International Scientific Committee of Ozone Therapy

Madrid Declaration on OZONE THERAPY

(3rd Edition)

March 22th, 2020

"For the Unification of Criteria in the Practice of Ozone Therapy"

MADRID DECLARATION ON OZONE THERAPY
(3rd edition)
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1st edition: Approved at the "International Meeting of Ozone Therapy Schools" held at the Royal National Academy of Medicine in Madrid on **June 4th, 2010**, under the auspices of AEPROMO (Spanish Association of Medical Professionals in Ozone Therapy).

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MADRID DECLARATION ON OZONE THERAPY
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The updating process of the 3rd edition of the Madrid Declaration took nearly one year. Since July 2019 ISCO3 issued several international calls asking ozone therapists to send their proposals and suggestions for the updating of the Madrid Declaration on Ozone Therapy. Last day to receive proposals: November 30, 2019. Based on the proposals received the authors wrote different drafts until agreeing on the text. Then it was submitted for discussion among the ISCO3 members. After an intensive exchange of comments, suggestions, and revisions, finally, the 27 members of ISCO3 unanimously approved the 3rd edition of the Declaration on March 22, 2020 and it became an official document of the committee.

ISCO3 wants to thank all those who participated and/or supported the committee in the challenging efforts of updating the Declaration.

WHO IS ISCO3?

ISCO3 is a scientific non-profit association founded in 2010 and its activities are governed by the Spanish legislation.

ISCO3 is an independent scientific medical body from national and international associations or federations of ozone therapy; and commercial companies. As a consequence, its twenty-seven members do not represent any or various national or international ozone therapy associations, or commercial companies. Its members act within ISCO3 only in their own capacity.

ISCO3 has been created with the clear intention that it has to turn into an international scientific authority due to the composition of its members; and that its recommendations may become a source of reference to all those who practice this medical therapy.

The members are elected every five years. ISCO3 issued an international call at the beginning of July 2019 asking health professionals from any place, directly related to the practice of ozone therapy, to apply. The deadline set was October 31, 2019. Based on the applications received the current members have been elected for the five years period that stared on January 1, 2020 and will end on December 31, 2024. The new members elected the Governing Board of the committee integrated by the President, the Vice President, and the Scientific Secretary.

Main Objectives of ISCO3

To determine the scientific and medical merits of each particular application of ozone, as well as a code of good practice, in order to create a standard practice and prevent the possibility of malpractice.

To standardize each particular application of ozone based on scientific evidence.

To achieve these objectives ISCO3 has the following three tools:

- The approval and publication of the Madrid Declaration on Ozone Therapy. First publication in 2010, and two updates in 2015 and 2020.
- The approval and publication of different papers on subjects related to ozone therapy.

- The ISCO3 *Ozone Therapy International Library*, with all published and presented papers on ozone therapy. It is online, of free access, and is updated frequently.

DISCLAIMER

ISCO3 documents (including the Madrid Declaration on ozone Therapy) are recommendations and serve as a source of guidance and reference tor all those who practice ozone therapy. They are not mandatory. It is up to each ozone therapist to use his or her own clinical judgment in applying the recommendations issued by ISCO3.

All technical publications of ISCO3 are under ISCO3's name, including codes of practice, safety procedures and any other technical information. The content of these publications, were obtained from reliable sources, based on technical information, published research, from the experience of the members of ISCO3 and from others at the date of their approval.

ISCO3 does not guarantee results and assumes no liability or responsibility in connection with the references for the use of information or suggestions contained in ISCO3's publications.

ISCO3 has no control whatsoever with regards to performance or non-performance, misinterpretation, proper or improper use of any information or suggestions contained in ISCO3's publications by any person or entity (including ISCO3 members) and ISCO3 expressly assumes no liability in connection thereto.

ISCO3's publications are subject to periodic review and users are advised to obtain the latest edition.

MADRID DECLARATION ON OZONE THERAPY
3rd ed., 2020
Official document of ISCO3

Taking into account since the discovery of ozone by the Dutch physician Martinus van Marum in 1781 and synthesized by the German chemist Christian Friedrich Schönbein in 1840, its medical use has increased in different parts of the world and health professionals are showing more interest in ozone's benefits and how it works. Accordingly, with the increase in the number of ozone therapists all over the world, the number of patients reaping benefits from ozone has risen. Although great efforts and advances have been made since the approval of the 1st edition of the Declaration in 2010, the consolidation of ozone therapy has not been easy. Resistance is still found within the medical community and ozone's recognition in the legal field will require more coordinated efforts.

Recalling that pre-clinical research and clinical trials on the use of ozone therapy have been carried out in different countries, with considerable scientific rigor, obtaining results that support its practice using systematic medical protocols.

Bearing in mind that the preclinical studies, genotoxic, toxicology and clinical studies carried out, endorse the safe application and the generally innocuous character of this medical therapy under a wide range of doses. More detail in ISCO3 official document: *Ozone Therapy and its Scientific Foundations.*[1]

Emphasizing that research and clinical experience with medical ozone are making progress despite various obstacles. However, the main and permanent challenge for researchers and for ozone therapy associations is the lack of accessibility to adequate financial resources that are essential to conduct the required scientific research.

Stating that, it is absolutely necessary to work with specific objectives, and in a unified way to ensure a practice of great precision with standardized safe clinical protocols.

Recognizing that there is variance that the medical community wishes to standardize, and that progress has already been made, it is necessary to continue with the development of medical definitions of procedures and protocols determining the best applications where necessary, as well as a code of good practice, in order to overcome more efficiently the possibility of malpractice.

Welcoming with great satisfaction that ozone therapy practice has been regularized in the following 13 countries so far: Greece: 1991 and 2014. Ukraine: 2001 and 2014. Italy in the regions of Lombardy (2003), Emilia-Romagna (2007) and Marche (2009), and favorable court decisions have been taken by the Administrative Court of Lazio (1996 and 2003). China: 2005. Russia: 2005 and 2007. Spain: Between 2007 and 2012 through Directives issued in 15 autonomous communities; and that ozone therapy is used in 20 pain treatment units of the public health sector.[5] Cuba: 2009 and 2015. Sultanate of Oman: 2010. Emirate of Dubai of the United Arab Emirates (UAE): 2011. Portugal: 2013 to 2018. Turkey: 2014. Brazil: Dentistry in 2015; and Medicine in the public health sector in 2018. México: in the state of Nueva León (2018). Important efforts are being deployed

in other countries towards regularization. So, it is likely that other countries may follow. More details can be found in ISCO3 official document: *Ozone Therapy and Legislation - Analysis for its Regularization.*[2]

Taking into account that the International Scientific Committee of Ozone therapy (ISCO3), as the repository of the Madrid Declaration on Ozone Therapy and as the author and responsible body for its updating, invited ozone therapists around the world to send their proposals to improve it. ISCO3 received a high number of proposals and based on these and the internal contributions and edits provided by the members of the committee, ISCO3 approved the 3rd. edition on March 22, 2020. Through this exchange and subsequent revisions, the entire ozone therapy community had an opportunity to participate in a serious medical scientific exchange with the objective of having a global document to apply to patient care and improve clinical outcomes by health care professionals.

Considering that the updated version of the 3rd. edition of the Declaration reflects the advances in the field of ozone therapy, provides tools for the correct application to patients, and reflects a great amount of unanimity among the community of ozone therapists from around the world.

The International Scientific Committee of Ozone Therapy (ISCO3)
has adopted the following
CONCLUSIONS

First. To approve the "**Therapeutic Ranges for the Use of Ozone**" and the two addendums detailed in the annex of this Declaration.

Second. To increase the exchange of knowledge, research, and experiences, both positive and negative that occur in the field of ozone therapy, to further the expansion of this knowledge benefits that this therapy has. To stimulate the publications of research results in specialized medicine journals. To study the scientific background of scientific papers collected in the free access online ISCO3 *Ozone Therapy International Library*.[3]

Third. To encourage health researchers to increase their creative efforts, so that ozone therapy continues to demonstrate its therapeutic benefits with safety and effectiveness under the development of controlled clinical trials.

Fourth. To continue creating Standardized Operative Procedures, according to good clinical practices for each procedure, taking into account new developments, with the view of increasing the quality and making diverse homogeneous treatments.

Fifth. To make systematic efforts to ensure that each scientific congress/meeting to be organized adopts conclusions that reflect the progress made and sets achievable and realistic targets, sharing the findings and aims to encourage and promote research to deepen the understanding of ozone therapy. To work towards the harmonization and unification of criteria at the international scientific level.

Sixth. To encourage the different associations to work in their own countries where the ozone therapy has not yet been regulated to get it properly regulated and therefore to enjoy a legal status.

Seventh. To encourage the edition of textbooks, the organization of theoretical and practical courses on ozone therapy in a systematic way, in order to standardize the practice of ozone therapy based on scientific evidence; this will result in more efficient medical health care delivery which will benefit the patients and improve outcomes.

Eighth. To encourage that training courses may follow the same guidelines and standards issued by internationally competent organizations such as ISCO3. [4]

The International Scientific Committee of Ozone Therapy (ISCO3)
has adopted the following
RECOMMENDATION

That the ***Therapeutic Ranges for the Use of Ozone*** and its addendums detailed in the annex to this "Madrid Declaration on Ozone Therapy" (3rd. ed., 2020) serve as a reference to ozone therapists to apply safe and effective dosages of ozone while minimizing adverse events.

These ***Therapeutic Ranges for the Use of Ozone*** and its two addendums are the summary of scientific research in different countries and are the result of many years of experience and clinical practice.

ACKNOWLEDGEMENTS
The International Scientific Committee of Ozone Therapy (ISCO3)

Express its most sincere gratitude and recognition to **Dr. Velio Bocci** (passed away in 2019) and **Dr. Nabil Masouff** (passed away in 2015) for the significant and important contributions they have made in the application of ozone therapy in the fields of research, teaching, information and patient care, to the point that in the history of ozone therapy they must be considered among its most important pioneers.

Express its most sincere gratitude and recognition to **Dr. Adriana Schwartz** for her great vision in detecting the need to draft a document that unifies criteria and for the initiative to write under the international consensus the first Madrid Declaration in 2010. Dr. Adriana Schwartz through her dedication and commitment has continued being the leader in the process of updating the 2nd and 3rd edition of the Madrid Declaration on Ozone Therapy along with **Dr. Gregorio Martínez Sánchez**.

Express its most sincere gratitude to **AEPROMO (Spanish Association of Medical Professionals in Ozone Therapy)** for its secretarial, administrative, organizational and logistical support in the process of making possible the publication of the 3rd edition of the Madrid Declaration on Ozone Therapy.

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ANNEX AND ADDENDUMS TO THE MADRID DECLARATION ON OZONE THERAPY (3rd ed.)

WHICH IS AN INTEGRAL PART THEREOF

Approved by
The International Scientific Committee of Ozone Therapy (ISCO3)
on March 22, 2020

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THERAPEUTIC RANGES FOR THE USE OF OZONE

ACRONYMS

ACD-A: *Anticoagulant Citrate Dextrose* Solution A
ACE: Angiotensin-converting enzyme
ADL: Activity of Daily Living
AE: Adverse Effects
AMP: 5'-adenosine monophosphate
AMPK: AMP-activated protein kinase
b.w.: Body Weight
CBV: Circulating Blood Volume
CE: European Community
COPD: Chronic obstructive pulmonary disease
CT: Computed Tomography
DIV: Direct Intravenous Injection of Ozone
DOAC: direct oral anticoagulants
DPG: 2,3-Diphosphoglycerate
EBOO: Extracorporeal blood oxygenation-ozonation
EBM: Evidence Based-Medicine
ESA: Erythropoiesis Stimulating Agents
EU: European Union
FDA: US Food and Drug Administration
FIO: Italian Federation of Ozone Therapy
G6PD: Glucose 6 phosphate dehydrogenase
GED: Gas Exchange Device
HBO3: Hyperbaric Multi Passes Method
HIV-AIDS: Human Immunodeficiency Virus Infection and Acquired Immune Deficiency Syndrome
HNE: 4-hydroxy-2,3-transnonenal
INR: International Normalized Ratio
IO3A: International Ozone Association
IP: Intraperitoneal
MAH: Major Autohemotherapy
MiAH: Minor Autohemotherapy
MOG: Medical Ozone Generator
NOAC: New Oral Anticoagulants
NIH: National Institutes of Health (USA)
O_3/O_2: Medical Ozone
O_3SS: Ozonized Saline Solution
O_{3X}: Ozone Therapy
PTFE: Polytetrafluoroethylene
PV: Peroxide Value
PVDF: Poyvinylidene difluoride
RIO_3: Rectal insufflation of Ozone
ROS: Reactive Oxygen Species
SH: sulfhydryl group
µg/NmL: Microgram/ Normalized milliliter of ozone concentration
USP: United States Pharmacopoeia
UV: Ultra Violet
VTD: Venous Thromboembolic Disease

1. THERAPEUTIC BASIS

Ozone therapy (O_{3X}) is a complementary medical treatment that uses an oxygen-ozone mixture (95%-99.95% of oxygen and 0.05%-5% of ozone), generated by a certified medical device,[1] as a therapeutic agent and a medical protocol to treat a wide range of diseases. Depending on the route of application ozone can act by 1) direct oxidation or 2) by an indirect pathway: The response depends on the modulation of nuclear transduction mechanisms and signals such as Nrf2-NFkB and protein synthesis.[2-4] As a result, the dose concept in ozone therapy is based on its hormetic response, and this is crucial to manage the equilibrium between the pro-inflammatory/anti-inflammatory response. Currently (May/2020) in the MedLine (Pub Med) database there are 3329 papers relating to ozone therapy of which 251 clinical trials, 169 randomized controlled trials, 24 systematic review and 18 meta-analysis studies, supporting the use of ozone in medicine. Additionally, at ClinicalTrials.gov (database of clinical studies at the National Institutes of Health of EEUU) there are 37 registered studies found for "ozone therapy".[5]

Ozone Therapy is a complementary therapy, not an alternative therapy. Ozone therapy is an adjunctive therapy and should be performed *along with* and not *instead of* the allopathic medicine. Understanding the difference between *complementary* and *alternative* is critical for the practitioner of ozone therapy. The application of ozone therapy complements other allopathic treatments such pharmaceutical interventions and surgical procedures and does not replace them as an alternative.

The US National Center for Complementary and Integrative Health (NCCIH) at the National Institutes of Health (NIH) clearly states the difference between the complementary and alternative medicine. "When describing these approaches, people often use 'alternative' and 'complementary' interchangeably, but the two terms refer to different concepts: If a non-mainstream practice is used **together with** conventional medicine, it's considered *complementary*. If a non-mainstream practice is used **in place of** conventional medicine, it's considered *alternative*." [6]

The US National Cancer Institute at the National Institutes of Health also specifies the difference. Alternative medicine: "Treatments that are used instead of standard treatments (…) For example, a special diet may be used instead of anticancer drugs as a treatment for cancer." [7] Complementary medicine: "Treatments that are used along with standard treatments, but are not considered standard (…) For example, acupuncture may be used with certain drugs to help lessen cancer pain or nausea and vomiting." [8]

The European Parliament interested that the European Union adopts a legislation on non-conventional medicines also sets up the difference between complementary and alternative medicines: "whereas a given medical or surgical treatment applied instead of another may be described as *alternative* and a treatment used to supplement another treatment may be described as *complementary*" (letter D). Based on this capital difference "3. [The European Parliament] calls on the Commission, in formulating European legislation on non-conventional forms of medicine, to make a clear distinction between non-conventional medicines which are *complementary* in nature and those which are *alternative* medicines in the sense that they replace conventional medicine."[9]

Ozone therapy has to be practiced as a complementary, adjunctive or palliative treatment for various diseases. Ozone therapy is part of the techniques of new technologies that complement and facilitate conventional treatments. It is another tool in the medical arsenal of the physician.

Ozone Therapist. The doctor who practices this therapy is called an ozone therapist. The word therapist comes from the Greek (*therapeutes*) composed by the verb *therapeuein* and meaning: care for, attend, alleviating, hence the word therapy refers to the person who is dedicated to curing diseases, in this case with oxygen-ozone mixture.

Practitioners should limit their practice to the field of their proficiency. This means: physicians have to be in charge of human medical treatment or clinical trials; dentists have to treat diseases and conditions of the oral cavity; veterinarians have to treat diseases, disorders and injuries in non-human animals; Biochemists, pharmacists, biologists will participate in the molecular, preclinical and clinical research. In case of clinical research, the direct interaction with patients will be the responsibility of a physician. Nurses and technicians will act in accordance to the instruction of the corresponding doctor. Ozone therapy is a medical act and has to be performed by a physician.

Medical ozone generator (MOG). Within the European Union the ozone generators are medical devices falling within class IIb. The Council Directive 93/42/EEC indicates that "as a general rule" medical devices should "bear the CE mark to indicate their conformity with the provisions of this Directive to enable them to move freely within the Community and to be put into service in accordance with their intended purpose".

As a consequence, it is highly recommended that the buyer verifies the "CE declaration of conformity". In other countries it is very useful that the buyer first verifies if the ozone generator has authorization issued by the governmental institution dealing with medical devices. For more details see ISCO3 *Guidelines and Recommendations for Medical Professionals Planning to Acquire a Medical Ozone Generator.* Madrid, 2019. International Scientific Committee of Ozone Therapy.[10]

The MOG must produce ozone exclusively from medicinal grade, at least 99, 5% pure oxygen, coming from a medical quality certified container, e.g. a high-pressure cylinder. The oxygen for industrial purposes does not qualify for medical use, because the requirements of hygiene, filling speed, internal bottle humidity and sterility of these cylinders are different from the medical ones. Machines using room air, including oxygen concentrators, do not qualify for ozone therapy, because this leads to the production of substances different to ozone.

The MOG must allow the measurements of precise ozone concentrations (1 *µg*/mL-80 *µg*/NmL), and must generate a homogeneous oxygen-ozone mixture. The concentration expressed in *µg*/NmL and must have a margin of error equal or better than ±10%. No other substances besides O_2 and O_3 may be present in the produced gas mixture.

Medical grade oxygen (O_2) should fit the quality standard of the local Pharmacopoeia. If local Pharmacopoeia is not available, the reference Pharmacopoeia should be: European Pharmacopoeia,[11] United States Pharmacopoeia,[12] Japanese Pharmacopoeia[13] or Russian regulation GOST 5583-78 (Industrial and Medical Oxygen).[14]

According to these Pharmacopoeias,[13] the medical ozone generator (MOG) must be able to generate the therapeutic, i.e. homogeneous oxygen-ozone mixture with a range of ozone concentration between 1 *µg*/NmL and 80 *µg*/NmL. No other substances besides O_2 (medical grade oxygen) and O_3 (ozone) can be present in the produced gas mixture. To ensure the accuracy of the ozone concentration, the calibration of the MOG should be done regularly, once a year.

Concentrations. The measure of ozone gas concentration should be compensated for temperature and pressure. The pressure of 1 atm (760 mmHg, 1.10325 bar, and temperature of 0°C; 273.15 K) should be taken as the standard. Those conditions should be referred to as "International Standard Conditions" and the unit "normalized ozone concentration" preferably expressed as *µg*/NmL.[1] This is the only unit recognized by the International Ozone Association-IO3A.[15] The concentration expressed in *µg*/NmL must have a margin of error equal or better than ±10%.

Dose effect relationship. Ozone therapeutic indications are based on the knowledge that low physiological doses of ozone may play important roles within the cell.[16,17] The dose / effect relationship of ozone is hormetic.[18] The hormetic response of ozone is not a hypothesis, it is a fact demonstrated clinically and experimentally.[3,4] The interaction of ozone mediators (mainly H_2O_2 and 4-hydroxy-2,3-transnonenal, HNE) with nuclear factor and the consequent induction of a therapeutic response is now well established by scientific data.[2,3,19] Low doses of ozone are able to modulate biochemical pathways through known molecular redox master switches such as Nrf2/Keap1 or NF-kB/IkB as an adaptive response. In that way, low doses stimulate cell protective pathways and nuclear transcription without altering cell viability;[16] on the contrary high doses can be genotoxic.[18,20-22]

The use of non-appropriate range dosage of ozone therapy may cause serious side effects, ranging from tissue necrosis[18,20] to a potential induction of cancer that may develop during chronic ozone exposure or due to high doses exposure.[21]

At the molecular level, different mechanisms of action have been described to support the clinical evidence of ozone therapy.[21] Data summarized in this document are based on more than 3000 scientific books and papers listed in the free access online ISCO3 *Ozone Therapy International Library.*[23]

There are <u>therapeutic, non-effective and toxic concentrations</u> of ozone. It has been proven that concentrations of 10 *µg*/NmL or 50 *µg*/NmL and even smaller, have therapeutic effects with a wide security margin. So it is now accepted that the therapeutic ozone dosage for **systemic treatment** [Major Autohemotherapy (MAH), Ozonized Saline Solution (O_3SS), rectal insufflation (RIO_3), vaginal, etc.], ranging between a total ozone dose of (5.0-6.0) mg per treatment and concentrations ranging from 10 *µg*/NmL to 50 *µg*/NmL, are safe and effective.[22]

The total ozone dose is equivalent to the gas volume (mL) multiplied by the ozone concentration (*µg*/NmL) (Dosage= Volume x Concentration). The Dose is not given by kg body weight but by dose dependent response and the concentration can be expressed as well in *µg*/NmL or as mg/NL of ozone.[24] We strongly advise applying the *up-dosing system*, as Dr. Bocci stated, "*start low, go slow*".[25]

Studies, involving the calculation of the ozone dose based on body weight, are ongoing. All therapeutic dosages are divided into three types, according to their mechanism of action (Table 1).

a) Low doses: These doses have an immunomodulatory effect and are used in diseases where there is suspicion that the immune system is very much compromised. For example, in cancer, for the elderly and for debilitated patients, etc.

b) Medium doses: They are immunomodulatory and stimulate the antioxidant enzyme defense system. They are most useful in chronic degenerative diseases such as diabetes, atherosclerosis, Chronic Obstructive Pulmonary Disease (COPD), Parkinson syndrome, Alzheimer, and senile dementia.

c) High doses: They have an inhibitory effect on the mechanisms, which occur in autoimmune diseases such as rheumatoid arthritis and lupus. They are especially employed in ulcers or infected injuries and are, also, used to prepare ozonized oil and water.

Materials to be used. All materials used must be disposable and ozone resistant e.g.: glass, silicone, stainless steel 316, fluoropolymer plastics, PTFE polytetrafluoroethylene (Teflon®), PVDF polyvinylidene difluoride (Kynar®), Fluorocarbon (Viton®), laboratory-grade glass, titanium, and polycarbonate.

The action mechanism. Most of the local routes of application of ozone act through direct oxidation. However, the systemic route use of ozone is based essentially on the following mechanisms:

Redox bioregulator. At low doses, systemically applied ozone reacts with biomolecules to generate second mediators (e.g. hydrogen peroxide and 4-hydroxyalkenals).[2] Second messengers induce a signal transduction through the oxidation of -SH residues. The response depends on the modulation of nuclear transduction mechanisms such as Nrf2 or NFκB and protein synthesis resulting in a regulation of the antioxidants Nrf2 pathway, or an immunomodulation through NFκB.[2-4] There is solid molecular evidence of this mechanism at clinical and preclinical levels.[26-30]

Pain relief: Local injection of ozone reduces the pain at least by the following molecular mechanisms: Direct oxidation of pain mediators or pain receptors,[31] inhibition of purinergic receptors P2X3 and P2X7;[32] modulation of caspase pathways,[33] inhibits tissue autophagy (through the inhibition of LC3B and Beclin1) and apoptosis (through the inactivation of Caspase 3, phosphodiesterase 2A and NFκB p65 signals).[34] Activates the 5'-adenosine monophosphate (AMP)-activated protein kinase (AMPK).[35]

Tissue oxygenation: The systemic route of ozone administration is useful in hypoxic and ischemic syndromes. Ozone leads to correcting the altered hemostatic-hemorheological parameters improving blood flow and the release of O_2 from hemoglobin into the tissues.[36-38] The mechanisms involved, at least in part, increase the 2,3 diphosphoglycerate (DPG) pathway.[39]

Table 1. Guidelines for ozone concentration / volume, according to the most common routes of administration (Tab. 1A, Local Routes; Tab. 1B, Systemic Routes).

Table 1 A. Local Routes

Most common routes of application: LOCAL					
Method	O_3/O_2	Levels			**Remarks**
		Low	**Medium**	**High**	
Auricular	C. (μg/NmL)	4	10	20	In dynamic application: Flushing manually, very slowly, using a siliconized syringe of 50 mL for 5 min.
	V. (mL)	50	50	50	
	Dose (mg)	0.2	0.5	1	
Bags	C. (μg/NmL)	30-20	50-40	80-60	20-30 min. The area must be moistened before treating it.
	V. (L)	Depending on the bag dimension			
	Dose (mg)	Depending on the bag volume			
Paravertebral	C. (μg/NmL)	10	15	20	See ISCO3 (2014)[40] *and* ISCO3 (2016)[41] for details.
	V. (mL)	5-20			
	Dose (μg)	50-200	75-300	100-400	
Intradiscal. Lumbar	C. (μg/NmL)	25	30	35	For cervical spine column, 3-5 mL volume of ozone.
	V. (mL)	10	10	10	
	Dose (μg)	250	300	350	
Subcutaneous	C. (μg/NmL)	5	8	10	Maximum 100 mL/session. 200 ml for cellulite.
	V. (mL)	1-2			
	Dose (μg)	5-10	8-16	10-20	
Subcutaneous infiltration of hands (glove technique)	C. (μg/NmL)	20	10	5	Infiltrate twice a week or till the pain has resolved (around 6 sessions).[42]
	V. (mL)	10-40			
	Dose (μg)	50-200	100-400	200-800	
Intra-articular joints (shoulder, knee, hip, elbow, ankle, etc.)	C. (μg/NmL)	5	10	15-20	See Reference: ISCO3 (2014)[40] *Ozone in Non-Rheumatic Locomotor System Pathologies*, for details.
	V. (mL)	5-20			
	Dose (μg)	25-100	50-200	75-100 300-400	

Note. Not recommended route: inhalation (high risk of toxicity, see text for details).[43]
Legend. C: concentration; V: volume; 1 mg = 1000 μg.

Table. 1 B. Systemic Routes.

Most common routes of application: SYSTEMIC					
Method	O_3/O_2	Levels			**Remarks**
		Low	**Medium**	**High**	
Major Autohemotherapy (MAH)	C. (μg/NmL)	10-20	20-30	35-40	In some cases (autoimmune diseases and viral infections), it could be assessed that up to (50-60) μg/NmL has proved to be safe and with a greater capacity of induction of cytokines. Venous blood volume may be estimated multiplying 1.2 by patient b.w.[44]
	V. (mL)	50 - 100			
	Dose (mg)	0.5-1.0 1.0-2.0	1.0-1.5 2.0-3.0	1.75-2.0 3.5-4.0	
Minor Autohemotherapy (MiAH)	C. (μg/NmL)	5-10	15-20	30-40	5 mL blood is removed intravenously and drawn into a 20 mL disposable syringe (already containing the same amount of ozone-oxygen mixture).[45]
	V. (mL)	5			
	Dose (μg)	25-50	75-100	150-200	
Vaginal	C. (μg/NmL)	10-15	20-25	30-35	Dynamic flow, flushing flow 0.1-0.2 L/min for 10 min.[46]
	V. (L)	1-2			
	Dose (μg)	10-15 20-30	20-25 40-50	30-35 60-70	
Rectal Insufflation (RIO_3)	C. (μg/NmL)	10-15	20-25	25-30	Major concentrations of 40 μg/mL can hurt the enterocyte. The only exception is ulcerative hemorrhagic colitis, beginning with a high concentration of 60-70 μg/mL / and 50 mL Vol. Once the bleeding diminishes, reduce concentration.[47]
	V. (mL)	100	150	200	
	Dose (mg)	1.0-1.5	3.0-3.75	5.0-6.0	
Extracorporeal blood oxygenation-ozonation (EBBO)	C. (μg/NmL)	0.1	0.25	0.4	Optimum flow 30-35 mL / min.[48]
	V. (L)	18			
	Dose (mg)	1.8	4.5	7.2	
Sauna*	C. (μg/NmL)	5	8	10	20-30 min, 10-15 with ozone, followed by 10-15 min with water vapor / Temp. 40-45°C.[49]
	V. (mL)	Depending on the design and type of sauna			
Acupuncture/ reflexology	C. (μg/NmL)	6	7	9	Acupuncture points: O_3/O_2 is injected intradermally or subcutaneously into each acupuncture point. Trigger points: O_3/O_2 is injected intramuscularly into each trigger point.[50]
	V. (mL)	0.1 - 0.3			
	Dose (μg)	0.6-1.8	0.7-2.1	0.9-2.7	
Ozonized Saline Solution (O_3SS)	C. (μg/NmL)	0.4	0.8	2	Requires saturation of the solution with constant bubbling for 10 min before the i.v. administration. The I.V. infusion has to be done under constant bubbling of ozone to assure the concentration is stable.
	V. (mL)	200 mL			
	Dose (μg/kg) b.w.	1	2	5	

Note. * Local way or it can be also used for systemic effect.[49]
Not recommended routes: direct intra-venous, hyperbaric ozone application, intraperitoneal ozone, high dose ozone therapy, multi- pass methods.[43]
Legend. C: concentration; V: volume; b.w.: body weight; 1 mg = 1000 μg.

2. OZONE THERAPY (O_{3x}) BASIC PRINCIPLES

The two basic principles that must be taken into account before any ozone treatment process is implemented are the following:

a) Primum non nocere: Before anything else, do no harm.

b) Stagger the dose: Always start with low doses, and increase them gradually.[25,51]

The exception will be in infected ulcers or injuries, where the reverse will be applied. In this case, start with a high concentration, and diminish it according to the improvement in the patient's condition. See table 1 for details. Higher ozone concentrations are not necessarily better, in the same way that it occurs with all the medical drugs.

2.1 Contraindications

Administration of ozone is contraindicated when it is administered systemically because of:

1. Glucose-6-phosphate. dehydrogenase deficiency (favism, acute hemolytic anemia).*
2. Toxic Hyperthyroidism - Basedow Graves status.
3. Thrombocytopenia less than 50.000 and serious coagulation disorders.
4. Severe cardiovascular instability.
5. Acute alcohol intoxication.
6. Acute infarct of myocardium.
7. Massive and acute hemorrhage.
8. During convulsive states.
9. Hemochromatosis.
10. Patients receiving treatment with copper or iron by i.v. administration.

* The prevalence of Glucose 6 phosphate dehydrogenase (G6PD) deficiency varies among ethnic groups with overall lower frequency in the Americas (3.4%), Europe (3.9%), and the Pacific (2.9%) as compared to sub-Saharan Africa (7.5%), the Middle East (6.0%), and Asia (4.7%).[52] Test of G6PD is recommended prior to O_3 therapy to avoid complications.

2.2 Warning

Pregnancy. Ozone therapy has been used with good results in the treatment of different diseases associated with pregnancy such as: gestosis,[53-55] placental insufficiency,[56] fetal growth retardation,[57] cervical ectopy,[58] preeclampsia.[55,59] However, it should be avoided during the first trimester of pregnancy (0-13 weeks), a critical time for development of the embryo and fetus.

Competitive sports. Systemic methods of application of ozone therapy may impact the muscle oxygenation,[60] by inducing an augmentation in increased 2,3 diphosphoglycerate.[39] The use of systemic ozone therapy in competitive sport must be authorized by the physician in charge and justified from the medical point of view in order to avoid a legal conflict.

"The Hematology Module, introduced in 2009, aims to identify the improvement in oxygen transport, including the use of ESA [erythropoiesis stimulating agents] and any form of blood transfusion or manipulation. The Hematology Module analyzes a panel of blood doping biomarkers that are measured in the blood sample of an athlete."[61]

Patient under treatment with anticoagulants. Concomitant systemic administration of ozone and anticoagulant may modify the coagulation pathway,[62] this implies the need for frequent monitoring of the International Normalized Ratio (INR). If the new direct-acting oral anticoagulants (ACOD) or new oral anticoagulants (NACO) indicated for the treatment of venous thromboembolic disease (VTD) are used, they are fully compatible with ozone and do not require INR controls (Rivaroxaban (commercial name: Xarelto®), Dabigatran (Pradaxa®), Apixaban (Eliquis®), Edoxaban (Lixiana®).[63]

2.3 Interactions with Ozone

During the treatment with ozone, antioxidant supplements may be used (e.g. vitamin C and vitamin E). However, the presence of these compounds in high concentrations in the blood interferes with the ozone's action as an oxidating agent and in the good course of the therapy. Consequently, oral vitamins or antioxidants, should never be given during treatment. They should be given before or after the ozone therapy only. The time of suppression depends on the bioavailability of each specific antioxidant. It is recommended that intravenous antioxidant therapy, such as vitamin C or glutathione, neither be administered, before nor during, but only after ozone therapy.

Avoid mixing medications such as homeopathy products, procaine, magnesium sulfate, glutathione, vitamin C, etc., with ozone; or simultaneous administration with systemic administration of ozone, for example mixing them with ozonized saline solution (O_3SS), or in the bottle of ozonized blood during the autohemotherapy, or mixing them in the same syringe. Ozone can oxidize and inactivate them or generate toxic compounds.

Ozone increases the effects of ACE Inhibitors. Treatment with ozone in patients under anticoagulation therapy such as Coumadin/heparin must be done under control of INR. Patients receiving treatment with copper or iron cannot receive ozone treatment.

Synergetic effect with other oxidative therapies (U.V., H_2O_2 etc.). We suggest that two or more oxidative therapies should not be perform in the same therapeutic act. This would increase the patient's oxidative stress. Complementary effects can be expected in association with laser therapy, magnetic therapy, acupuncture, diathermia and physiotherapy.

2.4 Adverse Effects

Most of the side effects reported could be related to *mala praxis*: administration technique, administration route, concentration of ozone administered, etc.

Grade of reported adverse effects (AE) according to NIH (2010) criterion.[64]

2.4.1 Grade 1 Mild

Asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.

- Some patients reported a brief and transient feeling of local heat and slight pain during the ozone injection.[65]
- Hematoma at the ozone infiltration site in one patient.[66]
- Four patients reported the sensation of itching on lips and tongue at the end of the session, three patients described nausea and a bad taste in the mouth during re-infusion of ozonized blood, and one patient suffered dyspnea during the administration of the therapy.[67,68]
- Onset of euphoria after the application of ozone using the oxygenation and extracorporeal ozonation of blood (EBOO) in 15 patients treated for skin lesions secondary to arterial ischaemia.[69]

2.4.2 Grade 2 Moderate

Minimal, local or non-invasive intervention indicated; limiting age-appropriate instrumental Activities of Daily Living (ADL).

- Onset of reduction of sensitivity in the legs of two patients in the group treated with ozone and corticoids that remitted in two hours.[70]
- Five patients reported lumbar and leg pain after the ozone injection that resolved spontaneously; and eight patients showed mild corneal irritation and reversible dyspnea after the administration of ozone.[71]
- When ozone was administered by rectal insufflation, cases of bloating and constipation were reported.[69]

2.4.3 Grade 3 Severe

Medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care.

- Vertebrobasilar stroke.[72]
- One acute bilateral vitreoretinal haemorrhage.[73]
- One case of meningeal irritation.[74]
- Three cases of viral hepatitis.[75]
- Series of cases of serious infectious complications during spinal degenerative pathologies treatment.[76]
- Acute prevertebral abscess secondary to intradiscal O_3/O_2 chemonucleolysis.[77]
- Vasoconstriction during fast reinfusion using major autohemotherapy.[78]
- Purulent complication secondary to O_3/O_2 therapy for the treatment of lower back pain.[79]
- A case report of spondylodiscitis after O_3/O_2 therapy for treatment of a cervical disc.[80]

- A case report of severe headache following O_3/O_2: Pneumocephalus after epidural approach for the treatment of lumbar hernia.[81]
- A case report of thunderclap headache caused by an inadvertent epidural puncture during O_3/O_2 therapy for patient with cervical disc herniation.[82]
- Hard adhesions between the soft tissues and bony structures in patients who received O_3/O_2 injections by the intraforaminal approach.[83]

2.4.4 Grade 4

Life-threatening consequences; urgent intervention indicated.

- One case of gas embolism was reported in the peri-ganglionic venous plexus, involving the vertebrobasilar artery that manifested clinically as local pain for several minutes, and which cleared in a few days.[84]
- One case of embolism in spinal cord infarction and myocardial infarction following the intradiscal O_3/O_2 therapy.[85]
- One case of a syncopal episode. Upon awakening, was found to have ataxia, aphasia, hemiparesis, and left sixth nerve palsy after a paravertebral ozone injection.[86]
- On case of cardiopulmonary arrest and pneumoencephaly developing after epidural O_3/O_2 therapy.[87]
- Myocardial infarction after ozone therapy (major autohemotherapy).[88]
- Ischemic stroke after an O_3/O_2 therapy (Anton syndrome).[89]
- A case report of O_3/O_2 therapy induced sinus arrest in a hypertensive patient with chronic kidney disease (treated with major autohemotherapy).[90]
- A case report of transient cortical blindness after intradiscal O_3/O_2 therapy.[89]

2.4.5 Grade 5

Death related to adverse effects.

- Four cases of death by gas embolism after the administration of ozone by direct intravenous injection.[91-93]
- One case of death following ozone application by autohemotherapy to treat psoriasis.[94]
- One case of death by fulminating septicemia following ozone therapy for lumbar disc herniation.[95]

2.5 Toxicity

Ozone should never be inhaled. This route is forbidden. Ozone is not toxic when used in the adequate range dose (Table 1), and by trained professionals with the right clinical protocols. Fatal cases are the result of *mala praxis*.

2.6 Pediatrics Dosages through Rectal Insufflation

Systemic application in children only by rectal route.

- The concentrations to be used depend on the grade of the oxidative stress of the patient and the pathology to be treated (Table 2A).
- The volume to be administered depends on the age of the patient (Table 2B).
- To perform the rectal insufflation a catheter is introduced (1-2) cm inside the anal sphincter.[47]

Table 2. Pediatric dosages by rectal insufflations

Tab. 2 A. According to the oxidative stress

Weeks of treatment	Concentration O_3 (μg/NmL)		
	Oxidative stress		
	Low	Moderate	Severe
First	20	15	10
Second	25	20	15
Third	30	25	20
Fourth	35	30	25

Tab. 2 B. Volumes to be administered according to patient's age

Age of the patient	Volumes to be administered (mL)
28 days-11 months	15-20
1-3 years	20-35
3-10 years	40-75
11-15 years	75-120

The dosage changes every five sessions. Cycles of 15-20 sessions are indicated every four-five months during the first year. Later the patient will be evaluated to determine the frequency of the cycles for the second year.

3. MAIN ROUTES OF APPLICATION

Medical ozone can be applied locally or parenterally. In order to attain a synergistic effect, the various routes of application of ozone can be used combined or alone.

3.1 Recommended Systemic Routes of Application

The routes of application described below have been proven to be effective and safe. They are the result of many years of research and clinical experience, with more than 3,000 documented publications. Consult the free access online ISCO3 *Ozone Therapy International Library*.[96]

We welcome the therapeutic range indicated by the guidelines of the Russian Ozone Therapy Association, published in its *Handbook of Ozone Therapy* (2008);[97] the guidelines published by

the Ozone Research Centre, scientific unit of the Cuban National Centre for Scientific Research, in its book *Ozone Basics Aspects and Clinical Applications* (2008);[22] the *Guidelines for the Use of Medical Ozone* published by the German Medical Society for the Use of Ozone in Prevention (2009);[98] the significant contribution from Dr. Velio Bocci in the book *Ozone: A New Medical Drug* (2010),[51] the most recent publications: The book *Clinical Ozone Therapy Manual* of Adriana Schwartz (2017),[24] and the G*uidelines and Good Practices in Ozone Oxygen Therapy*. Consensus Conference (FIO, 2018).[99]

3.1.1 Major Autohemotherapy (MAH)

Major Autohemotherapy (MAH) is a treatment that involves mixing the blood of the patient with medical grade ozone (O_2-O_3) and its immediate reinfusion by intravenous infusion.[44] The volume of blood to use varies between 50 mL and 100 mL. However, blood volumes greater than 200 mL must be avoided to prevent any risk of hemodynamic disturbances, especially in elderly or unbalanced patients.

It is necessary to define the volume of blood to be extracted. This is done based on the weight of the patient being treated. Hemodynamic/hypovolemia disorders with a loss of 15% of total circulating blood volume (CBV) are not considered. In the case of MAH, a withdrawal of 1.5%-2% of total circulating blood seems to be conservative. A person of 85 kg has CBV of 65 mL/kg x 85 kg = 5 525 mL of blood. The 2% corresponds to 104.5 mL blood withdrawal.

Ranges of a safe blood collection are: 1.2 mL/kg to 1.3 mL/kg, with the limit of 150 mL in individuals of 150 kg.

For example: a person of 85 kg; 1.2 x 85 = 102 mL blood to be extracted. These dosages have been shown to be safe and effective.[100] They activate cellular metabolism and have immunomodulatory and anti-oxidant effects. It should be emphasized that each route of application has a minimum and a maximum dosage as well as concentration and volume to manage.[44]

Perfusion set. Within the European Union (EU) plastic-based devices, intended to contain blood, must meet the UNI EN ISO 15747:2005. All containers and devices used in O_{3X} must be ozone-resistant and must not release phthalates because these substances are toxic to the organism. For that reason, it is preferable to use glass for MAH. The plastic bags for MAH must be ozone-resistant and certified for blood collection by the EU or FDA (US Food and Drug Administration). No other modification to perform ozonized blood transfusion is allowed.

Ozone concentrations for systemic uses range from 10 *µg*/NmL to 40 *µg*/NmL; concentrations of 70 *µg*/NmL-80 *µg*/NmL and above should be avoided because of the increased risk of hemolysis, reduction of 2, 3 DPG and anti-oxidant and a consequent inability in activating immune-competent cells.

Anticoagulant. It is most advisable to use ACD-A Anticoagulant Citrate Dextrose Solution A, USP (2.13% free citrate ion), or citrate sodium 3.8% in a proportion of 10 mL per 100 mL of blood to be ozonized. Heparin is not advisable because it can induce thrombocytopenia[101]

and platelet aggregation.[102] The safe and effective proportion of ACD-A ranges from 7 mL -10 mL per 100 mL of blood.

Frequency of treatment. The number of treatment sessions and the ozone dosage administered will depend on the general condition of the patient, age and main disease. As a general rule, every five sessions the dose of ozone is increased and it is given in cycles that vary between 15 and 20 sessions. From the clinical point of view, an improvement of the patient occurs between the fifth and tenth session; and it is considered that after the twelfth session the antioxidant defense mechanism has already been activated. In the case of rectal insufflation the treatment is given daily, from Monday to Friday or three times a week. In the case of MAH or O_3SS, it can be administered two to three times a week. Cycles can be repeated every 5-6 months.

3.1.2 Minor Autohemotherapy (MiAH)

Minor Autohemotherapy (MiAH) is a treatment that involves mixing the blood of the patient removed intravenously (5 mL-10 mL) without anticoagulant, drawn into a sterile, pyrogen-free disposable syringe (already containing the ozone-oxygen mixture, 10 *μg*/NmL to 40 *μg*/NmL). The blood and ozone mixture is intensively shaken and slowly reinjected intramuscularly in the ventrogluteal region along with the gas.[45] MiAH is an immune stimulant therapy, comparable to "auto-vaccination".

Indications. Its main indication is in all dermatological diseases. As an auto vaccine in psoriasis, dermatitis, eczema, acne vulgaris, allergies and furunculosis. As an adjuvant in cancer or in chronic debilitating pathologies.

Method. 5 mL of blood is removed intravenously and collected into a 20 mL disposable syringe prefilled with the same amount of ozone-oxygen mixture (5 mL) without anticoagulant. Intensively shake for 30 s and slowly inject intramuscularly along with the gas.

Cycles: of 5-10 treatments once a week.

3.1.3 Ozonized Saline Solution (O_3SS)

Ozonized Saline Solution (O_3SS) is a widespread practice in Russia and developed by the Russian school of ozone therapy in the city of Nizhny Novgorod (Volga Federal District).[103] O_3SS is supported by pre-clinical studies,[104-106] more than 92 clinical trials[107,108] (mainly published in Russian language) and an estimated number of 500 university theses. It is probably the most extensively and scientifically supported application method of ozone therapy, but its practice is mainly confined to Russia,[97,109] although its application has boomed also in Latin America, United States and Spain. Its efficiency is testified by the results of a high number of scientific research studies submitted at the eighth Practical Scientific Conferences which took place in Russia from 1992 to 2014.[110] Some recent studies appearing in the international data base demonstrated the applicability of this method in clinic e.g. in Acute appendicitis,[111] Brain trauma,[112] Diabetic foot,[113] Obstructive jaundice,[114] Vulvar leukopathy,[115] Delayed fetal growth[57] and Lymphovenous failure of lower limbs.[116]

A team of researchers led by Prof. S. Razumovsky, a major world expert in the chemistry of ozone, found out, through an investigation of the processes of the decomposition of ozone in aqueous media, that the decomposition of ozone in the aqueous solution of NaCl is not accompanied by the formation of products different from the oxygen, and no noticeable amounts of hypochlorites and chlorates were observed in particular. This is significant for the medical applications of ozonized isotonic solution.[117]

At the Scientific Research Center of the Nizhny Novgorod Medicine Academy, Russian scientists, under the leadership of the academician A. Korolev, successfully developed the method of ozonized saline solution in October 1977. In April 1979, for the first time in the world, a cardioplegic ozonized solution in the coronary system of a patient with congenital cardiac injury was administered. In November 1986, the first extracorporeal ozonized blood during placement of a prosthetic mitral valve was conducted.

Ozonized saline solution may be prepared by four methods

- **First method**. The three needles: Requires constant bubbling of ozone to ensure the solution is constantly saturated with ozone gas.
- **Second method**. The two needles: The solution is saturated for 10 min and requires rapid transfusion due to the decrease of the concentration over time.
- **Third method.** Is a combination of methods using two and three needles. In this case, the ozonized saline method takes two needles and intravenous infusion followed by periodic bubbling ozone from a special tank. The ozone concentration in saline solution is stable. This requires special equipment.
- **Fourth method.** Spain has designed the Dual Kit, a closed system device, classified as a medical device, with a seal of the European Union (CE), free of phthalates and used both for ozonized blood and for saline solution.

Recommended dose of ozone. The ozonized saline solution (O_3SS) is carried out with very low ozone concentrations which are calculated according to the weight of the patient.

Low ozone dose: 1 *μg*/kg.
Medium ozone dose: 2 *μg*/kg.
High ozone dose: 5 *μg*/kg.

Calculation of the ozone gas concentration to prepare O_3SS

Please note that the dissolved ozone concentration is 25% of the ozone gas concentration in the saline solution.[118] This has to be taken into consideration when calculating the concentration set at the ozone generator: it has to be multiplied by 4 in order to get 100% of the dose needed.

Dose Formula.
Dose (*μg*) = dissolved ozone concentration (*μg*/mL) · Volume (mL) saline solution.
Example: Patient's weight = 80 kg; Saline solution volume = 200 mL.

Low ozone dose.
Total Dose per session= 1 *μg*/kg · 80 kg= 80 *μg*.

Dissolved ozone concentration in saline solution=80 *μg*/200 ml = 0.4 *μg*/NmL.
Ozone concentration to mark from the generator = Dissolved ozone concentration in Saline Solution · 4 = 1.6 *μg*/NmL.

Medium ozone dose. 2 *μg*/kg.
Total Dose per session= 2 *μg*/kg · 80 kg= 160 *μg*.
Dissolved ozone concentration in saline solution= 160 *μg*/200 mL= 0.8 *μg*/NmL.
Ozone concentration to mark from the generator = Dissolved ozone concentration in saline solution · 4 = 3.2 *μg*/NmL.

High ozone dose. 5 *μg*/kg.
Total Dose per session= 5 *μg*/kg · 80 kg= 400 *μg*.
Dissolved ozone concentration in saline solution = 400 *μg* · 200 ml =2 *μg*/NmL.
Ozone concentration to mark from the generator = Dissolved ozone concentration in saline solution · 4 = 8 *μg*/NmL.

The upper limit of the concentration of ozone in the ozonized saline solution is 2 *μg*/NmL; exceeding this limit is dangerous and can cause phlebitis. The exceptional cases are severe sepsis and severe viral infections. In such cases, the concentrations may be increased up to 5 *μg*/NmL to 8 *μg*/NmL.

Note. The volume of saline solution used for one procedure is (200-400) mL. The number of procedures for one cycle of treatment is 6 to 10. Procedures are conducted daily or every other day.

Low doses (0.4 *μg*/NmL) are used to stimulate the immune system and for diseases of the cardiovascular diseases. Also, for obstetrics, to prevent toxicity in the first trimester of pregnancy and fetal hypoxia in the third trimester. Widely used in prevention and adjuvant treatment for cancer.

Medium doses (0.8 *μg*/NmL) are used for detoxification in endo-toxemia and chronic inflammatory diseases of different etiologies.

High doses (2 *μg*/NmL) are used in the treatment of infectious (bacterial and virus) diseases, as well as in skin and burn diseases. Also, in autoimmune diseases where it is needed in immunosuppressive doses.

3.1.4 Extracorporeal Blood Oxygenation-Ozonation (EBOO)

This method is used in Italy, Russia, Ukraine, Malaysia, and rarely in some Latin American countries, mainly to treat severe peripheral arterial disease, coronary disease, severe dyslipidemia, Madelung disease, deafness of vascular origin, necrotizing fasciitis, septicemia infection resistant to antibiotics, ischemic stroke, chronic heart failure and viral hepatitis C. The method EBOO is an advanced variant of the MAH. The EBOO is similar to a hemodialysis, amplifies the therapeutic benefits reported of MAH by treating a greater volume of blood (4 L/h) at a lower ozone concentration (<1 *μg*/mL). The procedure EBOO represents a simultaneous oxygenation and ozonation of blood which is transferred from one vein system of the patient

to a gas exchange device (GED), and then from GED into another venous system. Upper and lower veins can be used for this procedure.

There are two basic procedures of the EBOO.[48]

The first method is based on GED of microporous, ozone-resistant, polypropylene hollow fibers with an external diameter of 200 μm, a thickness of 50 μm, and a membrane surface area of 0.22 m. Concentration of the oxygen-ozone mixture is around 99% and 1%, respectively. During this procedure the blood of the patient is transferred inside the hollow channels, and the ozone-oxygen mixture surrounds the channels from the outside.

The second method is based on the use of rotor and film GED (consisting of a glass bottle revolving horizontally and an immovable cork where are three nipples made of ozone-resistant polypropylene). If the procedures last more than one hour, it is necessary to introduce to the patient an extra dose of heparin (1 mL, 5 000 IU) in one hour. The procedure is completed by blood displacement from the lines and GED, using saline solution and removal of intravenous cannulas.

Note. Modern dialyzers used for hemodialysis are made of polysulfone, cuprophan and other non-ozone-resistant materials. The use of such devices for EBOO is provoking a risk of undesirable products of ozone-dialysis in the blood.[93,119-121]

3.1.5 Rectal Insufflation

The Rectal insufflation of ozone is a systemic route.[47] The gas is quickly dissolved in the luminal contents of the bowel, where mucoproteins and other secretory products with antioxidant activity readily react with ozone to produce reactive oxygen species (ROS) and lipid peroxidation products. These compounds penetrate the muscular mucosa and enter the circulation of venous and lymphatic capillaries.[122] This non-invasive technique can be used without risk in pediatric and elderly patients, and on patients with difficult access of veins for MAH. Generally, this is well tolerated and allows scaling doses similar to those used by MAH.

In chronic illnesses, the proper dosage of medical ozone produces temporary oxidative stress tolerance, so patients require repeated cycles of ozone therapy (20 sessions one/daily, constituting one cycle). It is recommended to increase the dose in each consecutive cycle, repeated at a 3 to 4-month interval in the first year. If there is more than six months between each cycle, doses must be the same as in the first cycle. Beneficial results are reported following rectal dosing (low, middle and upper middle doses). High doses will only be used after two cycles of ozone therapy with an interval of three months each.

The range of concentration is (10–5) μg/NmL.
The range of volume is (100–200) mL.
Concentrations higher than 40 μg/NmL can hurt the enterocyte.

3.1.6 Vaginal Insufflation

Considering the speed of capillary flow, as well as the fact vagina is a wide, clean, moist and well-vascularized receptacle, vaginal insufflation is a systemic route and is even a more effective route than MAH and RIO_3.[123]

Ozone concentrations of (10-30) *µg*/NmL and a volume between (1-2) L at a continuous flow rate of 0.1 L/min to 0.2 L/min for 10 min are used. A vaginal wash with ozonized water must be carried out previously. For this application an ozone destructor device and a special vaginal device are needed to acquire the equal, proper and safe distribution of the gas to the folds of the vaginal mucosa.[46,124] It is advised the use of a lubricant gel after the ozonization due to dryness effect of the ozone in the mucosa.

3.2 Recommended Routes of Application with Local Effect

3.2.1 Intramuscular, Paravertebral and Intra-Articular Injection

For details see: ISCO3 (2014). *Ozone in Non-Rheumatic Locomotor System Pathologies.*[40]

3.2.2 Paravertebral Intramuscular Injection

The **classical paravertebral infiltration** is performed by locating the upper part of the spinous process and injecting the cervical and dorsal column with 5 mL of ozone at (10-20) *µg*/NmL, 1.5 cm laterally from the spine/column, with a (0.8 x 40) mm needle.[41,125-127]

The infiltration for lumbar spine is made 2.0 cm from the spinous process, and 10 mL at the same concentration. The distribution of the needle is always bilateral, lateral or 2 cm above and 2 cm below the hernia. A depth from 2 to 4 cm should be considered when taking into account the constitution of the patient and/or the area to be treated (smaller in thin patients and in the dorsal region; and greater in obese patients and in the lumbar region).

Local anesthesia (1 mL procaine or 1 mL lidocaine) in the muscle is optional. This may reduce the pain caused by ozone. When an anesthetic is used, it is placed in a space higher than the infiltration of ozone to prevent its oxidation. The patient receives it with a strong burning sensation.

A practical approach for the treatment is done twice a week for the first two weeks. Once clinical improvement is achieved, the treatments should be spaced to once a week, for four to six weeks. And then, one session every 15 days until one cycle of 20 sessions is completed; these can be shortened once the symptoms have disappeared. Different frequency of administration has also been used, and a randomized controlled trial showed beneficial effect after 5 sessions a week for three weeks.

The recommended needle sizes for this procedure are (0.4 x 40) mm to 30G (0.3 mm) x 1½ (40 mm). In some cases, and with expert hands, longer needles may be used.

It is important that the physician adequately examines the muscles within the lumbar-sacral region and the sacroiliac articulations, to detect inflammation at this level or *trigger points*

in that zone, above all in patients with disc arthrosis that do not respond adequately to the paravertebral infiltrations. If these points are detected they must be infiltrated. Concentration: (10-20) *µg*/NmL. Volume: (5-20) mL. Dose: (50-400) mg.[128]

3.2.3 Hernias

Paravertebral Deep Injection

For this injection it is necessary to use a longer needle, 0.4 mm or 0.5 mm x 90 mm spinal needle to inject over the *laminae*, close to the foramen, or around the *facet* joint. Cervical/dorsal hernias: concentration (10-20) *µg*/NmL, volume (3-5) mL and for Lumbar hernias: the concentration is (10-20) *µg*/NmL, and a volume of (7-10) mL.[129]

3.2.4 Intradiscal Treatment

In general, only one intradiscal infiltration should be performed under mobile radiologic arch or fluoroscopic control or CT.[130] The patient has to be under sedation (not general anesthesia) and with an antibiotic prophylactic therapy on the same day of the procedure. In some cases, the intradiscal infiltration can be repeated within (2-4) weeks.

For **lumbar discolysis**, a (5-10) mL mixture of oxygen - ozone at a concentration of (25-35) *µg*/NmL is used.[131,132] All animal models have shown annulus disruption secondary to concentrations of 50 *µg*/NmL or greater, so it is advisable not to use concentrations over 40 *µg*/NmL.[133] The needle used is Chiba 25G x 3 1/2 (0.5 x 90) (regular) or 22G (0.7 x 203) mm (over weight patients).

For **cervical discolysis**, (2-3) mL with ozone at a concentration of (25-35) *µg*/NmL is used.[131,132] The needle used is Chiba 25G X 11/2" (0.5 X 40 mm).

The discolysis with ozone, although effective after only one treatment, requires specific infrastructure (for radiological control), and anesthetist and experienced personnel in the execution of the technique. Despite the fact that the paravertebral technique requires more sessions, it is equally effective and has a minimum level of risk.

3.2.5 Sacral Hiatus/Transluminal Peridural Infiltration

An infiltration is performed in the peridural space twice weekly, with previous identification of the peridural space by echography guide. A mixture of oxygen-ozone in a volume of (10-20) mL at a concentration of (10-20) *µg*/NmL is used.

The transluminal peridural method or through the sacral hiatus route is an alternative to consider in the treatment of herniated discs with ozone therapy, despite being an indirect method compared to the intradiscal. The reasons are:

- With this method, neither the patient nor the operator is exposed to the risk of radiation.
- Ozone acts over both the disk and the damaged root upon deposit of the gas in the peridural space at the level of the conflict zone disco-radicular.
- It requires few material resources and equipment, making it a less expensive but still effective method.

- Compared to the paravertebral, this indirect method requires fewer sessions and is very useful in the presence of multiple disc hernias.
- The success rate frequency is above 70%.
- It requires minimum time to recover.
- It can be performed on patients with major associated diseases.

In all cases, the three commented techniques require strict asepsis, sterility measures and an informed written consent.

3.2.6 Intraforaminal infiltration

Concentration range: (10-20) *µg*/NmL.
Intraforaminal approach for cervical injection under Radiological control: requires 5 mL volume and a 25G X 11/2" (0.5 X 40 mm) cervical needle can be used.[134,135]

Intraforaminal for lumbar injection: (7-10) mL and a Chiba 25G x 3 1/2 (0.5 x 90) (regular) or 22G (0.7 x 203) mm (over weight patients) lumbar needle can be used.[135-138]

3.2.7 Intra-Articular Treatment

Concentration: (2-10-20) *µg*/NmL

The Volume used depends on the articulation size: Fingers: (1-2) mL, others: (5-20) mL.[139-141]

3.2.8 The Gloves Technique (Emphysema subcutaneous technique)

Subcutaneous Infiltration of hands: (10-40) mL of oxygen-ozone mixture at (5-20) *µg*/NmL of concentration, with a 30G (0.3 mm) needle. This infiltration is efficient in the treatment of neuropathic pain and osteoarthritis.[42]

3.2.9 Gasification in Plastic Bag

Ozone bagging or *gasification in plastic bag i*s a local way of application of ozone. It consists of filling an ozone-resistant plastic bag with the O_3/O_2 mixture, creating a micro environment around the wound, allowing the body tissues to keep in contact with the gas mixture.

Concentrations range of (80, 70, 60, 40, 30, 20) *µg*/NmL are used for periods of (20, 10, 5) min, depending on the stage and evolution of the wound. A (60-80) *µg*/NmL is used only in purulent septic infections and for a very short time and for no more than 5 min. Once the infection is controlled and the healthy granulation tissue appears, the frequency and the concentration of the procedure has to be reduced to accelerate and induce the healing process.[142-144]

Note. It is necessary to moisten the area and to remove all the air from the bag by vacuum before insufflating the gas into the bag. At the end of the procedure, the remaining ozone gas must be suctioned before removing the bag.

3.2.10 Subcutaneous (Mesotherapy) Application

This application is used for cosmetic purposes in acne[145] and cellulitis. In cellulitis, never use a volume larger than 200 mL per session, one injection every (5-10) cm in skin fold and in a

volume of (2-3) mL per point. Concentration of 15 *μg*/NmL to 20 *μg*/NmL with a 27G (0.3 mm) needle. Cycles of 15-20 sessions, twice a week.[145,146]

These results are better if they are associated with ozone rectal insufflation, MAH[24] or O_3SS[145] applied twice a week.

3.2.11 Ozone Suction Cup

Using concentrations ranging from 15 *μg*/NmL to 60 *μg*/NmL, with a variation in the duration of the treatment between 5 to 20 min. Using suction cup, vacuuming is necessary to remove air and ozone from the bell. Vacuum increases the blood flow and ozone can react better.

3.2.12 Insufflation in Fistulas

The practitioner must always be sure, first that no communication exists with the respiratory tract. It is important to keep in mind the possible gas build-up in a closed cavity, blocked or cystic, to avoid dangerous or painful increases in pressure, for example, in cutaneous, perianal and surgical fistulas. A fistula wash with ozonized water must be carried out previously to insufflate the gas. Within the duration of 5 min to 20 min, the concentration of the oxygen-ozone mixture used is (10-80) *μg*/NmL.[147]

3.2.13 Ophthalmologic

In ophthalmological cases (keratitis, corneal ulcers, conjunctivitis and ocular burns),[148] a special glass attachment adapted to the contour of the eye is used. Due to the burning sensation of the topical application of ozone in gas or in form of ozonized oil, it is recommended to use anesthetic eye drops before the application of the ozone. The concentration of ozone is between (20–30) *μg*/NmL, application time 5 min, two to three sessions per week. Sub-conjunctival injection, previous placement of an anesthetic eye drops, with a volume between 1-2 mL per eye, in the bottom of the sac, at a concentration of 10 to 35 *μg*/NmL *μg*/mL with a volume of (1-2) mL.

Ozonized oil at (200-400) IP: Ozonized oil due its bactericidal and virucidal properties is advisable to apply in the form of eye drops four or five times a day after topical anesthesia for the ocular burning that occurs as when the ozone gas is applied.[24,149] The application of ozonized solution (10 *μg*/NmL) is useful as antiseptic of the ocular surface prior to ophthalmic surgery.[150]

3.2.14 Insufflation Vesico-Urethral

For vesico-urethral it is advisable not to use gas directly, but to irrigate with ozonized bi-distilled water. The vesical mucose membrane is too sensitive to the oxidative properties of ozone, especially in interstitial cystitis.

Ozonize 500 mL of bi-distilled water at 20 *μg*/NmL concentration, during 10 min at continuous flow of 200 mL/min[151-155] Proceed to irrigate leaving 50 mL of the ozonized water inside the bladder at the end of the procedure.

3.2.15 Intra Prostatic

In acute or chronic bacterial prostatitis, the treatment consists of 5 mL of O_3/O_2 at 20 *µg*/NmL in the peripheral area of each lobule, needle 27G x 2. One session every week per 10 weeks.[24] In benign prostatic hyperplasia 40 mL of O_3/O_2 at 30 *µg*/NmL is injected into the prostate (20 mL in each lateral lobe).[155,156]

3.2.16 Otic Route

Check that the eardrum is intact. Due to the drying properties of ozone, it is recommended to moisten the ear canal and the eardrum membrane before applying the ozone.

For insufflation, a syringe or a special headset with an ozone destructor device can be used, or performing otic insufflation with a modified stethoscope with silicone tubes, connected between them with a "Y" and female Luer lock connector of Kynar, to assemble the syringe filled with ozone at the concentrations described. It has to be manually and slowly administered, so that the ozone can be absorbed in the ear canal and on the tympanic membrane. If there is minimal leakage of ozone, the administration should be done much more slowly. It will not be necessary to connect this device directly to the ozone machine.

Concentration: (10-25) *µg*/NmL; Application time: 5 min.[157-159]

Indications: Otitis, dermatitis of the ear canal, sinusitis and circulatory problems of head and neck.

3.2.17 Intratonsillar Infiltration Route

Infiltrate at the anterior and rear pillar of both tonsils; two to three points with 2 mL to 3 mL of ozone at concentrations of (10-20) *µg*/NmL are used. Four to five sessions are required. In case of nasal polyps, infiltrate directly into the polyp tissue a volume of 2.0 mL at a concentration of 50 *µg*/NmL.[160] Inhalation[161] is performed only in a state of hydrosol of ozonized oil using an ultrasonic generator of hydrosol of ozonides, forming particles of about 5 µm (volatile organic compounds). Never inhale ozone.

3.2.18 Ozone Micro Doses in Trigger and Acupuncture Points

As a general rule the trigger points are located in the muscles and are often deep, so the application has to be intramuscular and the volume can be between (3-5) mL depending on the anatomical place, and the concentration is between (6-9) *µg*/NmL.[50]

For acupuncture points or reflexology areas, the application is intradermal and fluctuates between (0.1 to 0.3) mL and up to 1 mL (maximum) of the gas mixture of O_3/O_2 with concentrations below (6-9) *µg*/NmL.[50,162]

3.2.19 Topical Application of Water, Oil and Ozonized Creams

Ozone in water and ozonized oil are applied on ulcers, dirty traumatic lesions, chronic torpid ulcers, bed sores, burns, herpetic lesions, psoriatic lesions, fungal infections, insect stings, in dental infections, as a surgical cavity cleaner and in several infected lesions at different

concentrations: high, medium, and low, depending on what it is intended to achieve (to disinfect, to regenerate) and on the type of tissue (Table 3).[163-166]

The preparation of ozone in water is carried out by using a glass cylinder, filled about ¾ with bi-distilled water through which the gas mixture has to be bubbled continuously for at least (5–10) min to achieve saturation. The unused ozone flows out via silicone tubing into a destructor and is converted to oxygen (Table 3A).

The study of the physicochemical properties of ozonized vegetable oils has great importance for their characterization and identification. To determine the quality of ozonized products, analytical methods of peroxide,[167] acidity[167] and iodine values,[168] relative density and viscosity are usually carried out. The peroxide value represents the quantity of peroxide expressed in mL equivalents of active oxygen contained in a 1,000 g sample ($mEqO_2$/kg). This index will be used for dosages criterion (Table 3 B).[169]

Since oil steam diffusion in the high-voltage pipes is unavoidable, the ozonization of oils must never be executed with a medical generator. Otherwise, the result would be the production of several toxic substances and the danger of explosion. The recommended method to assay Peroxide values is as it is described in the European Pharmacopoeia,[11] modified by Zanardi *et al*. (2008)[170] and standardized by ISCO3.[169] In addition, other quality control assay as the acid values[167] or the iodine values[168] are currently standardized by ISCO3.

3.2.20 Sauna Cabin or Quasi-Total-Body Exposure to Ozone

Ozone saunas combine (hyperthermia) steam vapor (40-42)°C with O_2/O_3 at lower concentrations (5 *μg*/NmL). The time of exposure in saunas is 10 min followed by 10 min of only steam exposure. Bag for quasi-total-body exposure use O_2/O_3 at lower concentration (5-10 *μg*/NmL). In this case, the median time of exposure is 20 min. It is claimed that it serves for detoxification, aesthetic medicine, stress relaxation and muscle tension, improve blood circulation, total body infective diseases, and psoriasis. Clinical trials using those methods are needed.

Table 3. Dose ranges of ozone in water and ozonized oils.

Table 3 A. General specifications to prepare ozone in water.

O_3 in bi-distilled water					
Method	Specifications	Levels			**Remarks**
		High	**Medium**	**Low**	
Ozone / water 1. Local treatment, essentially use high O_3 concentrations 2. For ingestion, low O_3 concentrations are used.	O_3 C_g. (μg/NmL)	80	60-40	20-10	Final concentration of ozone in water (bi-distilled water) usually corresponds to 1/4 (25%) of the O_3 bubble concentration at 20 °C. Estimate bubble time is (5-10) min at flow 3L/h. Those parameters are variable, depending on the O_3 flow and type of bubble device.
	V. H_2O (mL)	V. of water depends on the area to be treated (see practical examples below).			
	Final O_3 C_w. (μg/NmL)	20	15-10	5-2,5	
Ozone / water (Practical examples) 1) For external use	O_3 C_g (μg/NmL)			80	
	Volume of bi-distilled H_2O (mL)			500	
	Bubbling time (min)			10	
	Final O_3 C_w. (μg/mL)			20	
	E.g. of applications		Ulcer, bed sores		
2) Ingestion	O_3 C_g. (μg/NmL)			10	
	Volume of bi-distilled H_2O (mL)			250	
	Bubbling time (min)			5	
	Final O_3 C_w. (μg/mL)			2,5	
	E.g. of applications		Gastric ulcer		

Note. The ozone in water must be maintained in a tightly closed glass bottle with a silicone or Teflon® cap, possibly in the refrigerator. If it is kept at 5°C, the ozone concentration is halved in some 110 h, but at 20°C the ozone half-life is only 9 h.[51]

Legend. C_g: ozone gas concentration; C_w: ozone concentration in water; V: volume.

Table. 3 B. Specifications of ozonized oil.

Ozonized Oils					
Method	Specifications	Levels			**Remarks**
		High	**Medium**	**Low**	
Ozonized oil	PV ($mEqO_2$/kg)	800-1200	600-400	400-200	Recommended method to assay peroxide values in the method is as it described in the European Pharmacopoeia,[11] modified by Zanardi *et al*. (2008),[170] & standardized by ISCO3.[169]

Indications

1. 400 IP: For oral administration in post-surgery[171] and diseases of the intestinal tract like *Helicobacter pylori*. In facial revitalization, rosacea, acne and stimulation of granulation.
2. 400-600 IP: In wounds, trophic ulcers and burns under clear and frank granulation, canker sores.

3. 600 IP: In vaginal mucosa (vulvo-vaginitis), rectal (hemorrhoids), nasal, trophic ulcers in epithelialization phase, care of the scalp and skin.
4. 800-1200 IP: In severely infected wounds and ulcers, gingivitis, alveolitis, herpes simplex, herpes zoster, psoriasis lesions.

Notes. Some commercial formulations include enhancing skin penetrating agent which are appropriated for intact, uninjured skin: In psoriasis, viral diseases and fungal infestation of the skin, onychomycosis, furunculosis, and abscess.

The oils must be kept in dark glass bottled under refrigeration of 4ºC. Ranges of dose based on peroxide index are indicative and based on a summary of current data available.[172] The lack of quality control of peroxide values induces bias in the current available studies, e.g.:

1) Sunflower ozonized oil (peroxide value 75 $mEqO_2$/kg–100 $mEqO_2$/kg) reduces symptoms related to skin burns, and is effective in preventing the post-lesional hyperpigmentation.[173]

2) Topically applied ozonized sesame oil for acute cutaneous wound healing in mice indicate that both low (<1,000 $mEqO_2$/kg) and high doses (>3,000 $mEqO_2$/kg), as expressed in terms of peroxide value, delay cutaneous wound healing. “Middle” concentration (about 1,500 $mEqO_2$/kg) has the most beneficial effect in accelerating the wound closure ratio.[174]

Legend. PV: peroxide values.

3.3 Application Routes not Recommended for not Being Safe

3.3.1 Direct Intravenous Injection of Ozone (DIV)

Its application is strongly discouraged due to the risk of gas embolism which can occur even in the case of using a slow infusion pump and volumes of 20 mL.[43] The complications of stroke range from a simple axillary bubbling sensation, then cough, a feeling of retrosternal weight, dizziness, to changes in vision (amblyopia), hypotensive crisis, with signs of cerebral ischemia (paresis of the members) and death. It is important to note that five patients died as a result of a gas embolism after administration of ozone by direct intravenous injection.[91,92,175] In addition, there has been a reported case of death due to air embolism during the use of ozone in the treatment of psoriasis.[94]

It ought to be kept in mind that oxygen solubility at 37°C is only about 0.23 mL per 100 mL of plasmatic water, and therefore, venous plasma cannot dissolve oxygen quickly enough, leading to the formation of a gas embolus.

A simulation of the effect of DIV in a preclinical study using mouse and rabbit models, came to this conclusion: “The preclinical results obtained provide evidence that the implementation of direct intravenous ozone is highly risky, because of the severe adverse effects and the mortality that can result, so its use is not justified in humans.” [43] In clinic, there are only three case reports supporting the benefit of this method.[176-178] Due to the lack of homogeneity in the terminology used in ozone therapy, a bibliographic search using the keyword “intravenous ozone” may lead to the appearance in 15 papers.[179-193] However, the reading of the “materials

and methods" section, reveals that what the authors have called "intravenous ozone" is the classic MAH or the administration of O_3SS. In any case they do not use the ozone gas directly into the vein.

Gaseous embolism symptoms are evident in patients undergoing DIV. Despite the theoretical discussion, on whether oxygen (the main component of O_3/O_2) may be embolic gas or not, the fact is that there are reports of deaths by the application of this method.[91,92,175]

Furthermore, except inside clinical trials approved by an Ethic Committee or an Institutional Review Board, there is no justification for putting the patient and the therapy at risk when there are other methods which are safe, have been tested and are effective, such as major autohemotherapy (MAH), minor autohemotherapy (MiAH), Ozonized saline Solution and rectal insufflation.

The use of ozone for the purpose of sclerosing veins (which is also not effective for these purposes), means a direct intravenous gas infusion, therefore it is also not advisable.

3.3.2 Intra-Arterial Injection

Except inside clinical trials approved by an Ethic Committee or an Institutional Review Board. Its application is strongly discouraged due to the risk of gas embolism.

3.4 Application Route PROHIBITED

Inhalation route

Being highly toxic, the inhalation route is absolutely prohibited. The anatomical and biochemical characteristics of the lung make it extremely sensitive to oxidative damage by ozone.[21] On the other hand, it has been demonstrated that during ozone inhalation the down regulation of several genes involved in the antiviral response and in the regulation of type I interferons it takes place, which, in the long term, predisposes to an increase in the susceptibility to opportunistic infections.[194]

3.5 Application Routes that Have not Received Total Consensus

3.5.1 Injection of Ozonized Water

Intra joint injection of ozonized water (practiced essentially in China) involves the joint injections of ozonized water at 22 μg/NmL. Validity of the procedure needs to be demonstrated by clinical trials. Pre-clinical findings suggest that tumor growth is suppressed after the treatment with ozonized water because it facilitates the treatment of the antitumor drug (cisplatin) by increasing blood perfusion.[195]

3.5.2 Injection of Ozonized Glucose Solution

There is no clinical or preclinical evidence for the use of ozonized glucose solution. The reaction between ozone and glucose generates unknown aldehydes, which are potentially toxic to the organism.

3.5.3 Hyperbaric Multi Pass Method (HBO3)

The hyperbaric multi pass method uses extremely high doses of ozone and heparin. As with DIV, HBO3 has no pre-clinical or scientific clinical evidence. According to anecdotal evidence from patients or practitioners the main side effects are: loss of vision, lung disturbances, colored urine (hematuria due to hemolysis). It is well known that the association of heparin with ozone increases the activation and the aggregation of platelets.[102,196] This is the reason why the MAH uses a citrate-based anticoagulant. In the 10-step system consisting of extracting 200 mL of blood and adding 200 mL of O_3 at 70 μg/mL + (20 000–24 000) IU of heparin, the patient receives a total dose of 140 mg of ozone plus the heparin dose which is too high, that may exacerbate the main side effects of heparin: thrombocytopenia, mild pain, hematoma, hemorrhage, local irritation, erythema, increased liver aminotransferase, anaphylaxis and immune hypersensitivity reaction.[197] The observed side effects during the HBO3 are indicative of the toxicity of high ozone doses.

3.5.4 Intraperitoneal Ozone

It has been said that in cases of mesothelioma, peritoneal carcinomatosis or peritonitis, endoperitoneal or endopleural injections of up to 2500 mL of gaseous mixture with an ozone concentration of 10-20 μg/NmL can be performed.[198] This modality is rarely used and must be performed by a specialist.[198] There is not any clinical trial documenting its benefits. However, the use of this therapy in cancer is supported by a two pre-clinical study.[199,200] The administration of a drug in the pre-clinical study via intraperitoneal (IP) is of common use, because of the difficulty of approaching the veins of animals. IP is mainly considered an experimental method. The experimental model of cancer in rabbits, is done by the implantation of tumor cells into the rabbit´s ear, and consequently the marginal ear vein cannot be used for drug administration. This could mean that the result observed in the preclinical trial probably does not depend on the route of administration.

The use of IP in humans is not frequent, as it involves a very invasive method, it needs operating room conditions. Therefore, the benefits of ozone as adjuvant in cancer should be reached using other ways of administration such as the MAH,[201,202] with low side effects, low cost and low invasiveness compared with the I.P. (intraperitoneal hemorrhage, pain, etc.). Any cancer intervention should be approved by the patient and in consultation with an oncologist. The only fact available today about the role of ozone in cancer, is its role as adjuvant,[201,202] not as a cure. Promising or creating expectations of healing a patient with cancer is a serious lack of medical ethics.

The IP route is in the scientific experimental phase in animals - to which various tumor cell lines have been implanted – but results show that ozone is more cytotoxic to tumor cells than many of the chemotherapeutic drugs used, and without causing adverse effects. The research into this matter has been essentially undertaken at the Animal Medicine Laboratory of the Philipps-University of Marburg (Germany), by the then Medical Veterinarian Professor Siegfried Schulz.[199,203]

In addition, the intraperitoneal route was used to modulate the inflammatory response of acute lung injury in the cecal ligation/puncture infection model in rats, although there was no improvement on survival rates.[204]

It is exhorted that investigations in animals continue to be carried out. Experimental studies for the treatment of cancer using this route of administration in humans have not yielded convincing data so far.

However, the washing of the abdominal cavity intra-operatively in peritonitis with (5-10) L ozonized saline solution at a concentration of (4-6) μg/mL for 20 min, and with the placement of a silicone tube as drainage for further washing, has been used in human beings.[24]

4. PATHOLOGIES MORE APPROPRIATE TO BE TREATED WITH OZONE THERAPY

The diseases sensitive to the ozone treatment may be classified into three categories, according to evidence-based medicine (EBM). Evidence quality was assessed based on the source type (from meta-analyses and systematic reviews of randomized clinical trials) as well as other factors including statistical validity, clinical relevance, currency, and peer-review acceptance.

Grades of Recommendation. Levels of evidence were adapted from: The U.S. Preventive Services Task Force, and the Centre for Evidence Based Medicine, Oxford. Selected levels of evidence in ozone therapy were classified as:[205,206]

Level A. Good scientific evidence suggests that the clinical benefits of ozone substantially outweigh the potential risks. Based on systematic reviews with randomized controlled trials, systematic reviews with homogeneity of cohort studies or systematic reviews with the homogeneity of case–control studies.

Level B. At least fair scientific evidence suggests that the clinical benefits of ozone outweigh the potential risks. Based on individual randomized controlled trials (with a narrow confidence interval), cohort studies or case–control studies.

Level C. At least fair scientific evidence suggests that there are clinical benefits provided by ozone, but the balance between benefits and risks are too close. Based on expert opinions without explicit critical appraisals, case reports, or based on physiology, bench research, or “first principles”, or descriptive epidemiology.

4.1 Diseases in the Level A

a. Spinal diseases (disc herniation, spondylolysis, discarthrosis, lumbar and cervical pain, etc.).[126,128,207,208]

b. Knee osteoarthritis.[139,140,209-212]

For details see: ISCO3 (2014). *Ozone in non-rheumatic locomotor system pathologies.*[40]

c. Ozone therapy in the management of chronic radiation-induced proctitis.[69,213,214]

d. Ozone infiltration in plantar fasciitis.[215,216]

e. Ozone therapy in diabetic foot ulcer.[217-221]

4.2 Diseases in the Level B

Use of ozone in this category includes:

In orthopedic and traumatology:

a. Painful disorders of musculoskeletal soft tissue.[222]
b. Patellar chondromalacia,[223,224] Gonarthrosis.[225-228]
c. Tendinopathies (tennis elbow,[229,230] jumper's knee,[231] painful shoulder[230,232] and Rotator Cuff Tendinopathy).[233,234]
d. Quervain's tenosynovitis.[235,236]
e. Carpal tunnel,[237,238] Tarsal tunnel.[239]

Infectious diseases:

f. Dental caries and periodontal disease (see Addendum A for more details).
g. Osteomyelitis,[240,241] abscesses with fistula,[147] infected wounds,[242,243] chronic ulcers,[221] and burns.[244,245]
h. Acute and chronic infectious diseases, particularly those caused by bacteria resistant to antibiotics or to chemical treatments,[246] viruses (hepatitis,[247-249] HIV-AIDS,[250,251] herpes and herpes zoster infection,[252,253] papillomavirus infections),[254,255] fungi (mycosis and candidiasis fungi).[256]
i. Endometritis and vaginitis (Monilial and candida).[257]

Although the ozone therapy represents a useful support for the treatment of these diseases, it is worthy to underline that neither the ozone nor its metabolites, among which the H_2O_2, reach a germicide tissue concentration, because the free pathogens are protected by plasma antioxidants and intracellular viruses which are unattainable.

Ozonized solutions and ozonized oils have been used for the treatment of infection diseases topically and orally and their effectiveness have been proven in clinical trials for the treatments of:

a. Protozoan parasites: giardiasis,[258,259] leishmaniasis.[165]
b. Mycoses: Athlete's foot, Onychomycosis,[260] Tinea pedis.[166,256]
c. Bacterial infection: Helicobacter pylori,[261-263] Staphylococcus aureus.[264]
d. Viral diseases: Herpes zoster.[265]
e. Autoimmune diseases: pemphigus vulgaris,[266] psoriasis vulgaris,[164] atopic dermatitis.[267]
f. Periodontitis,[268-270] alveolar osteitis,[271] oral mucositis induced by chemotherapy,[272] necrotizing gingivitis,[273] pericoronaritis,[274] root canals,[275] oral lesions,[276] Osteonecrosis of the Jaw.[277]
g. Conjunctivitis, keratoconjunctivitis, and corneal ulcers.[278]
h. Perianal Crohn's fistulas,[279] urinary fistulas,[280]
i. Intestinal dysbiosis.[281]

j. Bartolinitis[24] and vaginal candidiasis.[282,283]
k. Bed sores, chronic wounds,[163,284] Diabetic foot ulcer,[285] infected burn wounds.[286]

Other diseases using ozone therapy, supported by clinical trials:

a. Diabetes.[287-290]
b. Chronic fatigue syndrome[291] and fibromyalgia.[292-294]
c. Sudden sensorineural hearing loss.[295]
d. Advanced ischemic diseases.[296-298] Lower limb arterial ischemia,[299,300] post-myocardial infarction rehabilitation,[301,302] and cerebral infarction,[303]
e. Age-related, macular degeneration (atrophic form).[304-306]
f. Anti-ageing,[307] and anti-oxidant.[308]
g. Ozone therapy in rheumatoid arthritis.[309]

Additional papers supporting these applications are available at the free access online ISCO3 *Ozone Therapy International Library*.[96]

4.3 Diseases in the Level C

For these pathologies the ozone therapy, either used only as an exclusive form or as support for a specific treatment, according to the cases, becomes a medicine/treatment with a high therapeutic success rate according to preliminary clinical reports.

These include:

a. Cancer-related fatigue.[310] Chemotherapy/radio therapy side effects.[311,312] The ozone therapy associated with orthodox treatments may accelerate and improve results.[313] However, ozone therapy has so far not been able to show a therapeutic effect on cancer. For all these pathologies ozone treatment should be integrated with the conventional treatment, and there is evidence of its utility,[213,311,314,315] but more precise studies are required.[316]
b. Asthma.[298,317]
c. Vestibulocochlear syndrome, Menière's disease.[318]

In the following cases the combination of orthodox treatments and ozone therapy, at least on theoretical grounds, shows that it may be useful but there is no real clinical evidence. The anecdotal evidence suggests the existence of therapeutic effectiveness but, in many cases the efficacy has been achieved by using various types of therapy, therefore the results are not reliable. In some studies, the combination of ozone therapy with another treatment has been evaluated, concluding that ozone therapy acts as a complement.

a. Autoimmune diseases: Systemic sclerosis,[319] multiple sclerosis,[4,320,321] rheumatoid arthritis,[309,322] crohn's disease,[323] chronic inflammatory bowel disease,[323] and lupus erythematosus.[324]

b. Lung diseases: Emphysema[325] and chronic obstructive pulmonary disease.[326,327]
c. Skin diseases: Psoriasis,[164,324,328] eczema and atopic dermatitis.[146,264] Acne:[329] Acne conglobata,[143] Acne scars,[330] Alopecia.[331]
d. Sepsis: Severe sepsis,[332,333] necrotizing fasciitis,[334] peritonitis,[335,336] burns,[244,337] maxillary infection,[338] suppurative otitis media,[339] tonsillitis,[340,341] frontal sinusitis,[342] cystitis.[153]
e. Respiratory diseases: Tuberculosis,[343] bronchitis,[344] respiratory failure,[345] rhinosinusitis.[346]
f. Gastro intestinal diseases: Cholelithiasis[248] and peptic ulcer,[347,348] gastrointestinal hemorrhage.[349]
g. Ophthalmology: Dry eye syndrome,[350] diabetic retinopathy,[351] endophthalmitis,[352] choroid diseases,[353] retinitis pigmentosa,[354] and chronic glaucoma.[355]
h. Nervous system disorders: Ethanol withdrawal.[356]
i. Pain: metatarsalgia,[357] and migraine.[358,359]
j. Pregnancy: Placental failure,[56] preeclampsia,[59] and infertility caused by fallopian tube adhesion.[360,361]
k. Vascular diseases:Ischemic heart disease.[362]
l. Cancer metastasis (as adjuvant or to reduce side effects of chemo or radiotherapy): Refractory hemorrhagic radiation proctitis.[69] Prostatic hyperplasia.[363]
m. Raynaud's syndrome.[364]
n. Chronic kidney failure.[365]
o. Liver diseases: hepatitis A, B, and C.[366-368]
p. Edematous fibrosclerotic panniculopathy.[369]
q. Thyroid-nodule.[370]
r. Neurology: Senile dementia,[371] and Parkinson's syndromes.[372,373]

Additional papers supporting these applications are available at the free access online ISCO3 *Ozone Therapy International Library*.[96]

5. GENERAL BASIS FOR TREATMENT

Not all patients respond equally to the small, controlled oxidative stress that is produced by ozone therapy. Therefore, the ozone treatment should always be applied in a gradual and progressive manner, starting with low doses and increasing gradually to avoid unnecessary risks, until a clinic diagnostic method for the oxidative stress is available, which allows the dose to be adjusted.

It is possible to measure and classify the state of the oxidative stress of the patient. Only one variable of the antioxidant/pro-oxidant system (as index of total antioxidant activities), is not advisable. Markers of bio-molecular damage (such as malondialdehyde, advanced product of protein oxidation, etc.), activities of enzymes (e.g. catalase, superoxide dismutase, glutathione peroxidase), antioxidants (e.g. glutathione) and indicators of the total antioxidant activity are recommended. Unfortunately, reliable methods or equipment for measuring oxidative stress are not available. Research in this direction is underway.

If the redox balance is not well known (antioxidants/pro-oxidants balance) and the patient is in an oxidative stress, an initial medium or high dose may damage cellular antioxidant mechanisms and aggravate the clinical picture. *It is therefore preferable to start with low doses and to increase according to patient response. This is the general practice rule.*

However, it is very important that the physician takes into consideration the nutritional status of patients (by anamnesis and anthropometric index). Food is the source of exogenous antioxidants and is of paramount importance in the clinical response of ozone. According to the patients' initial clinical state, the decision of whether they are eligible to receive the treatment with ozone or not would be made. In some cases, it will be necessary to improve the nutritious state of patients first before proceeding with ozone.

As with any medical treatment, patients may be divided into three types: Normo-responders, hyper-responders and hypo-responders. There are factors which cannot be controlled and that depend on the idiosyncrasy of the patient and the characteristics of how the disease manifests itself.

Ozone therapy is a *medical act* and should be practiced by medical doctors and implemented with scientific rigor. It can produce, with a low frequency, a minimum of adverse effects. For these reasons, we consider that the regularization of the ozone therapy carried out by the authorities should include the following requirements, and in cases where this has not been done the ozone therapists should apply them.

The medical centers where the ozone therapy is practiced should have mandatory sanitary authorization for its functioning and should abide by the following requirements:

1. To have a qualified doctor with training and recognized experience in ozone therapy. This will be the person responsible for the management of the treatment.
2. To use the appropriate equipment to generate and apply the ozone therapy. These should also have the required authorizations from the appropriate sanitary authorities. In the case of the European Community equipment should be marked with the CE. The equipment to generate ozone must be calibrated or revised periodically, according to the recommendation of the manufacturer, to avoid incorrect applications or concentrations.
3. To use medical oxygen provided by an authorized company.
4. To work with carbon mask, as a personal protection, during open applications of ozone (bags, dental, otic, vaginal applications, etc.)
5. To implement the various and appropriate protocols, according to the administration route chosen, in order to guarantee the quality of treatment. The protocols should be appropriately validated and recognized by the international scientific ozone therapy community.
6. To establish an informed written consent, which should be signed by the patient and the medical doctor responsible for the implementation of the ozone therapy, leaving a copy in the clinical record of the patient.
7. To have an appropriate airing and ventilation system.
8. To have lifesaving drugs, ventilation support equipment or an Ambu bag.

9. To take into account that the intra disk application of ozone should be done in a surgical room: In hospital or in an ambulatory unit for major surgery under fluoroscopic guidance.
10. The key to the therapeutic success depends on diverse controllable factors which include the scientific preparation and technique by the ozone therapist; the method that is employed; the quality of the ozone and the general application of the good clinical practices. The non-controllable factors depend on patient idiosyncrasy and on the state of the current illness.

5.1 Essential Requirements

To carry out any procedure, the described routes of application require technically qualified personnel, as well as a written informed consent, followed by strict measures of asepsis and sterility.

As in any other medical procedure, all the material used in ozone therapy, that it will be in contact with tissue or fluids of the patient, must be either disposed after only one use, or be sterilized (ex. surgical equipment); and the oxygen-ozone gas mixture must pass an antimicrobial sterile filter (<20 μm) before administration.

The ozone generator used should be in line with the recommendations of ISCO3.[10] Professionals should attend post-graduate education courses which include basic contents defined by ISCO3.[374]

5.2 Basic Rules to Perform Training in Ozone Therapy

1. All ozone therapy trainers should have diplomas in ozone therapy issued by recognized bodies, preferentially by universities or by experienced prestigious national or international organizations in ozone therapy.
2. The training curriculum should be accredited and approved by a recognized body such as universities or an experienced prestigious national or international organization in ozone therapy such as ISCO3.[374]
3. The practical training must necessarily be done in a controlled clinical environment that meets the current health legislation of each country.
4. All the disposable material used for training must comply with the rules listed in this Declaration.

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Addendum A
APPLICATIONS of OZONE in DENTISTRY

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THERAPEUTIC RANGES FOR THE USE OF OZONE IN DENTISTRY

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1. PREFACE

Since the early pioneering days of the Swiss dentist Dr. E Fisch (1899-1966), ozone application in dentistry has evolved and now is being used by a growing number of dentists worldwide. Due to the high disinfection and oxidation properties of ozone, scientists studied the use of ozone in various applications, mainly in water treatment, where the bulk of the fundamental ozone science as we know it today emerged. The promising results from the use of ozone in water treatment encouraged the expansion of its use to other applications, e.g. air and surface treatments, which are more relevant to healthcare and medical professionals.[1-2]

In parallel to the use of ozone in industry in the early twentieth century, scientists and physicians introduced the use of ozone in medical and dental applications. It was not until the last two decades of the 20th century that researchers began to uncover the precise mechanisms of ozone on mammalian cell physiology. With this improved understanding, the clinical guidelines became more relevant and precise and, in turn, led clinicians and researchers to re-examine the use of ozone in dental medicine.[3-4]

During this period, several national and international medical ozone associations were formed, the International Scientific Committee of Ozone Therapy (ISCO3) was established, and a multitude of scientific congresses and courses were held. Ozone therapy is now legally practiced in several countries around the world. ISCO3 published the *Madrid Declaration on Ozone Therapy*[5] in 2005, which is considered an international reference for both clinicians and legal authorities.

When reviewing the clinical applications of ozone in the medical and dental literature, it is important to distinguish between the use of devices that produce ozone from ambient oxygen and those that produce ozone from medical grade oxygen. This review contains literature from researchers and clinicians using both types of machines. The results and outcomes should be evaluated accordingly since the production of reactive nitrogen species by ambient ozone machines have been shown to be cytotoxic.[6]

Even though there has been a steady increase of healthcare professionals using ozone, ozone therapy has not yet reached the point where it is considered a mainstream treatment modality. Consequently, it is not reimbursed by social security programs or by private insurance companies. Two large issues that pose major obstacles to ozone therapy advancement are the shortage of public research funds and the reticence of pharmaceutical companies to invest in non-patentable modalities. Despite these challenges, several medical ozone therapies can, now, justifiably, be classified as evidence-based medicine. High quality published clinical trials, meta-analyses, and the collective clinical experiences have demonstrated ozone's efficacy in certain medical applications.[7-10]

A recent literature review of the clinical applications of ozone in dental and oral medicine reveals a diverse range of applications using various ozone modalities including gas, water and oil. While this review did not distinguish between medical and ambient ozone, the authors reported generally favorable clinical results for restorative dentistry, conflicting results for surgery and endodontics with little to no risk of complications. Again, this points out the risk of combing literature from ambient and medical ozone. Most of the literature citations in the

article referenced topical applications of ozone in the oral cavity. While invasive routes of ozone administration such a gas injections were shown to be effective for certain conditions, there is still a lack of consensus on concentrations, dosage and timing of such therapies.[11]

Dr. F. Sabbah (2018), reviewed the noticeable deviation regarding ozone gas parameters between clinical observations/expertise and scientific literature. The former is still leading the way on how ozone therapy and related equipment are being used in dental practice. [12]

Licensed dental professionals should use ozone only within their field of expertise and according to their respective legal authority's policy. Collaboration between medical and dental professionals is highly recommended in cases where systemic ozone administration is required for oral or maxillofacial conditions.

This dental ozone therapy section and general guidelines are not intended to be a substitute for a thorough training in the use of ozone in dentistry. It is recommended to acquire a basic knowledge in ozone science in general, specifically, the use of ozone in medicine and its mechanisms of action, biological effects, indications, contra-indications, modes of administration, precautions and safety of use, and be certified in ozone therapy supported by a scientific society or university.[13]

Several topics are well detailed in the medical section (i.e. ozone gas–ozonized water and vegetable oils generation, ozone equipment requirements). Therefore, only some variants better suited for dentistry will be mentioned in the dental section.

2. BASIC PRINCIPLES

2.1 Safety – Precautions

The use of ozone gas intra-orally is probably the most critical among all other ozone applications in industry and healthcare when it comes to inadvertent and accidental inhalation of ozone gas. Thus, it is paramount for the dentist and his/her staff to take all necessary safety precautions during the application of ozone gas intra-orally to avoid any accidental inhalation.

- Stop immediately the procedure if the particular odor of ozone is detected. Check for leaks and application modality.
- Whenever possible, use a silicone cup (i.e. a piece of 10, 8, 6 mm ø silicone tube adapted to the delivery handpiece) when applying ozone gas. If the handpiece is a single line type, puncture the silicone cup with an 18G needle and suction excess gas. If the handpiece is a dual line type, turn ON the dedicated suction and then apply ozone gas.
- At all times, use the dental unit high vacuum to suction any gas leaking outside the silicone cup or the treatment area even if the delivery handpiece has a dedicated suction pump.
- Custom thermoformed total arch trays must be well sealed with silicone impression material all around the edges. It is advisable to perform a hermetic test of the sealed trays (inside the mouth) by connecting the outlet port to the suction source and a 20 mL syringe filled with air to the inlet port of the tray. If the tray is properly sealed and hermetic, the suction could easily aspirate the air from the syringe. In case the syringe

plunger is not efficiently and automatically pulled in by the suction source, this means the tray was not properly sealed and the potential for leakage is high. Recheck and reseal as needed. This video demonstrates the trays hermetic seal test: https://www.youtube.com/watch?v=PrwFrLV7a7I

- Be certain that vitamin C in single gram increments and medical grade oxygen with a concentrator mask are always available in the dental office. In case of an accidental inhalation of ozone gas, mild to severe coughing may occur. If possible, have the patient consume the vitamin C in a cup of water. Next, have the patient breath the oxygen delivered at flow rate of 2 L/min. If coughing is still evident after 15 min, administer another gram of vitamin C. If two rounds of vitamin C administration fail to resolve the coughing or if oral administration is not possible, a doctor should be prepared to administer via sublingual, a misting inhaler, intramuscular or intravenous routes; however intravenous and intramuscular administration should not be attempted by an inexperienced doctor. If conservative measures fail to resolve complications of ozone treatment, emergency medical alternatives should be part of an office plan. For specific information on types of vitamin C and safe medical protocol and routes of administration, doctors should reference the text *Death by Calcium* by Thomas E Levy, M.D., J.D.

Supporting documents
First Aids in Ozone Therapy (Inhalatory exposition and accidental over dose).
Safety Information and Adverse Event Reporting Program Form.
https://isco3.org/officialdocs/

2.2 Ozone gas – Ozonated Water and Oils and Related factors

By definition, any liquid, gas or substance used in medical applications, especially for injections, must be of high purity. Ozone gas generators for dental applications should obey the local and/or international standards of quality and safety. Especially, the guidelines relate to the external and internal components of the unit that should be made of ozone-resistant materials.

A large variety of ozone gas generators for medical and commercial applications are available and can also be used for dental applications. In the dental setting, additional components are available and recommended to ensure the patient and operator's safety as well as to facilitate the proper therapeutic application.[14] Among these recommendations are:

- A handpiece with a flexible silicone cup to ensure precise ozone gas delivery to prepared teeth.
- A handpiece with a Luer lock connection to allow the use of cannulas to deliver precise amounts of gas or ozonated liquids to the gingival crevice.
- A foot pedal to allow controlled delivery of ozone gas to a dental ozone handpiece.
- High volume intraoral suction to ensure evacuation of excess unreacted ozone gas or liquids.

2.3 Oxygen Source Dilemma

Ninety percent of dental research and studies used ozone gas generators operating on ambient air and certified as EC medical devices, whereas a large majority of dentists use oxygen cylinders to generate ozone gas mixtures.

The controversy over medical ozone versus ambient ozone will continue to persist until dentists, dental researchers and manufacturers understand that the generation of reactive nitrogen species for clinical application is contraindicated. Unfortunately, studies that employ ambient ozone generators continue to be generated. It should be to no one's surprise that studies using these devices fail to effectively treat dental conditions such as periodontitis.[15] Oxygen concentrators with a high oxygen purity yield (≥95%) might be used in some topical dental procedures. However, during injections into soft tissues and in intraosseous procedures, a medical-grade oxygen source (99.9%) for ozone generation is still indicated. [14]

2.4 Ozone-Compatible Materials

The external and internal components of the ozone unit should be made of ozone-resistant materials. Between the materials that have good to excellent compatibility with the ozone gas are silicone, fluoropolymer plastics, PTFE polytetrafluoroethylene (Teflon®), PVDF polyvinylidene difluoride (Kynar®), Fluorocarbon (Viton®), laboratory-grade glass, 316 stainless steel, Flexelene, and titanium, among others.[16]

2.5 Ozonated Water

High purity waters (Distilled, reverse osmosis, ion exchange, nano-filtration technologies) are suitable for ozonated water production in dentistry. [17] Depending on the reaction vessel and ozone gas/water mixing technologies, concentration of dissolved ozone at saturation can be around ¼-⅕ th of the ozone gas concentration.[18] The solubility of ozone in refrigerated water (2-3°C) is greater than room temperature water, but the latter is almost three times more reactive than cooled ozonated water and is more convenient for intra-oral use in case of hypersensitivity to cold stimulus.[19] Ozonated water is commonly used in dental applications for surfaces disinfection and for patients' mouth rinse.

2.6 Ozonated Oils

Ozonated oil products for medical applications, including dental, must be labeled according to their peroxide index (PI), commonly expressed as mEq O_2/kg.[20] Choose the appropriate PI according to the clinical case and healing phase.

It is highly recommended for new ozone users to purchase and utilize ozonated oils that have documented components and a measurable PI rather than to attempt its production. Controlled industrial processes of production are required to prevent the generation of toxic secondary byproducts which, in turn, could produce unwanted clinical outcomes.

2.7 The Ct Factor Concept

The CT value, expressed in mg/min/L, is a kinetics calculation commonly used in the disinfection of public drinking water to calculate the lethality of chlorine or other disinfectants such as ozone. It is represented by the formula,

$$\ln(N|N_0) = \Lambda_{CW} C^n t$$

$(N|N_0)$ represents the survival ratio of the microorganism where N and N_0 are the logs of the colony forming units (CFU).
Λ_{CW} represents the Chick-Watson coefficient of specific lethality of the disinfectant.
C^n represents the concentration at a specific dilution represented by *n.*
t represents contact time.

While the formula is not specifically applicable to the disinfection of biological systems, the concept of varying concentration, volume and time (flow rate) can be applied in very general terms to achieve effective disinfection.

This is an important concept to understand in dentistry since there are a wide variety of organic and inorganic substrates that must take into consideration. For instance, understanding how ozone reacts preferentially with a lipid moiety versus a protein within a specific type of tissue would affect a clinician's decisions on concentration, volume and time (flow rate). A specific example of this would be the application of ozone gas in an infected extraction socket that bleeds versus one that does not. More ozone would be consumed by the active bleeding since the lipid component of the blood plus the introduction of additional new blood would consume more of the ozone before an antimicrobial action of the surrounding infected bone would be affected. The same applies in carious lesions and how much affected or partially infected dental tissues are left after excavation.

The concept of varying time and concentration is also useful when choosing ozone generators and the appropriate application equipment. For instance, a low ozone concentration generator might need more contact time to achieve similar results than a higher ozone output generator. This doesn't mean that high ozone concentrations are always better, some users prefer longer contact times with lower concentrations, or using higher flow rates. However, it is important to keep in mind the total dose that is being applied to the area of infection. This is represented by the formula:

Total Ozone Dose (mg) = Oxygen flow rate (mL/min) X
Ozone concentration (μg/NmL) X Time (min)/1000

The total amount of ozone application via total arch trays, ozonated water, ozone gas and ozonated oil, should be adapted to the severity of the clinical case and then reduced according to the progression of the healing process. While the general rule of applying ozone to chronic medical conditions is to proceed *low and slow*, the opposite is true for acute infections. In these cases, the general rule is to start with high ozone doses and then to reduce according to the healing progress.

2.8 Range of Ozone Gas and Ozonated Water Concentrations

Concentration, contact time and flow rate or volume are all related and should be adapted to the clinical case. The recommended concentration unit of ozone is *μg*/NmL. It is still difficult to recommend the best range of ozone concentrations or dose which are currently used in dentistry by various researchers and clinicians. Most of the initial dental ozone research and published clinical studies were done using the Healozone unit (Curozone) which delivers around 4 *μg*/NmL ozone gas in air at a flow rate of ~600 mL/min, and contact times of 30 sec up to 2 min. A newer version of this generator now delivers up to 32 *μg*/NmL in oxygen. A large number of dentists prefer even higher concentrations, up to (80-100) *μg*/NmL.[12]

Ozonated water concentrations and applied volumes also vary according to the clinical case. It is noteworthy to mention that ozonated water is considered to be more bio-compatible and less irritating to epithelial cells than the gas form (although no adverse events have been reported when ozone gas was applied for the duration commonly used in dentistry). Ozonated water concentration range between 4 *μg*/mL up to 20 *μg*/mL is used safely without any reported negative side effects.[12]

Irrigation of bone surfaces with ozonated saline solutions has probed to stimulate bone regeneration in an animal model. The saline solutions were ozonated between (10 to 40) *μg*/mL.[21] Fibroblasts response to ozonated saline solutions had also confirmed an increase of wound healing potential after the irrigation with a saline solution with a concentration of 8 *μg*/mL.[22]

By extrapolation from the recommended general guidelines for topical applications of ozone in medicine, and the significant positive results shown in dental studies where ozone gas, and ozonated saline and water were used, it is recommended to apply ozone gas and water whenever possible, and when indicated ozonated oils. Please note that ozone is considered to be an adjunct agent, not a substitute for other disinfectants and/or therapeutic agents commonly used in dentistry.

3. CLASSIFICATION OF DENTAL OZONE APPLICATIONS ACCORDING TO EVIDENCE-BASED MEDICINE

After reviewing 142 studies covering the major dental fields of ozone application where ozone gas–ozonated water and ozonated oils were used, separately or combined, significant positive results were retrieved and tabulated according to field of application (Table 1).[12]

Seventy five percent of the studies covering four major dental fields (Caries-Endodontic-Surgery-Periodontics) demonstrated had significant positive results with the use of ozone therapy.[12] In General, all dental fields combined showed a 76% positive results with the use of zone therapy.

It is noteworthy to mention that a majority of the studies where ozone gas was used, the generators operate on ambient air and produce low ozone concentrations. A reason why most of the research authors used these models is that they are EC medical device certified, which is a prerequisite in the European Union where the vast majority of research was conducted. By comparison, the authors have noted that many dentists commonly use pure oxygen to generate

ozone gas at higher concentrations and follow the same specifications and parameters as used by medical doctors in topical applications, which is also reflected in medical research covering this particular field of application (Table 2).[12]

Table 1. Major dental field of ozone application.

Field	#	SPR #	SPR %	SPR % All Fields	SPR % (a,b,c,d)		#	SPR #	SPR %
TMJ	5	5	100%	76%	75%	Restorative materials compatibility	36	31	86%
Surgery (a)	27	24	89%			Cytotoxicity	5	4	80%
Soft tissue lesions	8	7	88%			DUWL	4	4	100%
Periodontics (b)	16	12	75%						
Caries (c)	48	34	71%						
Endodontic (d)	34	23	68%						
Whitening	4	2	50%						
	142	**107**							

TMJ: Temporomandibular joint
DUWL: Dental unit water lines
SPR: Significant positive results
#: Number of studies

Table 2. Comparison of Ozone gas parameters used in topical applications in research and clinical applications.

Summary of ozone gas parameters	Research	Dentists
Concentration (*μg*/NmL)	0.2 – 4.2	10 - 100
Oxygen source	Ambient air	Pure oxygen
Dose (mg)	0.06 – 8.2	3 - 120

There is a discrepancy in ozone parameters between the ozone concentration used by researchers and clinicians. This makes it difficult to recommend the best therapeutic range for ozone gas use in dentistry. Some published studies used higher ozone gas concentrations in the range of 10-60 *μg*/NmL and showed significant positive results. One in vitro study compared the effectiveness of 4.2 and 53 *μg*/NmL ozone gas on endodontic microorganisms and found the higher concentration was more effective than the lower one.[21,23-26]

Besides the scientific evidence, ISCO3 *Madrid Declaration on Ozone Therapy* also serves as a solid foundation for legalization of ozone therapy, in this context the use of ozone in dentistry, and should reflect a concordance between research and clinical expertise, especially regarding ozone gas parameters.

It is with hope that future dental ozone studies take into consideration this finding. We would also hope that authors consider following the same guidelines, specifications and parameters as used in medical topical applications. We suspect that the overall 76% significant positive results would be vastly improved if there were a standardization of the clinical ozone generators and dental application equipment utilized in these studies. In order to ensure safety and minimize the risk of sanctioning by professional licensing organizations, ozone generators should be certified by a government-approved independent testing organization (CE, CSA, UL, FDA) as to their ability to consistently and reliably produce the concentration of medical grade oxygen/ozone stated on its readout. Likewise, application equipment (ozone trays and handpieces) should be constructed of ozone resistant materials that demonstrate minimal leakage and thus, inhalation by the patient.

For clinicians who wish details of a comprehensive literature review of dental ozone applications with over 250 references, refer to (Sabbah 2018).[12]

4. CLINICAL GENERAL GUIDELINES

As in medical topical ozone applications, it is recommended that dentists use ozone gas and ozonated water/saline solution whenever possible and to supplement with ozonated oils when indicated. Select the appropriate dose according to the clinical case and severity, starting with high doses then reducing them during the healing phase.[12,27]

Taking into consideration the ozone equipment commonly used in research and clinical practice and applied oxygen/ozone parameters, the following general guidelines cover both low and high ozone doses as reflected by research and clinical practice (Table 3).

Please note that these guidelines do not constitute strict protocols to follow. Clinicians should select the best ozone dosage and modes of applications according to each clinical case.

Table 3. General guidelines for the use of ozone gas, water and ozonized oil**.**

Clinical Case Severity	Ozone Parameters			
	O_2/O_3 gas μg/NmL	**Air/O_3 gas** μg/NmL	**O_3 Water** μg/mL	**Ozonated Oils** PI: mEqO_2/kg
Low severity	C: 5-20 Time: 30-60 s	C: 2-4 Time: 30-60 s	C: 4-8	PI: 500
Moderate severity	C: 20-40 Time: 30-60 s	C: 2-4 Time: 1-2 min	C: 8-15	PI: 800
High severity	C: 40-80 Time: 1-2 min	C: 2-4 Time: 3 min	C: 20	PI: 1,200
Injections Arthrocentesis	C: 5-20 V: 1-2 mL	N/A	C: 12-20 V: 100-200 mL	
Legend. C: concentration; **V:** volume; **PI:** peroxide index; **N/A:** not applicable				

Recommendations for dental ozone applications

Highly humidified ozone gas is more efficient than dry gas which is usually delivered from ozone generators and it will minimize dehydration of dentinal structures as seen with dry ozone gas. Re- humidification of dry ozone gas can be achieved by flowing the gas through a column filled with saturated ozonated water. Otherwise, rewet the dentinal structures with ozonated water during dry ozone gas applied for extended period of time.

It is more convenient to deliver large volumes of ozonated water with the dental unit air/water syringe than by using a manual syringe. Another advantage is that pressurized ozonated water has a higher transfer rate of ozone molecules to the target area than water delivered with a manual syringe. It is recommended to replace the rubber O-Rings of the air/water syringe with Viton O-Rings, and the PVC water tubes with Teflon.

Commercially available ozone-resistant pumps with variable speed are also a good choice and can deliver pressurized ozonated water up to 100 psi (689475 Pa).

4.1 Caries

Minimally invasive dentistry (MID) is becoming the standard of care, especially with the advancement of early caries diagnostic systems, selective atraumatic cutting instruments and bioactive restorative materials. The goal of MID is to implement early preventive measures, and in the case of invasive treatment, to selectively remove decayed or damaged dentin and enamel with minimal collateral damage of healthy structures and to attempt remineralization of affected tissues and regeneration of dentinal structures, thus maintaining overall tooth structures integrity and strength. Ozone plays an important role in this process by eliminating the causative pathogenic biofilm and its by-products. Even though there is minimal research on the effects of ozone on tooth pulp tissues and its ability to stimulate growth factors or even stem cells,[28,29] dentists' clinical observations and research in the medical field support the idea that ozone treatments might be effective at facilitating regeneration in early pulpal damage due to advanced decay. The possibility to avoid endodontic treatment in certain cases and regenerate a damaged dental pulp would represent an exciting and significant advancement in dental medicine.

4.1.1 Low Severity Clinical Case: Developmental Defects; Hypocalcified Fissures; Caries in Enamel Only; Partially Erupted Posterior Teeth.

Preventive treatment:
Ozonated water mouth rinse.
Fissures air prophy (sodium bicarbonate/Sylc).
Wash with ozonated water. Apply ozone gas followed by mineralizing agent.
Preventive bioactive sealant (Fuji Triage).

Invasive treatment:
Fluid (ozonated water) air abrasion.
Wash with ozonated water. Apply ozone gas followed by mineralizing agent.
Fill with Fuji IX or your preferred material.

4.1.2 Medium Severity Clinical Case: Caries in Coronal Third of Dentin

Anesthesia most probably not indicated.
Ozonated water mouth rinse.
Fluid (ozonated water) air abrasion / slow speed electrical handpiece (100 r·min-1) / hand instruments.
Cutting assisted with caries detector dyes / DiagnoDent.
Wash with ozonated water. Apply ozone gas followed by mineralizing agent.
Restore with Fuji IX, EQUIA, or your preferred adhesive material.

4.1.3 Medium-High Severity Clinical Case: Caries in Middle Third of Dentin

Assessment if anesthesia is needed.
Ozonated water mouth rinse.
Fluid (ozonated water) air abrasion / slow speed electrical handpiece (100 r·min-1) / hand instruments.
Cutting assisted with caries detector dyes / DiagnoDent.
Recommended to leave the affected slightly leathery layer dentin (bottom 0.5 mm).
Wash with ozonated water at demand during caries removal. Apply ozone gas.
Apply mineralizing agent.
Restore with Fuji IX, EQUIA, or your preferred adhesive material.

4.1.4 High Severity Clinical Case: Caries in Apical Third of Dentin.

Assessment if anesthesia is needed.
Ozonated water mouth rinse.
Fluid (ozonated water) air abrasion / slow speed electrical handpiece (100r·min-1) / hand instruments.
Cutting assisted with caries detector dyes / DiagnoDent.
Remove totally necrotic dentin (not sensitive) and leave <1 mm of the affected slightly leathery dentin layer.
Wash with ozonated water at demand during caries removal.
Apply ozone gas for 2 min or more. Apply mineralizing agent.
In case the caries lesion is very deep and to avoid pulpal exposure, it is recommended to perform 2 sessions treatment.

- Session 1: Debride all peripheral necrotic dentin and leave 1+mm infected/affected dentin over the pulp chamber. Flush cavity with large amount of ozonated water. Apply ozone gas and remineralizing agent.

  Fill with Tri-calcium silicates / GIC and reassess at 2-3-month interval.

- Session 2: Remove temporary filling, carefully debride non-remineralized dentin and apply ozone water / gas and remineralizing agent.

  Reassess at 3 months recall with X-Ray and clinical examination.

Please note that total arch ozone trays are recommended before and during the treatment, specifically in deep caries lesions.

4.2 Hypersensitivity: No Caries Involvement

Diagnosis – Risks factors assessment - Treatment planning according to clinical case -
Ozonated water mouth rinse.
Air prophy (Na bicarbonate–Sylc).
Wash with ozonated water.
Apply ozone gas. Apply mineralizing agent. Restoration if needed.

4.3 Root Canal Treatment

Ozonated water mouth rinse.
Cavity access–Canal(s) ID.
Flush cavity with ozonated water and apply ozone gas.
Proceed with your preferred chemical/mechanical shaping/cleaning technique.
Final rinse with large amounts (100-200 mL) of ozonated water (8+*μg*/NmL) using appropriate needles.
Activation with Ultrasonic or laser tips for improved debridement and disinfection.
Irrigate ozonated water-filled canal(s) with ozone gas (40-80 *μg*/NmL) for (1-2) min each canal via a handpiece or with a manual syringe (100 mL).
Keep the delivery tip freely moving inside the canal while suctioning excess gas.
In case a two session RCT is desired, fill the canal(s) with your preferred interim product.
Inject (1-2) mL at 5-10 *μg*/NmL in the peri-apical region. Repeat at subsequent recalls.

4.4 Regular Hygiene / Scaling and Prophy

Ozonated water mouth rinse.
Fill the scaler fluid bottle with ozonated water (if applicable) and proceed with the scaling procedure.
Irrigate at demand with ozonated water.
Ozonated oils if needed.

4.5 Periodontal Conditions

4.5.1 Mild Gingivitis

Ozonated water mouth rinse.
Fill the scaler fluid bottle with ozonated water (if applicable) and proceed with the scaling procedure.
Irrigate at demand with ozonated water.
Total arch tray ozone application may be required before initiating the cleaning/scaling procedure.
Apply ozonated oil and if needed slightly inside the sulcus at 400-600 IP with a sulcabrush.
Provide the patient with ozonated oil for home use. Apply once or twice daily for few days.

4.5.2 Periodontitis

According to the United States Centers for Disease Control (CDC), periodontitis is now considered a worldwide public health problem with an almost 50% prevalence in adults 20 to 64 and is found in over 70% of adults 65 and older. Besides its oral destructive consequences

and tooth loss, periodontal disease is linked to systemic chronic inflammatory diseases (atherosclerotic cardiovascular disease, diabetes, rheumatoid arthritis, cognitive impairment, obesity, metabolic syndrome, cancer, etc.).[30-31]

Topical and systemic ozone therapy may be extremely beneficial in alleviating the devastating damage, both local and systemic, of the periodontal biofilm and chronic inflammatory oxidative stress. It is unfortunate that ozone research on combined local and systemic administration is still lacking on this important health topic, and in our opinion, it should be a priority in future ozone studies. Medical and dental ozone therapists should collaborate in the treatment and management of periodontal disease which might prove to be a systemic chronic infectious disease, not an oral condition with systemic associations.

Total arch tray ozone application (gas-water).
Ozonated water-assisted Scaling/Root planning.
Pockets irrigation with ozone gas using an appropriate applicator (Ultradent capillary tips; 27G–25G blunt needle).
Ozonated oil application. Home use ozonated oil (PI 600-800) once or twice daily.
Reassessment – Decide if further sessions are indicated.

Please note that the total amount of ozone application via total arch trays, ozonated water, ozone gas, and ozonated oil should be adapted to the progression of the healing process. The general rule is to start with a high ozone dose and then reduce according to the healing progress.

4.6 Orthodontics

Irrigate thoroughly with ozonated water and apply ozone gas (20-30 μg/NmL; 30-60s) around each bracket. Repeat cycle each 3 months or as required. In presence of gingivitis, treat accordingly. Home use of ozonated oil (600 IP). It is easier to remove the orthodontic wires and elastics to apply ozone gas via a silicone cap and also to avoid any deterioration of non-ozone-resistant materials.

4.7 Oral Surgery

4.7.1 Pre-Surgical Conditioning

In situations where the medical status of the patient (diabetes; low immunity; medicines side effects; elderly) might affect the healing process or contribute to post surgery complications, a pre-surgery conditioning might alleviate such events. The tooth or teeth to be extracted and surrounding soft tissues, or even the total mouth, are treated with ozonated water and ozone gas using any application modality most suitable for the case. If direct ozone injection of the oral soft tissues is chosen, it is advised to apply the ozone in small (less than 1 mL) increments to avoid the possibility of introducing a large gas embolus within an arteriole.
The frequency of ozone application is adapted to the clinical situation of the patient.
Parenteral ozone administration by medical physicians might also contribute in the pre-surgery conditioning.

4.7.2 Ozone-Activated PRP/CGF

The effect of ozonated human platelet derived growth factors (PDGF) was examined by a dental research group (Anitua et al. 2015).32 Varying doses of ozone gas was added via continuous flow or single syringe method to anticoagulated and centrifuged blood samples. The results suggest that low ozone doses did not modify the properties and outcomes of PDGF, while higher doses alter the coagulation process of the fibrin and induce a destructive effect on morphogens and growth factors, reducing or inhibiting its biological potential.[32]

4.7.3 Tooth Extraction

Ozonated water mouth rinse.
Remove any existing plaque and infiltrate the sulcus with ozone gas
Proceed with tooth removal.
Flush the socket with ozonated water.
Cover the site with a gauze, use an applicator tip to irrigate with ozone gas (40-80 μg/NmL, 1-2 min) while suctioning excess gas. Fill the socket with few drops of ozonated oil. Home use ozonated oil once or twice daily and decrease application according to the healing phase.

4.7.4 Implants

Implants placement

Ozonated water mouth rinse.
Proceed with implant site preparation and irrigate with ozonated water/saline.
Infiltrate the site with ozone gas using an appropriate applicator while suctioning excess gas.
Lubricate the healing abutment threads with ozonated oil before insertion.
Home use of ozonated oil and reduce as healing progresses.

Final restoration placement

Thoroughly irrigate the implant well with ozonated water, dry, lubricate the abutment screw threads with ozonated oil before insertion. After screw tightening, wipe excess oil with alcohol dipped brush.

Periimplantitis

Non-invasive procedure if indicated
Ozonated water mouth rinse.
Irrigate with ozonated water and ozone gas using an appropriate applicator.
Use your preferred debridement technique and technology.
Place a few drops of ozonated oil inside the affected area. Home use of ozonated oil.
Inject (1-2) mL ozone gas (10-15 μg/NmL) around the targeted site.
Reassess at regular recalls and apply ozone as required.

4.8 Crowns & Bridges, Veneers

Ozonated water mouth rinse.
Pre-preparation: 30 s ozone gas using a handpiece/silicone cup or total arch tray.
Post-preparation: ozonated water/gas 1 min; apply mineralizing agent.

Pre-cementation: air prophy (Na bicarbonate–Sylc) / fluid ozonated water air abrasion, ozonated water/gas 1 min, mineralizing agent.
Post-cementation: ozonated water and oil in case of bleeding gums due to finishing/polishing procedure.
Prosthesis: rinse with ozonated water, ozone gas 1 min-ultrasonic bath with ozonated water.

4.9 Soft Tissue Lesions

Most mild soft tissue lesions, ulcers and wounds respond favorably with home use of ozonated water and oil (PI 600-800). In severe cases, in-office ozone gas/water application.

4.10 Ozone-Assisted Whitening

Air prophy / Isolation with light-cured dam.
Apply your favorite in-office high concentration hydrogen peroxide gel (15-20 min).
Rinse with ozonated water and apply ozone gas (5-20 *μg*/NmL-30 s/tooth or total arch tray 5 min).

4.11 Osteonecrosis of the Jaws OMJ/BONJ

In the event a surgical procedure is necessary in patients taking bisphosphonate medication, specifically by IV, or in patients at risk, a pre-surgical conditioning, as described above, might help to minimize the risks of ONJ.

Recent research showed that a combination of antibiotic therapy and application of ozonated oil were successful in treating the lesions without any surgical intervention. Ozonated water application and localized cleaning are also suitable throughout the treatment phase, as well as peri-lesion gas injections. Systemic ozone administration (provided by an MD) might be recommended.

4.12 Temporomandibular Joint Disorders (TMJ)

Temporomandibular joint and related muscles disorders are considered among the most common skeletal inflammatory and degenerative conditions, with a prevalence ranging from 15 up to 35% of the population. In 2020, according to the United States National Institutes of Health (NIH) website, the overall incidence of temporomandibular joint and related muscle disorders (TMJD) is between 5 to 12%. Some cited studies demonstrated an incidence in certain demographic groups of over 30%.

Compared to the high evidence-based supporting research in medicine, the limited number of published studies in TMJ showed significant positive results, whether by topical application over the joint, intra-articular injection of ozone gas (2 mL at 10-20 *μg*/NmL), or arthrocentesis using ozonated water lavage followed by gas injection.[12]

Future research in this field would help dental clinicians to distinguish between the beneficial effects of the TMJ intra-articular and para-articular ozone injections, as well as the topical applications as seen in some studies where ozone gas was topically applied over the affected TMJ

area. Even though there is a lack of research using ozone gas ear insufflation in TMJ, anecdotal observations by clinicians using this modality report beneficial results.

4.13 Dental Unit Water Lines (DUWL) – Whole Office Water Sanitation

DUWL is becoming a major concern for at-risk patients and dental staff exposed to contaminated dental unit water and aerosols via ingestion or inhalation, as well as potential infection of surgical wounds. Infection control agencies recommend that DUWL should not exceed the maximum allowable colony-forming-units (CFU) of drinking water, ranging from 100 to 500 CFU/mL. Reports of heavily biofilm -contaminated DUWL where a cohort of microorganisms (heterotrophic bacteria, fungi, amoebae, protozoa, coliforms, Legionella, pseudomonas viruses) were found and the total bacterial count was at hundreds of thousand CFUs.

Use of ozonated water in health care facilities and related industries is gaining more attention and is used for water and wastewater treatment, water in pharmaceuticals, hemodialysis water sanitation, and hospital laundry.

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OZONE THERAPY

(3rd Edition)

Addendum B

APPLICATIONS of OZONE in VETERINARY MEDICINE

ADDENDUM B
APPLICATIONS OF OZONE IN VETERINARY MEDICINE

THERAPEUTIC RANGES FOR THE USE OF OZONE IN VETERINARY MEDICINE AND CLINICAL APLICATION
Guidelines for Small Animals

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Index

1. INTRODUCTION

Veterinary ozone therapy is still a relatively new discipline. However, in recent years it has experienced an unprecedented boom. The veterinary ozone therapists of small animals, have accepted the challenge of being part (for the first time) of the 3rd. edition (2020) of the *Madrid Declaration on Ozone Therapy*. We do it from clinical experience, the publication of clinical studies in prestigious scientific journals and doctoral theses. In short and above all, we have the desire to propose a basis for action through the exercise of good practice, science and evidence

The principles of ozone application are essentially based on those described in the *Madrid Declaration on Ozone Therapy* in human medicine. Therefore, this application guide will contain only the specifications that differentiate the use of ozone in this branch of medicine

Given the characteristics of patients in veterinary medicine, the following considerations are taken into account:

-Maximum sterilization measures. The presence of hair facilitates the introduction of pathogens in blood, joints etc. Depilation is mandatory.

-Evaluate sedation when using certain routes that are known to cause pain.

-Perform applications in relaxed environments.

-Inform the owners of the animals about the therapeutic application of ozone; request and sign informed consent.

2. MAIN ROUTES OF APPLICATION[1-6]

The materials are governed by the same considerations of Human Medicine with the proviso that the dosage in veterinary is closely related to weight. Example: In the case of the major autohemotherapy (MAH), this is not usually done with a bag or a bottle since, due to the idiosyncrasy of the patients, weights ranging between 500g to 60 kg it make it almost impossible to do it. Therefore, the method also differs. Ozone concentrations and other specifications for each of the routes, are summarized in Table 1. On the other hand, the guidelines for treatment by pathologies are described in Table 2.

Acronyms
MAH: Major Autohemotherapy
MiAH: Minor Autohemotherapy
RIO_3: Rectal Insuffltation of Ozone
O_3SS: Ozonized Saline Solution.

Next, those routes of application that differ with those applied in human medicine are described.

2.1 Major Autohemotherapy (MAH)

Two syringes are used. One contains the anticoagulant and blood drawn and the other ozone. Using a 3-way key, transfer the ozone to the syringe that contains the blood. Mix and extract the excess gas. Infuse only ozonized blood. Start with low concentrations and increase at a rate of 5 *µg*/NmL every 2 to 3 sessions.

2.2 Rectal Insufflation

Female vaginal catheter is used in dogs and syringe alone or syringe with urethral catheter in cats. We will start with low concentrations and we will increase at a rate of 5 *µg*/NmL every 2 to 3 sessions.

2.3. Subcutaneous

Given the elasticity of the canine´s skin and feline species, the subcutaneous route is used at any point of the skin.

2.4. Intralesional

In dermatology: Application of small amounts of intradermal ozone spread throughout the lesion (concentrations are specified below).

In oncology: Intratumoral application of certain amount of ozone at various points of the tumor.

2.5 Not Recommended Routes

*The Direct Intravenous Injection of Ozone (*DIV), as in human medicine, is not recommended. *Intraperitoneal applications* are in the initial phase of study and will be considered in the future when more data related to safety is available.

Table 1. Main routes of ozone application in veterinary medicine.[1-8]

Most common administration routes in veterinary medicine					
Method	O_3	Doses			Observations
		High	**Medium**	**Low**	
MAH	C. (μg/NmL)	30-35	20-30	10-20	Sample Volume 1 mL/kg (blood)
	V. (mL/Kg)	1-1.5			
	Doses (μg/kg)	30-35 45-52	20-30 30-45	10-20 15-30	
MiAH	C. (μg/NmL)	30-40	15-30	10-15	Sample Volume 0.1-0.5 mL/kg (blood)
	V. (mL/Kg)	0,1-0,2			
	Doses (μg/kg)	3-4 6-8	1.5-3 3-6	1-1.5 2-3	
RIO_3	C. (μg/NmL)	30-35	20-25	10-15	
	V. (mL/Kg)	3			
	Doses (μg/kg)	90-105	60-75	30-45	
Intra-articular	C. μg/NmL	25	10	8	
	V. (mL)	0,5-10			
	Doses (μg)	12.5-250	5-100	4-80	
Para-tendon trigger point	C. μg/NmL	20	10	8	
	V. (mL)	0.5-10			
	Doses (μg)	10-200	5-100	4-80	
Paravertebral	C. μg/NmL	20	10	5	
	V. (mL)	0.5-10			
	Doses (μg)	10-200	5-100	2.5-50	
Subcutaneous	C. μg/NmL	20	10	5	
	V. (mL)	1-10			
	Doses (μg)	20-200	10-100	5-50	
Intralesional	C. μg/NmL	30-40	20-30	5-15	
	V. (mL)	0.5-20			
	Doses (μg)	15-20 600-800	10-15 400-600	2.5-7.5 100-300	
Vesico-urethral	C. μg/NmL	25	15-20	10	
	V. (mL)	5-50			
	Doses (μg)	125-1250	75-100 750-1000	50-500	
Bag	C. μg/NmL	40-70	30-40	15-30	
	V. (mL)	It depends on the volume of the bag			
	O_3	**High**	**Medium**	**Low**	
Oil	IP	800-1200	600-800	400	
Bi-distilled water	C. μg/NmL	40-75	30-40	20-30	Bubble time 10-15 min
	V. (mL)	It depends on the type of container			
	Doses (μg)	It depends on the type of container			
O_3SS	C. μg/NmL	2	0.8	0.4	Bubble time 10-15 min *(dissolved ozone concentration 25% of the ozone gas concentration)*
	V. (mL)	200-400 (NaCl 0.9%)			
	Doses (μg)	400-800	160-320	80-160	
	Doses (μg/Kg)	5	2	1	

3. FREQUENT PATHOLOGIES TREATED WITH OZONE

A summary by pathologies of the most frequent procedures in veterinary medicine that use ozone is described in Table 2.

Table 2. FREQUENT PATHOLOGIES TREATED WITH OZONE

Table 2.1. Digestive diseases [1,9-12]

PATHOLOGY	Administration routes				Observations
	MAH Min C- max C (*µg*/mL) Vol. Blood/ O_3/O_2 Sessions	MiAH Min C- max C (*µg*/mL) Vol. Blood/ O_3/O_2 Sessions	RIO_3 Min C- max C (*µg*/mL) Vol. Blood/ O_3/O_2 Sessions	Oil * PV Sessions	* in the absence of vomiting
Acute gastroenteritis, Canine parvovirus, Parasitic diseases, Immune-mediated gastrointestinal diseases, Pancreatitis	15-35 1-1.5 mL/kg 8	20-30 1.5 mL/10kg 8	10-30 3 mL/kg 12		
Chronic gastroenteritis	15-35 1-1.5 mL/kg 8-10	20-30 1.5 mL/10kg 8	10-30 3 mL/kg 12	400 (600**) 2-5 mL orally/day 1 gout/day 30 days	***Helicobacter pylori*

Note. MAH: Major Autohemotherapy. MiAH: Minor Autohemotherapy. RIO_3: Rectal insufflation of ozone. PV: Peroxide Value. Min C: Minimum concentration. Max C: Maximum concentration. O_3/O_2: Medical Ozone.

Table 2.2. Leishmaniasis [1,13]

Administrations routes					
MAH Min C- max C (*µg*/mL) Vol. Blood/ O_3/O_2 Sessions	MiAH Min C- max C (*µg*/mL) Vol. Blood/ O_3/O_2 Sessions	RIO_3 Min C- max C (*µg*/mL) Vol. Blood/ O_3/O_2 Sessions	Intralesional Min C- max C (*µg*/mL) Vol. Blood/ O_3/O_2 Sessions	Oil PV Administration	Bi-distilled water Min C- max C (*µg*/mL) bubbling time Sessions
20-35 1-1.5 mL/kg 8-10	30-35 1.5 mL/10kg 4-8	20-35 3 mL/kg 12-15	8-10 0.5 mL/kg 4-8	400- 600- 800 Twice-daily (until injury improvement)	60-15 10 min Until improvement

Note. MAH: Major Autohemotherapy. MiAH: Minor Autohemotherapy. RIO_3: Rectal insufflation of ozone. Min C: Minimum concentration. Max C: Maximum concentration. O_3/O_2: Medical Ozone. O_3SS: Ozonized Saline Solution.

Table 2.3. Hematology [1]				
PATHOLOGY	Administration routes			Observations
	MAH Min C- max C (μg/mL) Vol. Blood/ O_3/O_2 Sessions	MiAH Min C- max C (μg/mL) Vol. Blood/ O_3/O_2 Sessions	RIO_3 Min C- max C (μg/mL) Vol. Blood/ O_3/O_2 Sessions	
Anemias & Immune-mediated thrombocytopenias	10-35 1-1.5 mL/kg 4-8	20-35 1.5 mL/10 kg 6-9	10-35 3 mL/kg 9-12	Controversial use in MAH with hematocrit <20%

Note. MAH: Major Autohemotherapy. MiAH: Minor Autohemotherapy. RIO_3: Rectal insufflation of ozone. Min C: Minimum concentration. Max C: Maximum concentration. O_3/O_2: Medical Ozone.

Table 2.4. Liver diseases [1,14-16]					
PATHOLOGY	Administration routes				Observations
	MAH Min C- max C (μg/mL) Vol. Blood/ O_3/O_2 Sessions	MiAH Min C- max C (μg/mL) Vol. Blood/ O_3/O_2 Sessions	RIO_3 Min C- max C (μg/mL) Vol. Blood/ O_3/O_2 Sessions	O_3SS Min C- max C (μg/mL) Bubbling time Sessions	
Acute and chronic liver diseases	10-35 1-1.5 mL/kg 8-16		10-35 3 mL/kg 10-20	20-40 10 min 5	
Immune-mediated liver diseases		10-35 1,5 mL/10kg 8-16			It can be combined with MAH or RIO_3

Note. MAH: Major Autohemotherapy. MiAH: Minor Autohemotherapy. RIO_3: Rectal insufflation of ozone. Min C: Minimum concentration. Max C: Maximum concentration. O_3/O_2: Medical Ozone. O_3SS: Ozonized Saline Solution.

Table 2.5. Nephro-urology [1,2]						
PATHOLOGY	Administration routes					Observations
	MAH Min C- max C (μg/mL) Vol. Blood/ O_3/O_2 Sessions	MiAH Min C- max C (μg/mL) Vol. Blood/ O_3/O_2 Sessions	RIO_3 Min C- max C (μg/mL) Vol. Blood/ O_3/O_2 Sessions	Vesico-urethral Min C- max C (μg/mL) Vol. Sessions		
				Bi-distilled water	O_3/O_2	
Acute and chronic kidney disease	10-35 1-1.5 mL/kg 8- undefined *	10-35 1.5 mL/10kg 8-16 (immune-mediated)	10-35 3 mL/kg 10-20			* It depends on the chronicity of the process
Idiopathic feline cystitis	10-25 1 mL/kg 8-12	10-35 1,5 mL/10kg 4-10	10-35 3 mL/kg 10-20	40-65 1 mL/kg 5-7	15-25 1 mL/kg 6-10	

Note. MAH: Major Autohemotherapy. MiAH: Minor Autohemotherapy. RIO_3: Rectal insufflation of ozone. Min C: Minimum concentration. Max C: Maximum concentration. O_3/O_2: Medical Ozone.

Table 2.6. Oncology [1,10,17]					
Administration routes					Observations
MAH Min C- max C (μg/mL) Vol. Blood/ O_3/O_2 Sessions	MiAH Min C- max C (μg/mL) Vol. Blood/ O_3/O_2 Sessions	RIO_3 Min C- max C (μg/mL) Vol. Blood/ O_3/O_2 Sessions	Intralesional Min C- max C (μg/mL) Vol. Blood/ O_3/O_2 Sessions	Subcutaneous Min C- max C (μg/mL) Vol. Blood/ O_3/O_2 Sessions	
10-35 1-1.5mL/kg 8-undefined*/**	10-35 1.5 mL/10kg 5-15	10-35 3 mL/kg 10-20**	15-50 0.5 mL/point Undefined *	20-35 Peritumoral Undefined *	* The number of sessions depends on the process ** Cycles every 3 months

Note. MAH: Major Autohemotherapy. MiAH: Minor Autohemotherapy. RIO_3: Rectal insufflation of ozone. Min C: Minimum concentration. Max C: Maximum concentration. O_3/O_2: Medical Ozone.

Table 2.7. Dentistry [1,10,18]						
PATHOLOGY	Routes of administration					
	MAH Min C- max C (μg/mL) Vol. Blood/ O_3/O_2 Sessions	MiAH Min C- max C (μg/ mL) Vol. Blood/ O_3/O_2 Sessions	RIO_3 Min C- max C (μg/ mL) Vol. Blood/ O_3/O_2 Sessions	Intralesional Min C- max C (μg/ mL) Vol. Blood/ O_3/O_2 Sessions	Bi-distilled water Min C- max C (μg/ mL) Bubbling time Sessions	Oil PV
Periodontal disease	15-30 1 mL/kg 8-15	25-35 1 mL/10kg 8-10	20-35 3 mL/kg 8-15		5-20 10-15 min Once-daily until symptoms disappear	800-400 Until symptoms disappear
Feline gingivo-stomatitis	15-30 1 mL/kg 8-15	25-35 1.5 mL/10kg 8-10 *(main route)*	20-35 3 mL/kg 8-15 *(main route)*	8-15 0.1 mL/point 4-8	5-20 10 min Once-daily until symptoms disappear	800-400 Until symptoms disappear

Note. MAH: Major Autohemotherapy. MiAH: Minor Autohemotherapy. RIO_3: Rectal insufflation of ozone. Min C: Minimum concentration. Max C: Maximum concentration. O_3/O_2: Medical Ozone. PV: Peroxide Value.

Table 2.8. Endocrinology [1,19]				
PATHOLOGY	Routes of administration			Observations
	MAH Min C- max C (μg/mL) Vol. Blood/ O_3/O_2 Sessions	MiAH Min C- max C (μg/mL) Vol. Blood/ O_3/O_2 Sessions	RIO_3 Min C- max C (μg/mL) Vol. O_3/O_2 Sessions	
Hypothyroidism, Hypoadrenocortism Diabetes Mellitus	15-35 1.5 mL/kg 8-20	25-35 1.5 mL/10kg 8-16*	10-35 3 mL/kg 10-20	* MiAH in immunomediated

Note. MAH: Major Autohemotherapy. MiAH: Minor Autohemotherapy. RIO_3: Rectal insufflation of ozone. Min C: Minimum concentration. Max C: Maximum concentration. O_3/O_2: Medical Ozone.

Table 2.9. Ophthalmology [1,10,20-22]					
PATHOLOGY	Routes of administration				Observations
	MAH Min C- max C (*µg*/mL) Vol. Blood/ O_3/O_2 Sessions	MiAH Min C- max C (*µg*/mL) Vol. Blood/ O_3/O_2 Sessions	RIO_3 Min C- max C (*µg*/mL) Vol. O_3/O_2 Sessions	Oil PV	
Herpesvirus, Calicivirus Papilloma virus Corneal ulcers	10-25 1-1.5ml/kg 8	20-25 1.5 mL/10kg 8-10	10-25 3 mL/kg 10-15	800- 400 Once-daily until symptoms disappear	* MiAH in immunomediated

Note. MAH: Major Autohemotherapy. MiAH: Minor Autohemotherapy. RIO_3: Rectal insufflation of ozone. Min C: Minimum concentration. Max C: Maximum concentration. O_3/O_2: Medical Ozone. PV: Peroxide Value.

Table 2.10. Cardiorespiratory diseases [1,23]				
PATHOLOGY	Routes of application			Observations
	MAH Min C- max C (*µg*/mL) Vol. Blood/ O_3/O_2 Sessions	MiAH Min C- max C (*µg*/mL) Vol. Blood/ O_3/O_2 Sessions	RIO_3 Min C- max C (*µg*/mL) Vol. O_3/O_2 Sessions	
Feline Asthma, Herpes virus Calicivirus	20-30 1 mL/kg 4-8	25-35 1.5 mL/10 kg 4-8	20-30 3 mL/kg 12-15	In feline patients, the RIO_3 route is preferable to MAH
Pulmonary fibrosis	20-30 4-8		20-30 12-15	
Cardiorespiratory insufficiency	15-35 1 ml/kg 4-8	15-35 1.5 ml/10 kg 4-8	15-35 3 ml/kg 12-15	

Note. MAH: Major Autohemotherapy. MiAH: Minor Autohemotherapy. RIO_3: Rectal insufflation of ozone. Min C: Minimum concentration. Max C: Maximum concentration. O_3/O_2: Medical Ozone.

Table 2.11. Genitourinary diseases [1]

PATHOLOGY	Routes of application					
				Intravaginal / Vesico-Urethral		
	MAH Min C- max C (μg/mL) Vol. Blood/ O_3/O_2 Sessions	MiAH Min C- max C (μg/mL) Vol. Blood/ O_3/O_2 Sessions	RIO_3 Min C- max C (μg/mL) Vol. O_3/O_2 Sessions	Bi-distilled water Min C- max C (μg/mL) Bubbling time Sessions	O_3/O_2 Min C- max C (μg/mL) Vol. Sessions	Oil PV Administration
Prostatitis, BPH Cysts for and intra-prostatic Orchitis	15-35 1-1.5 ml/kg 4-8	10-30 1.5 ml/10 kg 4-8	15-35 3 ml/kg 12-15			
Vaginitis Pyometra Endometritis	15-35 1-1.5 ml/kg 4-8	10-30 1.5 ml/10 kg 4-8	15-35 3 ml/kg 12-15	15-60 10 min Once-daily until improvement	15-25 According to size Once-daily until improvement	800-600-400 Twice- daily until improvement

Note. MAH: Major Autohemotherapy. MiAH: Minor Autohemotherapy. RIO_3: Rectal insufflation of ozone. Min C: Minimum concentration. Max C: Maximum concentration. O_3/O_2: Medical Ozone. PV: Peroxide Value.

Table 2.12. Dermatology [1,10,14,16,24-26]							
PATHOLOGY	Routes of application						
	MAH Min C- max C (μg/mL) Vol. Blood/ O_3/O_2 Sessions	MiAH Min C- max C (μg/mL) Vol. Blood/ O_3/O_2 Sessions	RIO_3 Min C- max C (μg/mL) Vol. O_3/O_2 Sessions	Intralesional Min C- max C (μg/mL) Vol. O_3/O_2 Sessions	Bi-distilled water Min C- max C (μg/mL) Bubbling time Sessions	Bag C. min - C. max (μg/mL) Time Sessions	Oil PV Frequency
Dermatitis: bacterial, fungal, viral and parasitic	15-30 1-1.5 mL/kg 8-10		15-30 3 mL/kg 12-15		15-62 10 min Once-daily (until improvement)	20-65 10 min 10-12	1200-800-600 Twice-daily (depending of the degree of infection; decreased until healing)
Immune-mediated dermatitis Vasculitis Hyperkeratosis Anal fistulas	20-35 1–1.5 mL/kg 8-10	30-35 1,5/10kg 4-8	20-35 3 mL/kg 12-15	8-15 0.1 mL/point 4-8	15-60 10 min Once-daily (until improvement)	50-65 10 min 10-12	1200-800-600 Twice-daily (until healing)
Bacterial and fungal otitis					15-60 500 ml 10 min 8-16		Bacteria: 800-600 Fungus: 800-600 2/day/15 days
Otohematomas				20-30 Vol. extracted 1-3			
Wound healing				8-15 0.3- 1 mL/ point (until improvement)	15-60 10 min Once-daily (until improvement)		1200-800 (until granulation) 600-400 (until healing)

Note. MAH: Major Autohemotherapy. MiAH: Minor Autohemotherapy. RIO_3: Rectal insufflation of ozone. Min C: Minimum concentration. Max C: Maximum concentration. O_3/O_2: Medical Ozone. PV: Peroxide Value.

Table 2.13. Neurology [1,14,16,27-29]

PATHOLOGY	Routes of application							
	MAH Min C- max C (μg/mL) Vol. Blood/ O_3/O_2 Sessions	MiAH Min C- max C (μg/mL) Vol. Blood/ O_3/O_2 Sessions	RIO_3 Min C- max C (μg/mL) Vol. O_3/O_2 Sessions	Paravertebral Min C- max C (μg/mL) Vol. O_3/O_2 Sessions	Trigger point Min C- max C (μg/mL) Vol. O_3/O_2 Sessions	Subcutaneous Min C- max C (μg/mL) Vol. O_3/O_2 Sessions	Intradiscal Min C- max C (μg/mL) Vol. O_3/O_2	O_3SS IV Min C- max C (μg/mL) Vol. to infuse Bubbling Time Sessions
Herniated disc, discopondylitis	15-35 1-1.5 mL/kg 6-15		10-35 3 mL/kg 9-12	10-20 0.5-10 mL 8-10	5-20 0.5-5 mL 8-10	10-20 3-10 mL 8-10	30 1.5-2 mL/ disk	
Inmune-mediated encephalitis	15-35 1-1.5 mL/kg 6-9	15-35 1.5 mL /10kg 8-10	10-35 3 mL/kg 9-12			10-20 3-10 mL 8-10		0.4-2 10-30 mL/ kg 10 min 20
Ischemic vascular alterations	15-35 1-1.5 mL/kg 6-9		10-35 3 mL/kg 9-12					0.4-2 10-30 mL/ kg 10 min 20
Cognitive dysfunction	15-35 1-1.5 mL/kg 6-9	15-35 1.5 mL /10kg 8-10	10-35 3 mL/kg 9-12					0.4-2 10-30 mL/ kg 10 min 20
Degenerative myelopathy	15-35 1-1.5 mL/kg 6-9		10-35 3 mL/kg 9-12	10-20 0.5-10 mL 8-10	5-20 0.5-5 mL 8-10	10-20 3-10 mL 8-10		0.4-2 10-30 mL/ kg 10 min 20
Neuromuscular disorders	15-35 1-1.5 mL/kg 6-15	15-35 1.5ml/10kg 8-10	10-35 3 mL/kg 9-12		5-20 0.5-5 mL 8-10	10-20 3-10 mL 8-10		

Note. MAH: Major Autohemotherapy. MiAH: Minor Autohemotherapy. RIO_3: Rectal insufflation of ozone. Min C: Minimum concentration. Max C: Maximum concentration. O_3/O_2: Medical Ozone. O_3SS: Ozonized Saline Solution.

Table 2.14. Traumatology [1,10,14,16,30-32]							
PATHOLOGY	Routes of application						
	MAH Min C-max C (μg/mL) Vol. Blood/ O_3/O_2 Sessions	MiAH Min C-max C (μg/mL) Vol. Blood/ O_3/O_2 Sessions	RIO_3 Min C-max C (μg/mL) Vol. O_3/O_2 Sessions	Intra-articular Min C-max C (μg/mL) Vol. O_3/O_2 Sessions	Trigger point Min C- max C (μg/mL) Vol. O_3/O_2 Sessions	Subcutaneous Min C- max C (μg/mL) Vol. O_3/O_2 Sessions	Intralesional Min C- max C Vol. O_3/O_2 Sessions
Osteoarthrosis	10-35 1-1.5 mL/ kg 6-15		10-35 3 mL/kg 12-15	8-15 0.5-5 mL 3-6	8-15 0.5-5 mL 3-6	10-20 1-10mL 6-9	
Septic arthritis	10-35 1-1.5 mL/ kg 6-9	25-35 1.5 mL/10kg 4-8	10-35 3 mL/kg 9-12	8-20 0.5-5 mL 3-6	15-8 0.5-5 mL 4-8	10-20 1-10mL 6-9	
Tendinopathies					8-15 0.5-5 mL Until improvement	8-15 0.5-5 mL Until improvement	
Osteomyelitis	10-35 1-1.5 mL/ kg 6-15	10-35 1.5 mL/10kg 4-8	10-35 3 mL/kg 12-15			10-20 1-10mL 6-9	
Hygroma							15-25 Vol.Extracted 3-6

Note. MAH: Major Autohemotherapy. MiAH: Minor Autohemotherapy. RIO_3: Rectal insufflation of ozone. Min C: Minimum concentration. Max C: Maximum concentration. O_3/O_2: Medical Ozone.

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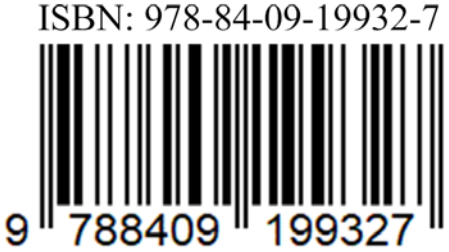

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