evidence for clinical application of ozone therapy includes: angiology, cardiology, pulmology, neurology, orthopedics, surgery, gastroenterology, nephrology, urology, gynecology, obstetrics, otorhinolaryngology, ophthalmology, dermatology and aesthetic medicine, gerontology, infectology, and stomatology. The most common conditions are mentioned above in the clinical indications of different ozone therapy methods. Ozone therapy has been successfully used in dentistry as well as in veterinary medicine (47).

Understanding the mechanisms of action and therapeutic effects of oxygen ozone therapy, along with ongoing research, continues to expand the clinical applications list.

4.2.9. Contraindications, interactions with other treatments and adverse effects

Systemic administration of ozone therapy is contraindicated in the following conditions:

- glucose-6-phosphate dehydrogenase deficiency (G6PD)
- toxic hyperthyroidism
- coagulation disorders, and thrombocytopenia below 50,000 /μL
- severe cardiovascular instability
- acute myocardial infarction
- stroke
- massive and acute hemorrhage
- hemochromatosis
- acute alcohol intoxication

- during convulsive states
- patients receiving i.v. treatment with copper or iron
- ozone intolerance

Testing for G6PD is recommended prior to ozone therapy in order to avoid complications, especially in ethnic groups where the prevalence is higher (39,47,57).

The following interactions with other treatments also need to be considered:

1. Antioxidative treatments using high doses of i.v. antioxidants leading to high concentrations of antioxidative compounds in the blood interfere with ozone's action as an oxidizing agent and are therefore not recommended within 24 hours of ozone therapy. Oral vitamins and antioxidants may be given prior to or after, but not during, M-AHT treatment sessions, depending on their pharmacokinetics.

2. A synergistic effect with other oxidative therapies can be expected, which will increase the patient's oxidative stress. Therefore, careful calculation of the dose depending on the oxidative capacity of the patient needs to be considered when two oxidative therapies are planned for the same day.

3. Mixing of any other substances in the M-AHT and O3SS bag or bottle is strictly forbidden, as ozone's oxidizing nature may create undesirable toxic compounds.

4. Treatment of patients undergoing anticoagulation therapy with coumadin or heparin must be carefully conducted controlling INR values. Other new oral anticoagulants are considered to be ozone compatible.

5. Ozone increases the effects of ACE inhibitors. The use of ozone therapy during the last two trimesters of pregnancy is allowed. The use of systemic ozone therapy in competitive sports is considered doping (39).

Adverse effects in ozone therapy are classified into five levels based on the NIH standard criteria: mild, moderate, severe, life-threatening, and death-related adverse effects (39). The majority of reported adverse effects are related to improper administration technique, route of application, and dose. Minor gastro-intestinal discomforts using RIO3, hypoglycemia, and skin irritations in the area of administration, most notably with the use of O3SS, have been reported. In comparison to 6.4% to 25% of all cases hospitalized worldwide due to intolerance to medical preparations, the complication coefficient of 0.7 cases per 100,000 sessions of ozone therapy admirably proves its safety (47).

4.3. Use of oxygen ozone therapy in COVID-19

Ozone therapy has valuable potential in the treatment of COVID-19 due to its oxygenating, immunomodulatory, anti-inflammatory, antiviral and oxidation-balancing properties.

Recent studies have shown an in-depth understanding of ozone therapy mechanisms at the molecular, subcellular, and cellular level through ozone derivatives acting as signaling and modulating molecules, provoking mitohormetic responses to oxidative stress. Figure 4.3.1. illustrates the complex molecular landscape and mechanisms related to ozone therapy in four major pathophysiological arenas in COVID-19: mitochondrial dysfunction, oxidative stress, chronic inflammation, and immune thrombosis. Ozone-generated bioactive mediators (H2O2, 4-HNE, and HO-1) and their three main pathways are presented in green (55).

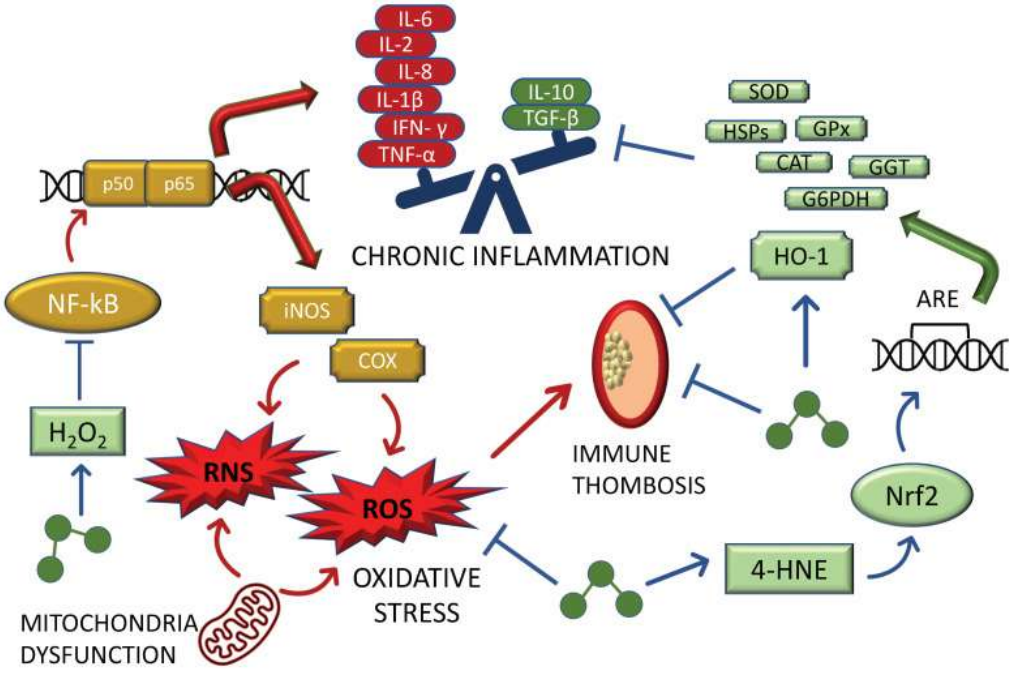

Figure 4.3.1. Molecular mechanisms of ozone therapy in COVID-19 (55)

The anti-inflammatory effect of ozone is achieved through the inhibition of the H2O2-mediated NF-kB pathway, modulating the immune response and counteracting hyper-inflammatory response and cytokine storm. Anti-oxidant effects work through the 4-HNE-Nrf2-mediated transcription of different anti-oxidant effector molecules. The anti-thrombotic effect acts through ozone induced heme oxygenase-1 (HO-1) and endothelial

nitric oxide (NO), hindering immuno trombosis. An antiviral effect is achieved by interfering with virus binding, invasion, and replication mechanisms via rapid Nrf2 activation and prevention of SARS-CoV-2 inhibiting effects (55).

Table 4.3.1. shows the results of the literature key word searches for "ozone therapy" and "ozone therapy COVID-19" on 17.10.2022. illustrating the current scientific activity supporting the use of ozone in medicine and in COVID-19.

Table 4.3.1. Results of the literature search on 17.10.2022.

Database / Regsistar	**Key word "Ozone therapy"**	**Key word "Ozone therapy COVID-19"**
PubMed - total	4,267	118
PubMed - clinical trials	317	9
PubMed - randomized control (RCT)	221	5
PubMed - reviews	548	28
PubMed - systematic reviews	70	5
PubMed - meta-analysis	36	2
Cochrane library	521	20
ScienceDirect total	10,102	371
ScienceDirect - review articles	1,923	144
ScienceDirect - research articles	3,729	101
ProQuest – scholarly journals	9,468	594
Springer - articles	2,848	112
Ebsco essentials – open access articles	1,272	79
ISCO3 library	3,805	64
MedRxv – preprints	52	33
Clinicaltrials.gov (USA/world)	72	7
Clinicaltrialsregister.eu (EU)	5	1
Chictr.org.cn (China)	30	1

27 articles were selected for the narrative review: 22 related to ozone therapy treatment of COVID-19, 3 related to ozone therapy as prophylaxis for COVID-19, and 2 related to treatment of long COVID-19. Reviewed studies included 968 participants using ozone therapy as an adjuvant to standard treatments, 2 as monotherapy and 378 using only standard therapy as a control group. The description of the ozone therapy protocols applied as interventions, clinical outcomes, and adverse effects was presented in table format and used for the assessment of efficacy and safety.

4.3.1. Ozone therapy in the treatment of COVID-19

22 original studies related to ozone therapy treatment of COVID-19 included 415 patients with all degrees of COVID-19 severity, from mild to critical, using ozone therapy as an adjuvant therapy to standard therapy and 2 patients using it as monotherapy. 190 patients served as the control group, being treated only with standard conventional COVID-19 therapy at the time. Standard therapy (ST) has been evolving over time and has included antiviral drugs, systemic corticosteroids, antibiotics, anticoagulants, oxygen therapy, symptomatic therapy, and vitamins depending on the severity of the disease and hospital protocols. Studies were conducted in 2020 and 2021 and consequently published, making a valuable contribution to the search for an effective treatment for COVID-19 in these times of great urgency and pressure.

The narrative review results are presented in four different sections according to the ozone therapy method used: M-AHT (10 studies), RIO3 (6 studies), SSO3 (5 studies), and a novel technique called inhalation of nebulized ozone (1 study). The majority of the studies were observational, and four were randomized controlled trials.

4.3.1.1. Major autohemotherapy (M-AHT) in the treatment of COVID-19

Ten studies were reviewed, including a total of 198 patients: 197 hospitalized with COVID-19 in the full range of disease severity, and 1 treated at home using M-AHT as monotherapy. 117 patients were included as a control group, receiving only standard conventional therapy (ST). Study types included: 2 randomized controlled trials (RCT), 2 prospective controlled studies (PCS), 2 case-control studies (CCS), 2 case series studies (CSS), and 2 case reports (CR). Table 4.3.1.1.1. visualizes the results of the literature review.

Table 4.3.1.1.1. Clinical studies of major autohemotherapy ozone therapy in COVID-19 treatment

Study type	**Study size pt/ control**	**Disease severity**	**Intervention protocol**	**Outcomes /results**	**Adverse effects**	**Reference**
RCT	48/44	Mild to moderate pneumonia - hospitalized	ST + M-AHT 1x daily for 3 consecutive days (200 ml blood, 32 ml sodium citrate, O3 40 µg/mL) - bag kit	↓ Leukocytes ↑ CRP ↑ Clinical improvement day 7 = Mortality rate = Need for ventilation = Hospital stay	NO	1. Sozio et al. (2021) Italy (62)

RCT	14/14	moderate to severe COVID-19 pneumonia -hospitalized	ST + probiotics + M-AHT 2x day 7 consecutive days (250 ml blood, 25 ml sodium citrate, O3 c.30 µg/mL) Total daily dose O3 15 mg - bag kit	↓ Need for ventilation ↓ Mortality rate ↓ CRP = Inflammatory markers = Lymphocyte sub-populations	NO	2. Araimo et al. (2020) Italy (63)
PCS	37/18	Mild to severe - hospitalized	ST + M-AHT 1x daily for 7 consecutive days, 100 ml blood, 30 µg/mL - bottle kit	↓ ICU hospitalization ↓ Mortality risk	NO	3. Çolak et al. (2021) Turkey (64)
PCS	9/9	Severe pneumonia - hospitalized	ST +M-AHT 2x day for 5 days (4 median), 200 ml blood, 35 ml ACD-A, O3 concentration 40 µg/mL - bag kit	↑ Recovery rate ↓ Time to clinical improvement (7 day median) ↓ Time to PCR negative ↓ Mortality risk ↓ CRP, D-dimer, ferritin, LDH = ventilator-free days	NO	4. Hernández et al. (2021) Spain (65)
CCS	30/30	Mild to moderate pneumonia - hospitalized	ST + M-AH 1x daily for 3 days, 200 ml blood, 35 ml sodium citrate,40µg/mL - bag kit	↓ SIMEU clinical phenotype ↑ significant clinical improvement = Hospital stay	NO	5. Tascini et al. (2020) Italy (66)
CCS	50/ historical control	Severe, ARDS - hospitalized in ICU	ST+ MAH 1x daily for 5 cons. days, 100-200 ml blood, 45 µg/mL - bag kit	↑fast improvment of respiratory indexes, SatO2%, PaO2/FiO2 ↓ inflamatory markers CRP, IL-6, ↓ thrombotic markers ↓ D-dimer, ALT, LDH	NO	6. Franzini et al. (2020) Italy (67)
CSS	3/0	Severe pneumonia, respiratory failure -hospitalized	ST+ M-AHT 2x day for 3/4/6 days, 200 ml blood, 35 ml ACD-A, 40 µg/mL - bag kit	↓ Hypoxia ↑ PaO2 ↓ FiO2 - no need for invasive mechanical ventilation ↓ LDH, CRP, D-dimer ↓ Hospital stay	NO	7. Hernández et al. (2020) Spain (68)
CSS	4/0	Moderate (1), severe (2), critical (1) - hospitalized	ST+ M-AHT 1-2x day for 1-5 days 100 ml blood, 25 ml anticoagulant, 40 µg/mL - bag kit	Chest x-ray and CT scan improvement, lesions resolved ↑ P/F ratio ↓ CRP, LDH,IL-6, D-d ↓ ICU stay ↓ Hospital stay	NO	8. Wu et al. (2020) China (69)

CR	2/2	Moderate to severe - hospitalized	ST + M-AHT 1x daily for 7 consecutive days, 100 ml blood, 20 µg/mL	Chest x-ray improvement ↑ CRP, LDH,IL-6 ↑ Recovery rate ↓ Duration of viral shedding ↓ Hospital stay	NO	9 Zheng et al. (2020) China (70)
CR	1/0	Moderate to severe – home treatment	Monotherapy M-AHT 1xdaily for 3 consecutive days for 2 weeks 135 ml blood, 15 ml sod. citrate 15-20 µg/mL - bag kit	↑ Clinical improvement ↑ SpO2 Chest CT scan improvement Full recovery	NO	10. Tricarico G (2021) Italy (71)

Symbols:↑ - increase, ↓ - decrease, = no change

M-AHT protocols ranged in number of days from 1–7, times per day from 1–2, blood volume from 100–250 ml, and ozone concentration from 20–45 µg/mL. The M-AHT technique used by the majority of studies was with plastic bag kit, and only one study used a negative pressure glass bottle.

Clinical parameters used in evaluation of the clinical course and therapeutic outcomes were: laboratory findings of inflammatory markers (C-reactive protein (CRP), Interleukin-6 (IL-6), ferritine (FER)), blood biochemistry parameters (ALT, LDH, urea), coagulation parameters (D-dimer, fibrinogen), a complete blood count and white blood cells differential (lymphocyte sub-population, neutrophil-lymphocyte ratio (NLR)), respiratory parameters (SPO2, PaO2, PaCO2, PaO2/FiO2 ratio) and imaging techniques (chest x-ray, CT scan, and MRI).

The clinical assessment scales used were the Italian Society of Emergency Medicine (SIMEU) phenotype class (1–5) and Taylor's Chest X-ray Severity Scale for Severe Acute Respiratory (SARS) Infection (1–5). Different studies also included mortality risk and rate, intensive care unit (ICU) admission risk, hospitalization time, recovery time, and time to a PCR negative result.

Results showed nominal improvements in the variables of different parameters and clinically significant improvements in all studies. Statistical significance was shown in only three studies (65-67), indicating the need for further research with a larger sample size and a more homogenous study design.

Studies showed clinical improvement in more than half of patients receiving adjuvant M-AHT therapy, compared to one third of those in the control group. Patients receiving M-AHT showed a faster improvement rate, especially in severe and critical cases, with immediate improvement of inflammatory markers after each ozone therapy session. A trend in the reduction of time to a PCR-negative test and hospitalization time was recorded. A significant reduction in mortality risk, especially in severe cases, was noted in case control studies. Laboratory and imaging parameters showed significant improvements. Adjuvant treatment has been started on different days since the COVID-19 diagnosis, depending on the clinical picture. The last case report listed (71) demonstrated that M-AHT can be safely and effectively used as a monotherapy at home. The patient received ozone therapy for three consecutive days, repeated for two weeks. The majority of the authors recommended starting with ozone therapy as soon as possible to counteract the SARS-CoV-2 infection and reduce the progression of the disease. No adverse effects were reported, confirming the high safety profile of M-AHT in COVID-19 as well.

4.3.1.2. Ozonized saline solution (O3SS) in the treatment of COVID-19

Five studies reviewed included a total of 152 patients with COVID-19 ranging from mild to critical grade: 151 hospitalized who received O3SS as adjuvant treatment and 1 treated at home with O3SS as monotherapy. Study types included two pilot clinical trials (PCT) and one of each (CCS, CSS, and CR). One CCS had 18 patients included in the control group. Table 4.3.1.2.1. lists the findings of the O3SS in the treatment of COVID-19.

Table 4.3.1.2.1. Clinical studies of ozonized saline solution (O3SS) in COVID-19 treatment

Study type	**Study size pt/ control**	**Disease severity**	**Intervention protocol**	**Outcomes /results**	**Adverse effects**	**Reference**
PCT pilot clinical trial	10/0	Moderate COVID-19 hospitalized	ST + vitamines + O3SS once daily for 8 days: 200 mL NaCl 0.45% . O3 concentration 2 µg/mL - i.v. 1h = 60 ggt/min - glass bottle kit	↑ Clinical improvement significant – resolution of clinical symptoms by day 10 - statistically significant: ↓ CRP, D-dimer, IL-6 ↑ SpO2/FiO x-ray improvement-clearnace of infiltrates SOFA score improvement ↓ LDH borderline	Minor: Pain at the site of injection in 4/10 Transiently raised ALT in 8/10 Headache in 1/10 Dilutional	1. Sharma et al. (2021) India (72)

				reduction ↓ shorter recovery time ↓ O2 supply Day 8 improvment from moderate to mild grade - no progression of disease severity	Hyponatremia in 1/10 NO moderate or severe	
PCT pilot clinical trial	25/vs historical control	Mild to severe COVID-19 pneumonia - hospitalized	ST+ O3SS: 200ml NaCl 0.9 % , O3 bubbling conentration 3-5 µg/mL once daily. i.v. 15-30 min, 10 consecutive days - down-titration: Day 1-5, O3 conc. 5 µg/mL= 250 µg daily dose Day 6-10, O3 conc. 3 µg/mL= 150 µg daily dose	↑ Clinical improvement significant. Symptoms reduction on Day 7-8. Complete resolution of dyspnea Day 10. ↓ D-dimer, fibrinogen, CRP, LDH, IL-6, ferritin x-ray improvement drastically Day 3-5 ↓ shorter recovery time ↓viral shedding time (median 8 days) no progression to critical disease severity = Hospitalization time ↓ Mortality rate 0	NO	2 Schwartz et al. (2021) Spain (73)
CSS	98/0	46 moderate, 6 severe, 16 critical hospitalized	ST+ O3SS for 5–10 days with 1–2 doses per day. - moderate 4-7 tretments - severe 5-10 treatments O3 concetration 35-45 µg/mL bubblig for 3-5 minutes through saline solution - i.v. 5-15 min	↓ shorter recovery time ↓ Hospitalization time 1. Moderate and severe - ↑ Clinical improvement rapid symptoms reduction on Day 2-3 in moderate, Day 3-5 in severe - no progression to critical disease severity - no need for respiratory support ↓Mortality rate 0 2. Critical stage - 6/16 receiving 5 doses, 1-2 day fully recovered - 10/16 deceased, did not live long enough to receive full 5 doses	NO	3. Razzaq et al. (2020) Iraq (74)
CCS	19/18	Mild to moderate hospitalized	ST + O3SS once daily for 10 days. 200 ml NaCl O3 concentration 2.2-2.4 mg/l - preparation - bubbling 1000-	Statistically significant improved sesne of well-being Day 3 while laboratory finding were similar to control group ↑ Clinical improvement, laboratory radiological	NO	4. Hammad et al. (2020) Russia (75)

			2400 µg/l for 15 minutes	and clinical findings Day 10 Significant improvments laboratory and MSCT findings Day 14		
CR	1/0	Moderate to severe COVID-19 pneumonia - home care	Vitamines + O3SS once daily for 7 days (following 10 days of RIO3 with no clinical results) O3SS down-titration: Days 1-3: 100 mL at 7 µg/NmL daily; Days 4-5: 100 mL at 5 µgN/mL, daily; Days 6-7: 100 mL at 3 µg/NmL, every other day.	RIO3 did not show satisfactory clinical improvement so it was followed by SSO3 ↑ Clinical improvement ↑ SpO2 ↓ O2 supply ↓ inflamatory parameters full recovery Day 7	NO	5. CachayMorales J. (2021) Peru (76)

The fifth study (76) is mentioned in the RIO3 and O3SS routes of application sections because both methods were used by the same patient. When RIO3 did not produce the desired clinical improvement, therapy was changed to O3SS, which produced rapid improvement.

Two methods of O3SS preparation and administration were used. The first method involves the preconditioning of saline solution with bubbling for 15–20 minutes until saturation is achieved prior to the infusion. The second one uses continuous bubbling during the infusion time as well. Recommended dosages and techniques of preparation with certified equipment and materials were used.

The authors' justifications for choosing the O3SS over the M-AHT include minimizing interaction with COVID-19 patients and avoiding potential problems with blood manipulation (72). Hypothesizing that because saline solution is a plasma expander, a larger amount of blood would be reached, potentially resulting in fewer treatment sessions (73). Historically, there has been a lot of clinical evidence to support the use of O3SS as an antiviral treatment.

The parameters used to evaluate the treatment outcomes were the same as those mentioned in the use of M-AHT and RIO3. Measurements were performed in a series on different days, demonstrating the dynamics of the disease and treatment results. Statistical analysis in PCT, CSS, and CCS studies, but not CR, revealed significant improvements in

primary and secondary end points. Clinical improvements were shown in all studies. The mortality rate was zero in all studies except in the group of critically ill patients in third study (74).

In addition to what was previously mentioned, the following scales were used in the reviewed O3SS studies: Sequential Organ Failure Assessment (SOFA) Score predicting ICU mortality based on laboratory and clinical findings (72). The scales for dyspnea (4 grades), weakness (3 grades), efficacy outcome ordinal scale with seven categories, and disease severity scale (4 grades) were well defined in the second study (73). The fourth study (75) used an adaptation of the the National Early Warning Score (NEWS) and the Hospital Anxiety and Depression Scale (HADS), as well as a specially designed questionnaire for COVID-19 symptoms.

Four minor, transient adverse effects were reported in the first study (72). Pain at the injection site was experienced by four out of ten (40%) participants. The liver enzyme alanine aminotransferase (ALT or SGPT) was briefly increased in eight out of ten patients (80%). Authors attribute the ALT rise to antiviral medications because levels return to normal upon end of treatment. One out of ten (10%) patients experienced a transient headache. One out of ten (10%) patients with borderline sodium levels on admission were diagnosed with mild dilutional hyponatremia. No adverse effects were noted in any other study.

The authors of the second study (73) were encouraged by the swift effectiveness of the O3SS, recording continuous daily improvements leading to full recovery with no adverse effects in all patients within ten days, resulting in the closure of COVID-19 unit. The fifth study used ozone therapy and vitamins at home without standard therapy. O3SS was applied once a day for 7 days with down titration showing rapid daily improvements.

Each study has highlighted the efficacy and safety of O3SS in the treatment of COVID-19, as well as the need for additional randomized clinical trials. Early beginnings of the ozonetherapy treatment have been recommended.

4.3.1.3. Rectal insufflation (RIO3) in the treatment of COVID-19

Six studies reviewed included a total of 52 patients (50 hospitalized, 1 outpatient, and 1 home care) with COVID-19 ranging from mild to severe grade who received rectal insufflation as adjuvant treatment. The RCT and CCS studies included 40 patients in the

control group who received only standard treatment. Two different ozone therapy methods were combined in two studies: RIO3 with minor autohemotherapy (Mi-AHT) (77) and RIO3 followed by O3SS (76). Study types included: 1 RCT, 1 CCS, 1 CSS, and 3 CR. Table 4.3.1.3.1. summarizes the findings of the RIO3-based literature review.

Table 4.3.1.3.1. Clinical studies of rectal insufflation (RIO3) in COVID-19 treatment

Study type	**Study size pt/ control**	**Disease severity**	**Intervention protocol**	**Outcomes /results**	**Adverse effects**	**Reference**
RCT	30/30	Mild to moderate COVID-19 hospitalized	ST + RIO3 2x day: 150 ml, 40 µg/mL + Mi-AHT* 1x day: 5 ml blood, 25 µg/mL for 10 days	↑ Clinical improvement significant ↓ CRP, LDH, ferritin ↑ SpO2 ↓NEWS score ↓ Time to PCR negative ↓ requirements for intensive care, no ICU admisions, no O2 therapy ↓ Mortality risk	NO	1. Shah et al. (2021) India (77)
CCS	14/14	Severe Bilateral COVID-19 pneumonia - hospitalized	ST+ RIO3 1x daily for 5-10 days, 150 mL gas volume, O3 concetration 35 µg/mL = 5.25 mg daily dose	↓ D-dimer, fibrinogen, urea, CRP, LDH, IL-6, ferritin ↓ Leukocytes ↑ Lymphocytes ↑ SpO2 , ↓ O2 supply ↓pneumonitis x-ray taylor score improvement ↓ Mortality rate ↓ Hospitalization time	Slight meteorism and bloating feeling - spontaneously resolved	2. Fernández-Cuadros et al. (2021) Spain (78)
CSS	4/0	Severe COVID-19 pneumonia hospitalized	ST+ RIO3 1x daily for 5 days,100 mL gas volume, O3 concetration 35 µg/mL	↑ SpO2 , ↓ O2 supply ↓ LDH, IL-6,CRP, ferritin D-dimer, fibrinogen, ↓ Leukocytes ↑ Lymphocytes x-ray taylor score improvement	NO	3. Fernández-Cuadros et al. (2020) Spain (79)
CR	2/0	1. Severe hypoxia - hospitalized 2. moderate hypoxia - outpatient	ST+ RIO3 1. case two, 2. case one session with large volume 2 l, 12.6 µg/mL, total dose 25.2 mg	Rapid clinical improvement ↑ SpO2	NO	4. Hendawy et al. (2021) Egypt (80)

CR	1/0	Severe COVID-19 pneumonia hospitalized	ST+ RIO3, 1x daily for 5 days 100 ml, 35 µg/mL	↑ SpO2 ↓ infammation x-ray taylor score improvement	NO	5.Peña-Lora et al. (2020) Spain (81)
CR	1/0	Moderate to severe COVID-19 pneumonia - home care	RIO3 once daily for 10 days up-titration: day 1-3: 100 mL, 25 µg/NmL daily; day 4 -8: 150 mL, 30 µg/mL; day 8-10: 200 mL, 30 µgN/mL. Following with O3SS* once daily for 7 days.	RIO3 did not show satisfactory clinical improvement so it was followed by SSO3 resulting in rapid clinical improvement * (see chapter for O3SS)	NO	6. Cachay-Morales, J. (2021) Peru (76)

*Studies including two ozone therapy methods.

A standard RIO3 technique uses a 50 ml syringe with an O2/O3 gas mixture slowly injected into the rectum via an ozone-resistant disposable catheter. The dosages used in five studies (76–79, 81) were consistent with international recommendations. In one study (80), a large volume of 2 liters of O2/O3 gas mixture was applied slowly under X-ray guidance. 2 liters of gas containing 12.6 µg/mL ozone resulted in a total ozone dose of 25.2 mg. The author's reasoning was to increase mucosal surface area by nearly filling the entire colon in order to achieve faster oxygenation improvement than seen when using the smaller volumes of 100–200 ml (39) or 150–500 ml (40). A patient with severe COVID-19 pneumonia who needed mechanical ventilation received two treatments, the second after x-ray verification of gas absorption. A patient with a moderate severity grade received only one treatment in an outpatient setting. Both patients' oxygen saturation improved rapidly with no side effects.

The same clinical parameters as mentioned in the use of M-AHT and O3SS were used as primary and secondary outcome variables in RIO3. Additionally, NEWS is used in the RCT study.

Clinical outcomes in all studies but one (76) showed improvements in the ozone therapy groups. Statistically significant improvements were stated in two studies, RCT (77) and CCS (78).

Interestingly, one study found no improvement after 10 days of daily RIO3, so the ozone treatment technique was changed to 7 days of O3SS, which resulted in significant clinical improvement (76). RIO3 was administered in home care for ten days beginning on the day of diagnosis, with the following up-titration regimen: days 1-3: 100 mL, 25 μg/mL daily; days 4–8: 150 mL, 30 μg/mL daily; and days 8–10: 200 mL, 30 μg/mL daily. The patient had mild diarrhea for two weeks prior to the diagnosis, which may have contributed to O3SS being a better route of application.

In all the studies reviewed, there were no adverse effects reported. Only one CCS mentioned slight meteorism and a feeling of bloating, which spontaneously resolved (78).

In summary, five studies reported improvements in O2 saturation, decreases in inflammation markers, and improvements in radiological score with no adverse effects in all severity grades of COVID-19.

4.3.1.4. Inhalation of nebulized ozone in COVID-19 treatment

This is a novel treatment considered a lung decontamination technique with a special inhalation device. A new prototype ozone-resistant nebulization device was built just for this clinical study, with ozone passing through distilled water and olive oil being delivered by a cold steam inhalator. Daily treatment in three sessions of 10 minutes at an interval of 5 minutes for five days with a dose of 0.2 ppm ozone gas. Olive oil serves to prevent irritation of the delicate respiratory mucosa susceptible to ozone damage. The known toxicity of ozone when inhaled prohibits this application method, but the novel technique and use of vaporized ozonized oil with a permissible ozone concentration for a brief period make it a worthy and courageous endeavor.

Table 4.3.1.4.1. Clinical study of inhalation of nebulized ozone in COVID-19 treatment

Study type	**Study size pt/ control**	**Disease severity**	**Intervention protocol**	**Outcomes /results**	**Adverse effects**	**Reference**
RCT	15/15	Mild to severe COVID-19 hospitalized	ST + inhalation of nebulized ozone, "lung desinfection treatment" 3 sessions of 10 min (0.2 ppm/ session) at	↓ significant reduction in hospitalization time ↓ CRP CT imaging score improvement ↓ PCR negative test	NO	1. Degniz et al. (2021) Turkey (82)

			5 min intervals, total 40 minutes daily for 5 days - specially designed prototype nabulizor with distilled water and olive oil	(median 5 days) = D-dimer, urea, LDH lymphocytes, leukocytes, platelets ↑ Clinical improvement		

The study resulted in a significant reduction in CPR inflammatory parameters, PCR test negative time, hospitalization time, and improved CT imaging. No adverse effects were noticed. Autors suggest that this lung desinfection technique reduces the incidence of COVID-19 pneumonia.

The oxidative preconditioning effect of ozone therapy, visible as a significant peak in the therapeutic effect curve after four to five ozone therapy sessions, has been well demonstrated. Therefore, it was sensible that the majority of study designs incorporate at least five consecutive ozone therapy sessions to achieve a significant therapeutic response (83).

In conclusion, all studies demonstrated significant efficacy and high safety of ozone therapy as adjuvant therapy in COVID-19 treatment with occasional, transient, minor adverse effects in the use of O3SS and RIO3, making ozone therapy a valuable and affordable tool in COVID-19 treatment.

Evaluating the use of ozone therapy as a monotherapy in COVID-19 treatment presented a challenge, as the majority of the cases remain unpublished. Patients who had good experience with ozone therapy prior to the infection, patients who were already receiving ozone therapy as wellness maintenance, ozone therapists, and their family members were the first ones to choose to treat COVID-19 solely using ozone therapy at home. Two published cases reviewed showed fast clinical improvements and full recovery after receiving ozone therapy (M-AHT and O3SS) at home in mild to moderate disease presentations (71,76). Other unpublished cases from clinical practice also showed the same results, providing a valuable contribution to lowering the burden on the health care system. Patients receiving ozone therapy regularly demonstrated preconditioning and a prophylactic effect. More initiative and research are needed in the use of ozone therapy as an alternative and monotherapy in COVID-19 treatment.

4.3.2. Ozone therapy in the prophylaxis of COVID-19

Prevention of COVID-19 plays an important role in the COVID-19 pandemic and has been investigated using a variety of approaches, particularly at the onset of the pandemic when neither specific chemoprophylaxis nor vaccines were available. Special precautions were taken to protect the essential healthcare personnel.

Three studies reviewed included a total of 398 healthcare workers and 21 nursing home residents using ozone therapy together with recommended chemoprophylaxis and vitamins. 171 healthcare workers served as a control group. Study designes used: one retrospective controlled cohort study (RetCCS), one retrospective cohort study (RetCS), and one case series study (CSS) with a case report (CR). The ozone therapy methods used were: O3SS and minor autohemotherapy (Mi-AHT). A retrospective controlled cohort study (RetCCS) conducted in India used nationally approved hydroxychloroquine (HCQ) as standard chemoprophylaxis at the time. All studies included the use of vitamins in different protocols: Vit.C, B12, D3, n-acetylcysteine (NAC), zinc, and multivitamine. Table 4.3.2.1. presents the results of the literature review.

Table 4.3.2.1. Clinical studies of ozone therapy in prohylaxis of COVID-19

Study type	**Study size pt/ control**	**Disease severity**	**Intervention protocol**	**Outcomes /results**	**Adverse effects**	**Reference**
RetCCS	Healthcare workers (HCW) 64/171	Healthy	ST (HQN) + vitamines + O3SS: 4 days in one month for 3 months. 200 mL NaCl 0.45% . O3 concentration 5 µgN/mL bubbling for 20 min., glass bottle - i.v. 1h = 60 ggt/min	↓Statistically significant decrease in rate of incidence of COVID-19 accross different risk of exposure zones red-orange-green: 4.6% vs. 14.03%	Minor: - Mild pain at the site of injectio Fatigue -Mild headache	1. Sharma et al. (2020) India (84)
RetCS	Healthcare workers (HCW) 320/ community prevalence	Healthy	8 Mi-AHT 1x day + 2 sessions Vit D3 + Vit B12 i.m. for 2 months.	↓ Significant decrease in rate of incidence of COVID 19: 2.19% vs 18.98%.	NO	2. Shah et al. (2020) India (85)

CSS/CR	Nursing home 35 participatnts (21 residents + 14 workers) / community prevalence	Healthy/ 1 asymptomatic	Mi-AHT 1x every 15 days till total of 8 (4 months), followed by 1 x month for 3 months, followed by 2x month for 3 monts = 17 Mi-AHT 5 ml blood, 30 µg/mL + Vit C, NAC	↓ Decrease in rate of incidence of COVID-19 – no incidence	NO	3. Ordóñez and de las Mercedes (2021) Spain (86)

Ozone therapy protocols were used according to standard recommendations. Doses applied in prophylaxis were higher than in treatment protocols, demonstrating better oxidative stress tolerance in healthy people and the results of the ozone oxidative preconditioning effect. The duration of the prophylaxis studies ranged from two to ten months.

All studies showed a significant decrease in COVID-19 incidence. The ten-month-long study in nursing homes demonstrated the safe and effective prevention of COVID-19 and other therapeutic benefits from ozone therapy, such as better glycemic control in diabetic patients and the absence of COVID-19 and other respiratory infections in comparison to other nursing homes. Considering the age and common comorbidities of elderly participants, the results were even more impressive. The author's rationale for using the Mi-AHT method was the auto-vaccination effect, supporting specific antibody production.

Only one study (84), whose authors acknowledged O3SS's mild temporary adverse effects (headache, fatigue, and soreness at the venepuncture site), reported these effects also in the COVID-19 treatment study (72), indicating that the adverse effects are related to the use of saline solution, which can irritate the site of injection.

As suggested by all authors, the encouraging clinical experience with ozone therapy as prophylaxis in COVID-19 provides a solid foundation for future research via larger prospective clinical studies. Effective prophylaxis is essential for reducing the global socioeconomic burden of treating acute and chronic COVID-19.

4.3.3. Ozone therapy in the treatment of Post-COVID-19 Condition (PCC)

Ozone therapy has also been contributing to post-COVID-19 prevention and treatment. Preliminary research is confirming efficacy and safety, prompting further investigation. Patients with post-acute COVID-19 were evaluated in two studies, with 134 patients receiving ozone therapy and 17 serving as controls. The study types reviewed were one multicenter CSS and one RCT. The methods of ozone therapy utilized were systemic: M-AHT and O3SS. Table 4.3.3.1. displays the findings of the literature review.

Table 4.3.3.1. Clinical studies of ozone therapy in the treatment of post-COVID-19 condition

Study type	**Study size pt/ control**	**Disease severity**	**Intervention protocol**	**Outcomes /results**	**Adverse effects**	**Reference**
Multicenter CSS	100/0	PASC post - acute sequelae of SARS-Cov-2	M-AHT SIOOT protocol: 2-3 x week for 2-3 weeks, 150/200 ml blood O3 conc. 40-50 µgN/mL - plastic kit	↓statistically significant reduction in fatigue ↓ pain ↓ discomfort in 67% of patients recovering to normal functionality	NO	1. Tirelli et al. (2021) Italy (87)
RCT	18 + 16 /17	Rehabilitation of patients with previous COVID-19 pneumonia	ST rehab + O3SS: 5 sessions 1. group (18) 1 x every day 2. group (15) every 2nd day O3 c. 2.0 mg/l	↑ Clinical improvement ↑ SpO2 ↓ O2 supply ↓ CRP, D-dimer better with daily O3SS ↑ higher effectiveness of rehabilitation	NO	2. Tsvetkova AV, et al. (2021) Russia (88)

The first study (87) examined the effect of M-AHT on the fatigue symptom using the Fatigue Severity Scale (FSS-7), revealing that 67% of patients regained normal functionality with a significant decrease in fatigue, pain, and discomfort.

The second study assessed the efficacy of O3SS as an adjunct to standard rehabilitation protocols during the rehabilitation program for patients recovering from COVID-19 pneumonia. O3SS was administered over the course of five consecutive sessions. The first group of 18 patients received O3SS daily, while the second group of 16 patients received it every other day. Daily use showed a better reduction in inflammatory parameters.

Positive study outcomes included significant clinical improvements, a decrease in inflammatory parameters and oxygen therapy use, an increase in oxygen saturation, and the overall efficacy of the rehabilitation program. There were no reported adverse effects in both studies.

Following positive results in the areas of prevention and treatment, preliminary studies of the use of ozone therapy in post-COVID-19 conditions provide excellent incentives, encouragement for additional research, and assistance in addressing the complexities of long COVID-19.

4.4. The role of the nursing professional in oxygen ozone therapy

Nurses traditionally have essential role in daily standard nursing care but also an important role in application of innovative and complementary therapies. Patient education and preparation, preparation and assistance in administration of the prescribed therapy, and monitoring of the effects are the roles of nursing professionals in ozone therapy.

Standard nursing skills are applied together with the specific skills and knowledge about ozone therapy: appropriate techniques in different routes of application, indications, contraindications, therapeutic effects, and safety. The five-step standard nursing care process (assessment, diagnosis, planning, implementation, and evaluation) and documentation are applied to ensure continuity of optimal, individualized care (89).

Periprocedural nursing care includes preprocedural instructions and preparation, procedural assistance and monitoring, and postprocedural monitoring and instructions.

The preparation phase begins with informing and educating the patient and their family about the prescribed procedure and its effects, as well as obtaining signed consent. Physical preparation and the proper positioning of the patient depend on the route of application, while psychological preparation and support continue throughout the procedure (57). The preparation of certified equipment and ozone-resistant materials is important to ensure the safe extemporaneous preparation of medicine in the form of the oxygen-ozone mixture. Assistance in preparation and administration of the prescribed therapy varies with the different routes of application. The monitoring of the patient during and after the procedure is completed with the follow-up instructions.

General infection protection and control practices, with the specifics of COVID-19 management need to be routinely applied (90). All infusion-related procedures require the use of aseptic techniques and sterile products.

The expiration dates of all materials to be used should be verified prior to the procedure (91). All materials used must be ozone resistant and disposed of after use: laboratory glassware, silicone, stainless steel, fluoropolymer plastics, polytetrafluoroethylene (PTFE), polyvinylidene difluoride (PVDF), fluorocarbon, titanium, and polycarbonate. Rubber has to be avoided, as it deteriorates when exposed to ozone. All autohemotherapy plastic kits must have dual certification: firstly, certification for blood collection in the EU through UNI EN ISO 15747: 2005; and secondly, for ozone resistance (39). Regular maintenance of MOG is required to ensure quality performance and the accuracy of the ozone concentration. An annual calibration is recommended (39).

In the case of ozone intoxication, the patient should be laid down and given humidified oxygen to breathe. To counteract the induced oxidative stress, the antioxidant capacity of the body should be strengthened using antioxidants administered intravenously or orally. A slow intravenous infusion of antioxidants, ascorbic acid and reduced glutathione (GHS), in a 5% glucose solution is recommended. Oral antioxidants, such as ascorbic acid, vitamin E, and N-acetylcysteine (NAC), can also be administered (40).

The safety of ozone therapy depends upon the use of the proper materials, a precise, standardized application technique, and an appropriate dose.

4.4.1. Major autohemotherapy (M-AHT or MAH) procedure

Currently, there are three types of M-AHT sets or kits based upon the type of mixing containers (glass bottle with negative pressure, plastic collapsible bag, and firm plastic "egg-shaped" container). There is also a difference in the number of lines used, from two to three. The use of three separate lines eliminates the risk of blood clotting if the same line is used to extract and reinfuse blood. The average duration of the M-AHT procedure is approximately 30 to 45 minutes, depending on the volume of blood treated and the condition of the patient.

4.4.1.1. M-AHT procedure with plastic bag infusion set with three lines

The materials required to perform M-AHT using a plastic bag with three lines using gravity is shown in Figure 4.4.1.1.1.

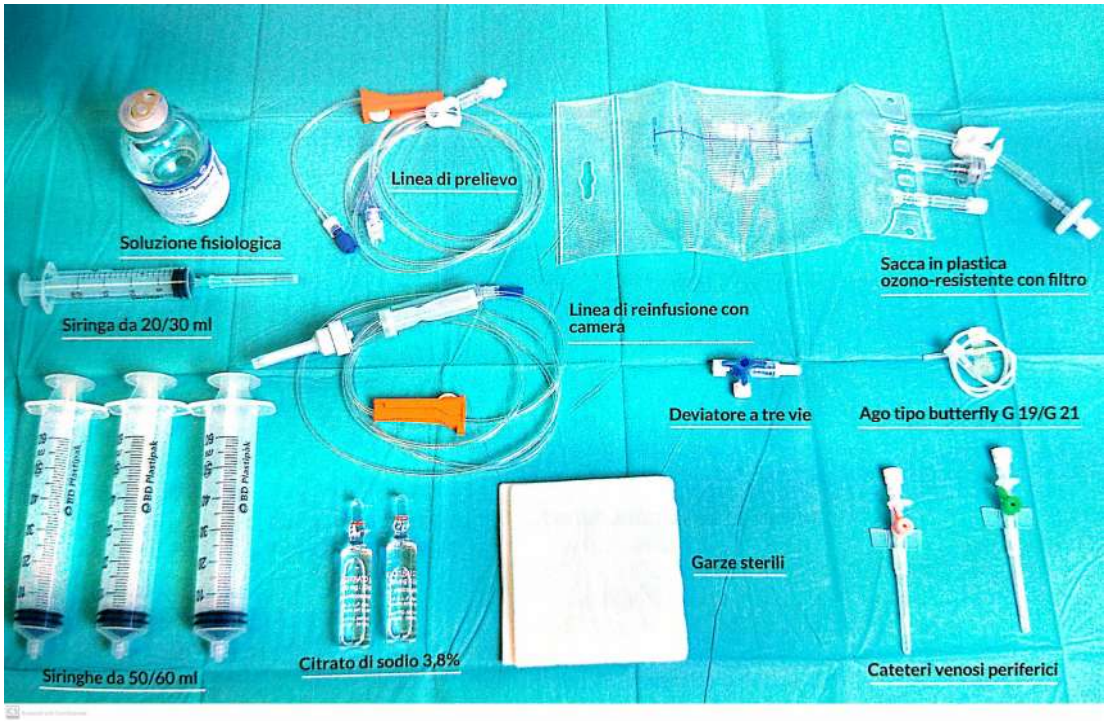

Figure 4.4.1.1.1. Materials for M-AHT plastic kit with 2-3 lines (57)

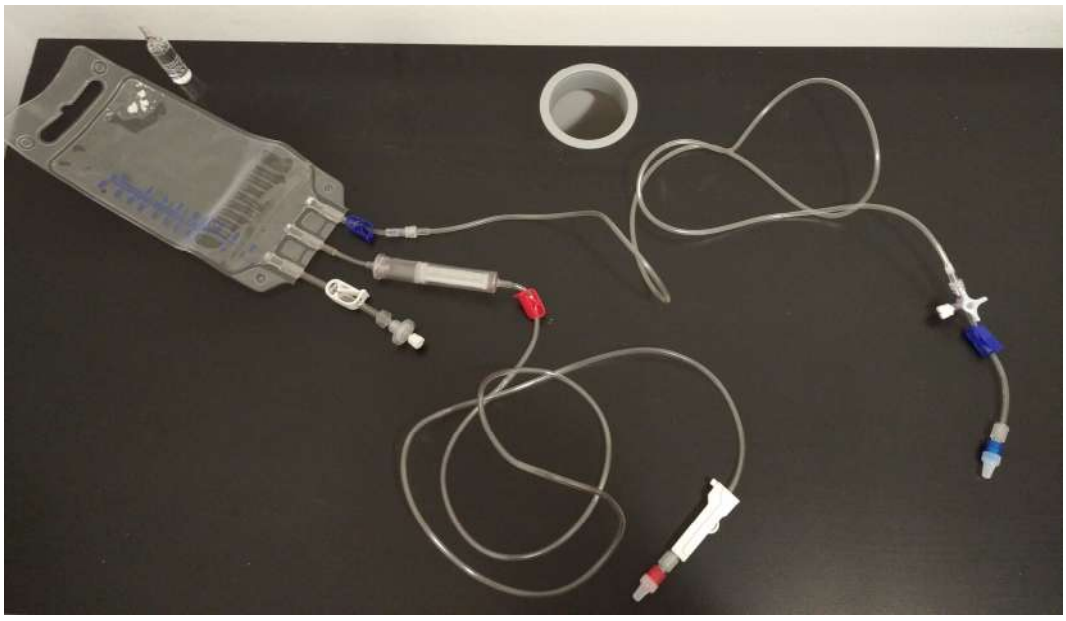

Figure 4.4.1.1.2. M-AHT plastic bag kit with three lines

Figure 4.4.1.1.2. shows the M-AHT plastic bag kit with all three lines. The lines have color-coded plastic clamps that match the color of the substance that is passing through them. The "blue line" has two blue clamps, one at the beginning, near the "butterfly" needle, and the other at the end, near the bag. This is used for venous blood extraction, which is naturally darker in color, corresponding to the blue color code. To keep the blood from clotting, it has to be primed with an anticoagulant from the beginning to the end. The "red line" has two red clamps, one above and one below the double filter chamber. The kit in this image does not have the red clamp near the bag; therefore, a separate small tube occlusion clamp was used to prevent blood from coming down into the filter during mixing. The "red line" is used for the re-infusion of treated blood, which after treatment becomes bright red, and it has a standard rolling regulator for controlling the infusion speed. A double-layered microfilter serves as a collector of possible microcoagulants and microbubbles. The "white line" has one white clamp above the gas microfilter (an antibacterial, ozone-resistant, hydrophobic filter with a porosity of 0.2 μm). It is used for the administration of the colorless oxygen-ozone gas mixture, hence the white color code. The bag has calibration markings and is used for mixing the blood with anticoagulant and gas. All connection ports for syringes are Luer-lock compatible.

The procedure using the M-AHT plastic bag kit with three lines, according to the author's experience, is explained in the following thirteen steps:

1. Patient evaluation: Prior to treatment, the patient’s medical documentation, laboratory results (complete blood count (CBC), creatinine, AST, ALT, and Glu), and other diagnostic tests are evaluated. Vital signs are checked (temperature, blood pressure, pulse rate, respiration rate, and SPO2 using a pulse oximeter) and documented. Indications for M-AHT are verified, and contraindications are evaluated.

2. Patient preparation: Standard infusion protocols are followed (91) and the patient is placed in a comfortable sitting, reclining, or lying down position.

3. M-AHT kit inspection: checking and tightening all the connecting parts and inspecting the seams and junctions on the bag.

4. M-AHT kit preparation: priming the blue line by injecting the anticoagulant starting from the beginning and clamping the first blue clamp to secure the first part of the line near the butterfly. Continue to inject the rest of the anticoagulant into the bag. Closing

the upper blue clamp and checking the seams of the bag for any leaks. Reopening the blue clamp for blood extraction.

5. Opening a venous access by using standard venepuncture technique for placing a peripheral venous catheter from G 18–G 20 or a butterfly metal needle G 19. Preferably using the basilica or cephalic vein of the forearm and butterfly type needle G 19. Securing, maintaining, and monitoring the venepuncture site is done according to standard nursing procedure.

6. Blood collection is achieved using gravity into the bag placed below the level of the patient's heart and arm to facilitate the blood flow. Assisted blood extraction with a separate syringe can be used when needed. The bag is placed on the blood collection scale and mixer, sometimes called the "rocking scale." A pre-set volume feature and automatic clamping of the tube when the desired volume is reached are commonly used. Alternatively, manual checking of the volume and weight and gentle mixing are used.

7. Medicine production: A prescribed oxygen-ozone gas mixture is produced by a medical ozone generator filling up a 50 ml plastic syringe.

8. Medicine application and mixing in the bag: gas mixture is injected directly into the bag via the white line. To ensure homogenous mixing and a continuous reaction, the gas is added incrementally depending on the volume of blood to be treated. A first syringe of 50 mL is added at 50 mL of blood, a second at 100 mL of blood, and the last one at the end of blood collection time. The last gas syringe is injected through the blue line to clean up the blood in the line, and the blue clamp near the bag is closed to prevent the return of the blood during mixing.

Blood may be mixed using a blood collector mixing scale or manually. Interestingly, a recent study showed that manual blood mixing is four times more effective in achieving homogenous gas diffusion (92). A recommended mixing time after blood is collected is 5-7 minutes. A small amount of foam is expected as the reaction of oxygen and ozone is in progress. It is emphasized that it has to be done gently to avoid hemolysis and superfluous foaming yet to achieve homogeneity (40). In practice, we found that gentle rocking of the bag in a horizontal figure-eight motion, as if drawing the infinity symbol "∞", visually produced the best results. As in many areas of technologically advanced modern medicine, the "human touch" is still optimal.

9. The maintenance of the peripheral venous access line: standard maintenance technique applies, rinsing the line with saline solution after the blood collection and prior to the reinfusion using a three-way stopcock.

10. Reinfusion of treated blood: Upon completion of the treatment time, the bag is mounted on the infusion stand with all clamps closed. The bag is visually inspected for the presence of clots or air bubbles, although the double filter in the drip chamber secures microfiltration. The upper red clamp is opened, the drip chamber with filter is half filled with blood, and the lower clamp is opened as shown in Figure 4.4.1.1.3. The red line is primed with blood and connected to the beginning of the blue line and the i.v. cannula via a three-way stopcock infusion port, as shown on Figure 4.4.1.1.4.

Figure 4.4.1.1.3. M-AHT plastic bag kit with three lines during reinfusion phase

Reinfusion speed between 90 and 120 drops per minute is regulated by a standard flow regulator with a roller clamp. During the reinfusion time, the bag is gently stirred every two minutes to ensure continuous mixing, prevent blood sedimentation, and keep the foam in the upper part of the bag. Figure 4.4.1.1.4. shows the venous access lines during the reinfusion phase. The red line is active, conducting bright, red, oxigenated blood, while the blue line was closed after blood collection.

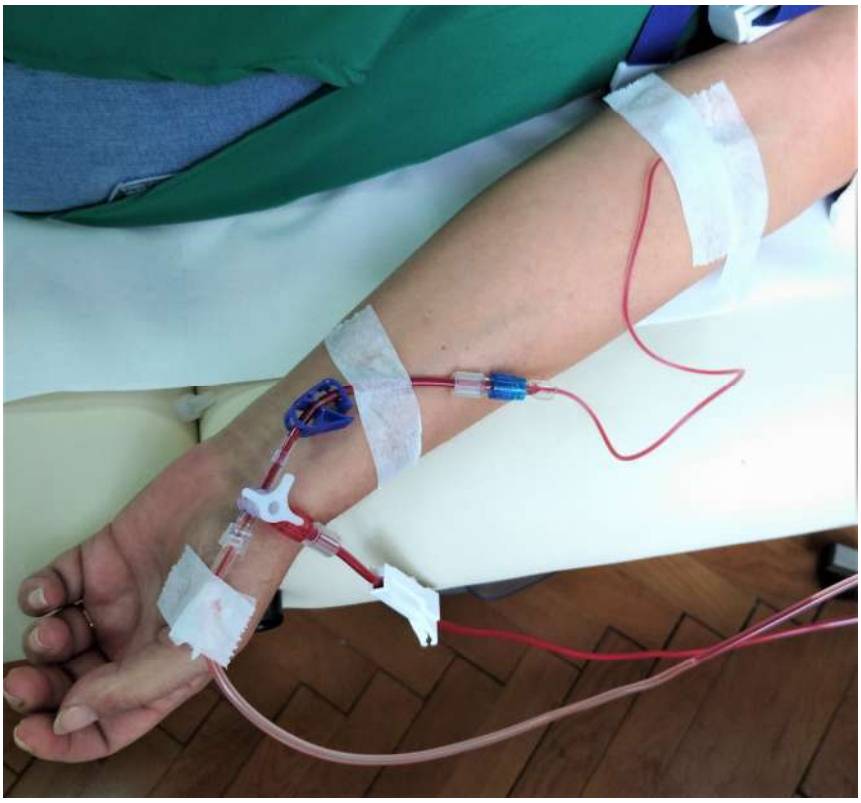

Figure 4.4.1.1.4. Venous access line during reinfusion phase

11. Disconnecting the infusion system: Upon the completion of reinfusion, the infusion system is disconnected and disposed of according to standard protocols. The metal needle has to be removed gently without pressing on the vein to avoid additional damage. A compression dressing is applied, and the patient is advised to remain still for five minutes until no signs of bleeding are verified. It has been observed in practice that ozone therapy aids the healing of the venepuncture site.

12. Patient monitoring: rising from a seated or lying position must be gradual. Furthermore, the absence of vertiginous or vagal symptoms must be confirmed. Throughout the procedure, the patient is talked to, observed, and any side effects are investigated.

13. Patient education and instructions: the patient is advised to report the effects of the treatment in the next 24-72 hours in order for the practitioner to access the oxidative burden for the adjustment of the next dose. There are three general initial reactions in regards to overall energy, activity level, and sense of well-being: no change, increased energy, and decreased energy.

4.4.1.2. M-AHT procedure with a negative-pressure glass bottle

Until 2009, the procedure of collecting and mixing blood in a glass bottle and delivering the gas combination with a glass syringe was the only one in use. Since then, the creation of ozone-resistant plastic has enabled the development of plastic kits. There are either 250 ml or 500 ml calibrated glass bottles used, with or without prefilled anticoagulant. The volume of gas and blood ratio, as well as the anticoagulant ratio, are the same as those described in the use of the plastic bag kit. The procedure steps are also the same, with the addition of a small amount of pure oxygen to adjust the pressure in the bottle and regulate reinfusion speed if needed. The glass bottle is mixed manually.

Figure 4.4.1.2.1. shows the blood collection phase using an negative pressure glass bottle, demonstrating the ascending flow of blood.

Figure 4.4.1.2.1. Glass bottle kit with two lines during blood collection phase

On Figure 4.4.1.2.2. a glass bottle is mounted on the infusion stand for reinfusion. It is noticeable that the same line and filter are used to collect and reinfuse blood, although this is not recommended.

Figure 4.4.1.2.2. Glass bottle kit with two lines during blood reinfusion phase

4.4.1.3. M-AHT procedure with firm plastic container with three lines

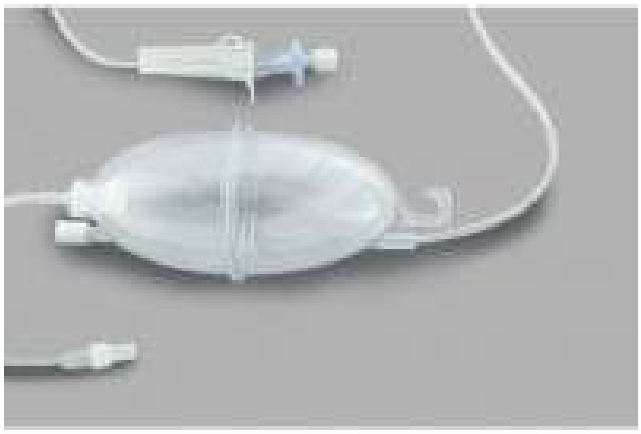

Figure 4 .4.1.3.1. M-AHT kit with firm plastic container and three lines (94)

The M-AHT kit with a firm plastic container in Figure 4.4.1.3.1. consists of a 300-ml calibrated plastic chamber and three access points for three designated lines, as in a plastic bag kit. The blood collection line is connected to the injection valve and is used first to inject the anticoagulant and then to collect blood. Next to it, on the bottom of the

container, is the reinfusion line with a blood filter built inside the plastic container. The third line is located on the top of the container and is designated for the gas. It is used for creating negative pressure during blood collection, delivering an oxygen-ozone gas mixture, and creating additional pressure during the reinfusion phase by adding pure oxygen as needed. The mixing of the blood is performed manually, with the same limitations as using a negative pressure glass bottle kit.

Figure 4.4.1.3.2. shows the M-AHT kit with a firm plastic container and two lines during the blood collection phase. Vacuum is produced by the ozone generator, as visible on the display, which shows a negative pressure of minus 420 using the gas line. A plastic container is placed on the infusion stand, midway up, above the level of the patient's arm and heart.

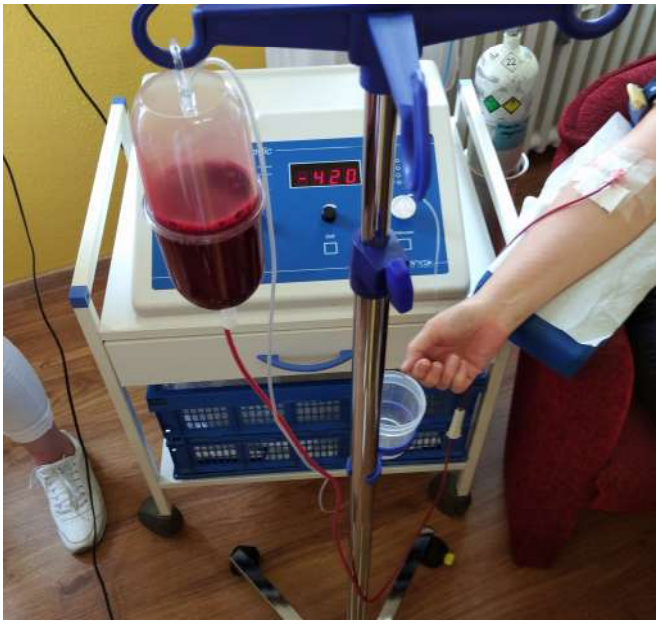

Figure 4.4.1.3.2. Firm plastic container kit with two lines during blood collection phase

Figure 4.4.1.3.3. shows firm plastic container M-AHT kit with two lines during the reinfusion phase. Oxygen-ozone mixture was delivered through the white gas line via a different, left-upper port on the ozone generator. Through the same port, additional oxygen to create pressure has been manually added as needed. A plastic container is placed high on the infusion stand to facilitate reinfusion primarily by gravity. The same line was used to collect and reinfuse blood, which is not recommended. The drip chamber with blood filter is not visible, as it is built inside a plastic container; therefore, the renifusion speed cannot be adjusted by counting drops.

Figure 4.4.1.3.3. Firm plastic container kit with two lines during reinfusion phase

4.4.1.4. A critical comparison of the three main M-AHT procedures

The following critical comparison points between the use of a plastic bag, a negative-pressure glass bottle, and a firm plastic container based on the author's own experience present valuable practical insights.

1. The pressure

The main difference between the use of a collapsible plastic bag using gravity for blood collection and reinfusion and other firm containers is the use of pressure. Negative pressure is used to facilitate blood collection and positive pressure to facilitate reinfusion. All glass bottles are manufactured with strong negative pressure, requiring pressure management skills throughout the whole procedure. Firm plastic container kits have the option of being used under pressure created by ozone generators and controlled by an ozone therapist, which allows for more flexibility than a glass bottle.

As the vacuum will draw in the anticoagulant, blood, and gas mixture, it has to be properly managed. Following the manufacturer's instructions, the lines connected to the bottle must pierce the silicon cap in the designated locations to prevent damage and material particles from entering the bottle.

Placement of the intravenous cannula and continuous monitoring of the venepuncture site must be performed with greater care to prevent vein collapse, endothelial damage, and possible needle retraction during blood collection. Utilizing a cannula with a smaller diameter increases the risk of hemolysis. The benefit of negative pressure is that blood can be taken out faster and in an ascending manner.

To keep the pressure in the glass bottle under control throughout the whole process, the clamps must be closed and opened in the right way. The line for transferring the gas mixture into the bottle has a bacterial filter, allowing for aseptic ventilation during reinfusion, and a roller clamp to regulate the flow of gas, counteracting the negative pressure to avoid abrupt sucking in of the gas mixture into the bottle.

Adding more pressure with pure oxygen during the reinfusion phase needs to be just right to avoid putting too much pressure on the blood filter, which is made to work at room atmospheric pressure. This would cause micro bubbles and micro bloodcloths, that would normally be filtered out, to form and pass through the filter. Additionally, excessive pressure can increase the saturation of oxygen in the blood and may cause discomfort in patients with patent foramen ovale, which is present in 25% of the general adult population (95). The collapsible plastic bag kit allows for manual assistance to achieve the same results where firm containers need to use pressure generated by a machine. For example, if the collection flow is too slow, blood can be drowned with the syringe and injected into the bag through the blue line using a three-way stopcock infusion port. Rolling down and gently squeezing the bag can provide gentle pressure to aid the reinfusion until the last drop.

2. The number of lines

In the use of a glass bottle, there are only two designated spots at the silicone glass top; therefore, only two lines may be used simultaneously. Changing the lines increases the possibility of cap damage and pressure change. The plastic bag and firm container are

designed with three designated access points with built-in or add-on lines, making them easier to use. The importance of using three separate lines has been emphasized previously.

3. Flexibility in volume and dose adjustments

The plastic bag and firm plastic container allow more flexibility in the amount of blood treated and precise dose adjustment, especially in the lower range. The pre-set vacuum bottle has more limitations, as it is programmed to collect one-half of the bottle's volume with blood. Furthermore, if the bottle comes with pre-filled anticoagulant, it is important to carefully remove the surplus and control the pressure to get the right ratios of all components. Delivery of the gas mixture in synchronicity with the collected volume of the blood to ensure homogenous treatment, is significantly easier with the plastic bag kit.

4. Mixing

The bag allows for more gentle mixing throughout the whole procedure, both with the blood collection mixing scale and manually. Mixing scale is not used with firm containers; therefore, there is a tendency to shake them more vigorously. This may be caused by a lack of tactile sense and reliance only on the visual change in blood color and foam formation.

5. Clinically significant observed phenomena

Three visual findings have been observed in practice using the plastic bag that have clinical significance and may direct further investigations. Firstly, any formation inside the bag (coagulation, foam, etc.) can be inspected and moved around by manually manipulating the bag. Secondly, the sedimentation rate can be observed in the closed blue line while the infusion set is mounted. Thirdly, the pressure inside the right atrium as a consequence of increasing pulmonary resistance can be indirectly assessed by measuring the difference between the level of the blood in the red line at the end of reinfusion by gravity and the level of the heart.

A glass bottle may be used to test the presence of the patent foramen ovale. Near the end of the reinfusion phase, when the oxygen pressure in the bottle is raised on purpose, the smaller amount of blood gets hyperoxygenated and may induce the fetal circulation response.

5. Waste disposal volume

Glass bottles and their separate lines generate more volume and weight of waste than plastic sets.

6. The cost

With the advent of ozone-resistant plastics, negative-pressure glass bottles with separate lines became more expensive to produce and dispose of.

The use of a three-line plastic bag gravity set is more flexible, easier to use, and safer from a practical standpoint, as there are no additional complexities related to pressure generation and adjustments, which are required when using vacuum glass bottles and optional when using firm plastic containers.

There are slight variations in the product design among different manufacturers, but they all have the required parts and use certified materials. The aim is to facilitate the procedure with maximum safety by using three tubing lines, special filters, and an adequate number and positioning of the clamps. Minimum invasiveness for the patient is achieved with only one venepuncture. It is noteworthy that the nurse practitioner played an important part in the development and improvement of the M-AHT plastic bag kit shown in Figure 4.4.1.1.2.

Nursing research showed that good clinical nursing practices in the application of ozone therapy play an important part in patient satisfaction and can be considered a therapeutic variable (96).

The nursing profession is inherently compassionate, pragmatic, and innovative; therefore, practical and scientific contributions to the profession should be systematically encouraged and valued. It is crucial to provide nursing professionals with ongoing support in the form of education and motivation so they can express their creativity and contribute both to the development of new and the improvement of existing nursing practices.

5. CONCLUSION

Coronavirus disease (COVID-19) is one of the most publicized, tracked, and researched infectious diseases in recent history. Alongside the official extension of the pandemic and the emergence of long COVID-19 challenges, the search for more specific, safe, and effective prophylactic, therapeutic, and disease management options continues.

Oxygen ozone therapy, being an old treatment modality, has been effectively and safely used in almost all clinical branches of medicine, including stomatology and veterinary medicine. The antimicrobial, anti-inflammatory, and immunomodulatory therapeutic effects of ozone therapy in the treatment of infections and diseases, together with general oxygenation and circulation improvement effects, have been successfully utilized in COVID-19 treatment. Mechanisms of action on the molecular and cellular levels and the therapeutic effects on cytokine modulation, oxidative stress modulation, improvement of circulation and tissue oxygenation, reparation of vascular endothelium, and the antithrombotic effect have been proven. Ozone, as a pro-oxidant molecule, inflicts transient oxidative stress on the organism, stimulating the antioxidant response in a hormetic dose-effect manner and requiring an individualized dosing and treatment approach.

All 27 studies reviewed demonstrated significant efficacy and safety of ozone therapy as an adjuvant therapy in COVID-19 treatment, prophylaxis, and post-COVID-19 conditions treatment. Recognizing the efficacy of ozone therapy in preventing disease onset and reversing disease progression emphasizes the importance of initiating treatment as soon as possible. Due to the heterogeneity of study designs, disease severity, and small sample size, more clinical trials are needed in order to standardize therapeutic protocols for different stages of the disease with different methods of application. The same need and rationale for further research extends to the use of ozone therapy in prophylaxis, treatment, and rehabilitation of post-COVID-19 conditions. Use of ozone therapy as a monotherapy in COVID-19 has been restricted to a limited number of cases successfully treated at home, the majority of which remain unpublished, indicating the need for additional research in this area as well.

Nursing professionals have an important role in the safe preparation of oxygen ozone medicine, the application of the ozone therapy technique, the education and preparation of the patients, and the monitoring of the effects. Meeting the challenges of the

individual clinical presentations of COVID-19 requires the nursing professional to be able to recognize the risk factors, signs, and symptoms when providing holistic, individualized care. Providing continuous education and training of health care professionals in ozone therapy is essential to maintaining the quality of care and high safety record.

The proven efficacy and safety of ozone therapy in COVID-19, as well as its affordability, have provided policymakers with additional evidence and arguments for integrating it into public hospitals and national health care systems in order to make it accessible to a larger population.

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7. ABBREVIATIONS

ACD-A: anticoagulant citrate dextrose solution A

ACE-2: angiotensin-converting enzyme 2

ALP: alkaline phosphatase

ALT: alanine aminotransferase

APTT: activated partial thromboplastin time

ARDS: acute respiratory distress syndrome

ARE: antioxidant response element

AST: aspartate aminotransferase

BUN: blood urea nitrogen

CBC: complete blood count

CBV: circulating blood volume

CCS: case-control study

CDC: Center for Disease Control

COPD: chronic obstructive pulmonary disease

CoV: coronaviruses

COVID-19: Coronavirus Disease 2019

CR: case report

CRP: C reactive protein

CSS: case series study

CT: computerized tomography

DIC: disseminated intravascular coagulation

DNA: deoxyribonucleic acid

EMA: European Medicines Agency

EBBO: extra-corporeal blood oxygenation

FDA: U.S. Food and Drug Administration

FER: ferritine

FIB: fibrinogen

FiO2: fractional inspired oxygen

GALT: gut-associated lymphoid tissue

GHS: reduced glutathione

Glu: glucose in blood

G6PD: glucose-6-phosphate dehydrogenase deficiency

gtt: drops (lat.*guttae*)

HADS: Hospital Anxiety and Depression Scale

HBV: hepatitis B virus

HCoVs: Human coronaviruses

HCQ: hydroxychloroquine

HCV: hepatitis C virus

HCW: healthcare workers

4-HNE: 4-hydroxy-nonenal

HO-1: heme oxygenase 1

ICD-10-CM: International Classification of Diseases, Tenth Revision, Clinical Modification

ICU: intensive care unit

IDLH: Immediately Dangerous to Life and Health

IL-1: interleukin-1

IL-6: interleukin-6

ISCO3: International Scientific Committee of Ozone Therapy

IV.: intravenous

Keap1: Kelch-like ECH-associated protein 1

LDH: lactate dehydrogenase

LOPs: lipid oxidation products

M-AHT or MAH: Major autohemotherapy ozone therapy

MAMs: mitochondria-associated endoplasmic reticulum membranes

Mi-AHT or MiAH: Minor autohemotherapy ozone therapy

MIS-C: multisystem inflammatory syndrome in children

MOG: medical ozone generator

MRI: magnetic resonance imaging

NAC: n-acetylcysteine

NAAT: nucleic acid amplification test

NEWS: National Early Warning Score

NF-κB: nuclear factor kappa B

NIH: U.S. National Institutes of Health

NLR: neutrophil-lymphocyte ratio

NO: nitric oxide

Nrf2: nuclear factor erythroid 2-related factor 2

OSHA: U.S. Occupational Safety and Health Administration

OT or O3: ozone therapy

O2/O3: oxygen-ozone therapy

O3SS: ozonized saline solution

PaCO2: partial pressure of carbon dioxide

PaO2: partial pressure of oxygen

PASC: post-acute sequelae of SARS-CoV2 infection

PCC: post-COVID-19 conditions

PCR: polymerase chain reaction test

PCS: prospective controlled study

PCT: pilot clinical trials

PEL: permissible exposure limit

PrEP:pre-exposure prophylaxis

PT: prothrombin time

PTFE: polytetrafluoroethylene

PUFA: polyunsaturated fatty acids

PVDF: polyvinylidene difluoride

RAS: renin–angiotensin system

RCT: randomized control trial

RetCS: retrospective cohort study

RetCCS: retrospective controlled cohort study

RIO3: rectal insufflation ozone therapy

RNA: ribonucleic acid

+ssRNA: positive-sense RNA

ROS: reactive oxygen species

RTLFs: respiratory tract lining fluids

RT-PCR: reverse transcription polymerase chain reaction

SARS-CoV-2: Severe Acute Respiratory Syndrome Coronavirus 2

SGPT: serum glutamic pyruvic transaminase

SIMEU: Italian society of emergency-urgency medicine

SIOOT: Italian scientific society of oxygen ozone therapy

SOFA: Sequential Organ Failure Assessment

SpO2: oxygen saturation

ST: standard conventional therapy for COVID-19

TNF: tumor necrosis factor

VIO3: vaginal insufflation ozone therapy

WFOT: World Federation of Ozone Therapy

WHO: World Health Organization

8. SUMMARY

Oxygen Ozone Therapy in the Treatment of COVID-19: Current State of Evidence on the Mechanisms of Action, Effectiveness and Safety

The COVID-19 pandemic presented humanity with numerous challenges, particularly in the fields of prevention and treatment. Oxygen-ozone therapy has been utilized in medicine for over a century, and its application in COVID-19 has been the subject of recent research and evaluation.

This narrative literature review thesis analyzed 27 primary research papers, presenting the current state of the evidence on the mechanisms of action, effectiveness, and safety of ozone therapy in COVID-19 prophylaxis, treatment, and post-COVID-19 condition treatment as adjuvant and monotherapy. Three of the most common systemic routes of ozone therapy application in COVID-19 were evaluated: major-autohemotherapy (M-AHT), ozonized saline solution (O3SS), and rectal insufflation (RIO3). All studies reviewed demonstrated significant efficacy and high safety of ozone therapy, with minor transient adverse effects reported with the use of O3SS and RIO3.

The main mechanism of action of ozone is through its derivatives, two main signaling molecules: reactive oxygen species (ROS) and lipid oxidation products (LOPs), which regulate numerous molecular pathways and result in the following therapeutic effects: antiviral, anti-inflammatory, antithrombotic, immune system modulation, modulation of oxidative stress, improvement of tissue oxygenation and circulation, and protection of the vascular endothelium.

The thesis explains that the role of the nurse is in the safe preparation of the oxygen-ozone mixture, assistance in the administration of the prescribed therapy using different routes of application, education and preparation of the patients, and monitoring of the effects. Major autohemotherapy technique is explained together with a critical comparison of the use of three M-AHT sets commonly used in clinical practice.

Key words: oxygen-ozone therapy, ozone therapy, major-autohemotherapy, COVID-19, nursing.

9. SAŽETAK

Ozonterapija u liječenju bolesti COVID-19: analiza literature o mehanizmu djelovanja, učinkovitosti i sigurnosti

Pandemija COVID-19 je pred čovječanstvo postavila brojne izazove, posebice u području prevencije i liječenja. Ozonoterapija se u medicini koristi više od jednog stoljeća, a njena primjena kod COVID-19 bila je predmet nedavnih istraživanja i evaluacija.

U ovom preglednom završnom radu deskriptivnom metodom analizirano je 27 izvornih istraživačkih radova u svrhu prikaza mehnizama djelovanja, učinkovitosti i sigurnosti ozonoterapije, kao adjuvantne i monoterapije, u profilaksi i liječenju akutnog i dugog COVID-19. Obrađena su tri najčešća načina sistemske primjene ozonoterapije kod COVID-19: velika autohemoterapija (M-AHT), terapija ozoniziranom fiziološkom otopinom (O3SS) I rektalna insuflacija (RIO3). Sve pregledane studije pokazale su značajnu učinkovitost i visoku sigurnost ozonoterapije, s blagim prolaznim štetnim učincima u primjeni O3SS i RIO3.

Glavni mehanizam djelovanja ozona je putem njegovih derivata, koje čine dvije skupine signalnih molekula: reaktivni kisikovi spojevi (engl. *ROS*) i lipoperoksidi tj. produkti oksidacije lipida (engl. *LOPs*). Signalne molekule reguliraju brojne molekularne putove i rezultiraju sljedećim terapijskim učincima: antivirusnim, protuupalnim, antitrombotskim, modulacijom imunološkog sustava, modulacijom oksidativnog stresa, poboljšanjem oksigenacije tkiva i cirkulacije, te zaštitom vaskularnog endotela.

U radu je objašnjena uloga medicinske sestre u sigurnoj pripremi smjese kisika i ozona, asistenciji u primjeni propisane terapije korištenjem različitih tehnika, edukaciji i pripremi bolesnika te praćenju terapijskih učinaka. Tehnika velike autohemoterapije objašnjena je zajedno s kritičkom usporedbom upotrebe tri M-AHT seta koji se uobičajeno koriste u kliničkoj praksi.

Ključne riječi: ozonoterapija, ozon terapija, COVID-19, velika autohemoterapija, medicinska sestra

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