

Original paper

Non-recommended routes of application in ozone therapy, a critical review

Dr. Gregorio Martínez Sánchez. PhD

Pharm. Dr., Ph.D., Free Lancet, Ancona, Italy

Dr. Adriana Schwartz. MD

MD, Spain. Gynecology and Obstetrics. Clínica Fiorela, Madrid

Dr. Roberto Quintero Mariño

Lawyer. Dr. in Political Sciences and International Relations

Dr. Fadi Sabbah

D.DS., Associate, Texas Institute for Advanced Dental Studies (TIADS)

By mistake we had published a draft of this paper, not the final version approved by the peer reviewer and by the editor. So the approved one appears now. We apologize for inconvenience caused. Issue date of the journal: May 27, 2018. Date of change of article: May 28, 2018

The editorial committee, in full agreement with the authors of the paper, has decided to make a modification in the title, changing "abusive" for "non-recommended" which actually reflects much better the intention of the authors. Our apologies for any inconvenience caused. Issue date of the journal: May 27, 2018. Date of modification of the title: June, 17, 2018.

Keywords

ozone therapy
direct intravenous
administration,
Hyperbaric ozone
application
High Dose Ozone
therapy,
Intraperitoneal
Ozone,
multi pass ozone
application.

Abstract

Ozone used within the determined therapeutic windows is absolutely safe and more effective than golden standard medications in numerous pathologies. However, there are practitioners who for the interest of increasing cost effectiveness and increasing speed in treatments, pretend to cure chronic diseases applying alternative administration ways, using high ozone doses, which are neither standardized, nor supported by pre-clinical / clinical data, nor evaluated toxicologically. In this review an analysis of the bibliographical data concerning efficacy and safety of this methods was analyzed. The search included a review of scientific articles and experimental results papers in the MEDLINE and Zotero ISCO3 Ozone Database, between the years 1980-2018. Descriptors: ozone therapy, toxicology, side effects were used. The primary (original articles) sources of information were located. The most representative emerging therapies: direct intra venous application (DIV), Robins method of direct intravenous ozone therapySM (RMDIVSM), Hyperbaric (HBO3) multi passes method, high dose ozone therapy (HDO), and Intraperitoneal Ozone (IPO3) are lack of clinical evidences and represent a serious risk for the human health. A rational clinical protocol fits to the good clinical practice and supported by and ethic committee is needed to prof it efficacy prior to be introduced in clinical practice. The emergent are not recommend for its clinical application (except patients enrolled in a clinical trial) until new scientific evidence of the benefit/risk ratio is provided..

Suggestion on how to quote this paper:

Martínez Sánchez, Gregorio et.al.. (2018). Non-recommended routes of application in ozone therapy, a critical review *Revista Española de Ozonoterapia*. Vol. 8, nº 1, pp 19-35

Autor para correspondencia: Dr. Gregorio Martínez Sánchez. MD, Madrid España. E-mail: <mailto:gregorcuba@yahoo.it>

Introduction

Ozone used within the determined therapeutic windows is absolutely safe and more effective than golden standard medications in numerous pathologies. However, there are practitioners who for the interest of increasing cost effectiveness and increasing speed in treatments, pretend to cure chronic diseases applying alternative administration ways, using high ozone doses, which are neither standardized, nor supported by pre-clinical / clinical data, nor evaluated toxicologically.¹

The Madrid Declaration on Ozone Therapy,² is an international consensus document, not legally binding instruments. The Declaration draws its authority from the degree to which it has been codified in, or influenced, national or regional legislation and regulations. This document emphasizes the following stamens:

“It is absolutely necessary to work with specific objectives and in a unified way to assure a practice with great precision and safety.

“There is variance that the medical community wishes to standardize, and that progress already has been made, that it should be taken into account; it is necessary to continue with the development of medical definitions of procedures and protocols determining the best applications where it is necessary, as well as a code of good practice, in order to overcome more efficiently the possibility of malpractice.”

In addition, this document is in line with two general principles of the medicine:

- 1) *Primum non nocere*: Before anything else, not to do any harm.
- 2) The ethical principles in medicine.³ These general principles should be taken into consideration in the clinical practice of the ozone therapy. As a consequence, any recommended application of ozone should be documented by pre-clinical and clinical trials.

Hence, the aim of this manuscript is to emphasize the rules for a secure practice of ozone therapy in the hope that ozone therapy will be used under scientific evidence base to avoid irreversible damage to the patients.

The search included a review of scientific articles and experimental results papers in the MEDLINE and Zotero ISCO3 Ozone Database, between the years 1980-2018. Descriptors: ozone therapy, toxicology, side effects were used. The primary (original articles) sources of information were located. Additionally, official documents of ISCO3 were consulted (In particular ISCO3/LEG/00/10 Not recommended application routes).⁴

Practitioners, devises and protocol, the key of a secure medical ozone therapy

Practitioner

As defined by the Madrid Declaration on Ozone Therapy² to carry out any procedure is required technically qualified personnel. Professionals should attend post-graduate formation courses which include at least the basic contents defined by ISCO3,⁵ or similar contents under the supervision of a local university or a scientific association of ozone therapy.

Practitioners should limit their practice to the field of their basic professional formation. This means: physicians will be in charge of human medical treatment or clinical trials; veterinarians should treat diseases, disorders and injuries in non-human animals; dentists should treat diseases and conditions of the oral cavity. Biochemists, pharmacists, biologists will participate in the molecular, preclinical and clinical research (in case of clinical research the direct interaction with patients will be responsibility of a physician). Nurses and technicians will act following the instruction of the corresponding doctor.

Devices / disposables

Generators used should be in line with the recommendations of ISCO3⁶ and the oxygen-ozone gas mixture must pass an antimicrobial sterile filter (< 20 µm) before injection. All materials used must be disposable and ozone resistant: glass, silicone probes, catheters and silicone tubes, connections of Kynar or stainless steel 316, and siliconized syringes.²

Protocols

Clinical protocols should be based in evidences (pre-clinical / clinical) that must be conformed to generally accepted scientific / ethic principles; hinged on thorough knowledge of the scientific literature and other relevant sources of information, adequate laboratories and, as appropriate, animal experimentation.

New routes of application of ozone therapy imply a clinical research. The design and performance of each research study involving human subjects must be clearly described and justified in a research protocol. Any clinical research in the area of ozone therapy must fit the same criteria set up for a regular drug. The proposed new routes of application must demonstrate some advantage over available treatment, such as:

- Showing superior effectiveness
- Avoiding serious side effects of an available treatment
- Improving the diagnosis of a serious disease where early diagnosis result in an improved outcome
- Decreasing a clinically significant toxicity of an available treatment
- Addressing an expected public health need

Basic pharmacology / toxicology mechanism of the ozone

Dose effect relationship

Higher ozone concentrations are not necessarily better, in the same way that it occurs with all the medicines. Ozone therapy has in general two main action mechanisms 1) Direct oxidation, with immediate effect of O₃ (e.g. inactivation of microorganism or pain mediator) 2) Surrogate effects, that involve the activation of a nuclear effectors (Nrf2 or NFkB) to induce a pharmacological response. In both, there is a therapeutic window. The knowledge of the therapeutic windows for each application route is described in the Madrid Declaration of Ozone Therapy² and it represents a summary extracted from the clinical experience of the main schools of ozone therapy, derived from the clinical practice, or from the experimental research.

The hormetic response of ozone is not a hypothesis, is a fact demonstrated clinically and experimentally.^{7,8} The interaction of ozone mediators (mainly H₂O₂ and 4-hydroxy-2,3-transnonenal (HNE) with nuclear factor induces a therapeutic response is now well established by scientific data.⁹⁻¹¹ Low doses of ozone is capable of utilizing known molecular redox master switches such as Nrf2/Keap1 or NF-κB/IκB to effect adaptive resistance. In that way, low doses stimulate cell protective pathways and nuclear transcription without altering cell viability;¹¹ on the contrary high doses can be genotoxic.¹²⁻¹⁵

The use of non-appropriate range doses of ozone in clinic may originate serious side effects, from tissue necrosis^{16,17} to a potential cancer that may develop during chronic exposition or during high doses exposition.¹⁸

Modalities of ozone administration

Ozone can be administered with great flexibility by different routes (e.g. extravascular blood oxygenation-ozonation, subcutaneous, intramuscular, intradiscal, intravaginal, intrauretral, vesical, etc.); but it **should never be used**:

- 1) **By inhalation.** Ozone oxidizes available antioxidants and reacts instantaneously with surfactant's polyunsaturated fatty acids (PUFA) present at the air – epithelial lining fluid interface to form reactive oxygen species that damages the respiratory system.¹⁹ Immediately after the exposition the first symptoms (headache, cough, dry throat, heavy chest, shortness of breath) became evident, and urgent aid measures should be taken.²⁰

There are two exceptions of administration of ozone or derivatives by inhalation.

- a) Small volume of ozone gas (O₂/O₃) at low concentration (6 µg/mL), only in apnea and conducted by a well-trained medical doctor, to treat sinus diseases.^{21,22}
- b) Volatile organic compounds generated by ozone. There are different methods, such as the bubbling of essential oils (e.g. essential oil of pine, thymus, eucalyptus, tea tree) or fixed oils (sunflower oil or olive oil), which generate derivatives that can potentially be used from the therapeutic point of view.^{23,24} In this case, the inhaled vapor is not O₂/O₃, but terpenes or other organic compounds.

2) **Directly injected** (intra-arterial injection or intra venous injection) as a gas mixture in the circulatory vessels because of the risk of provoking oxygen embolism, given the fact that the gas mixture never contains less than 95% oxygen.¹⁹ 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. The complications of stroke ranges 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 to death. It is important to note that at least five patients died as a result of a gas embolism after administration of ozone by direct intravenous injection.²⁵⁻²⁸ 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.²

Additionally, ozone is a very unstable gas, as the minutes pass, the concentration is lost. If it starts with 20 µg/mL, at the end of the 5 min, the concentration will end up in (10-14) µg/mL or even less. Taking into account the current scientific knowledge, the use of DIV (direct intra venous application) involves an unnecessary risk, which should not be done outside of a clinical trial, nor in a center with no capacity to solve potential complications. On the other hand, scientific societies should officially make clear their position against it (outside of its registered studies), to safeguard the image of ozone therapy in the event of accidents.

It would be enough that only one iatrogenic result produced for using DIV would originate a chaos in the ozone therapy professional community. So, it is of utmost importance to avoid in daily medical practice interventions that put the patient life in risk, especially if the procedure has not been scientifically proved. Any bad result would be detrimental for the ozone therapy.

A German paper describe a method use O₂ administration by i.v. According to this method they use a flow of 1 mL / min to about 3 mL / min in the vein. The starting dose is (10-20) mL and increases in the next 3 weeks by 10 mL each week. According to the authors, apart from the general improvement in oxygen availability, i.v. oxygen therapy causes eosinophilia, which can be valued as an increase in undetermined cellular immunological resistance. Furthermore, rheological qualities of the blood as well as diuresis are improved, the release of oxygen into the tissue is increased, and the blood pH is normalized. In addition, author suggest that compared with hyperbaric oxygen therapy, i.v. oxygen therapy seems to have less side effects. Application is less complicated, less expensive but probably of higher efficacy.²⁹ However, result was limited to the analysis of 20 patients and can't be reproduced by any other clinical trials. Because the potential risk of embolic event, potential benefit of this method should be verified in pre-clinical studies. This study was done only using O₂ nor O₃, promoter of i.v. O₃ use this paper to extrapolate the results and confuse the people. Is well know that the biological effects of O₂ or O₃ are completely different.

Emerging therapies in ozone therapy

With the term “emerging therapies” in ozone therapy, can be summarized a series of methods non-supported by scientific evidences which do not take into consideration any of the issues concerning the secure practice of ozone therapy. Nor even a “case report” can be found nor a pre-clinical data that support those aberrant variants. The only channels for the diffusion of the “success” of the therapy are Facebook, YouTube, personal web sites, testimonials from patients, etc.

Therefore, in most cases, they constitute serious cases of human experimentation that skip the elementary medical ethical standards.³ In most cases, the methods are advertised as "curative" or "resolving" chronic or terminal diseases (e.g. cancer or HIV). However, the list of diseases that “cure” these methods is endless. In contrast to appear as more effective methods than the traditional ones, there are reports of deaths and severe adverse events among patients who have been treated with emerging therapies. There is a permanent report in social media about serious side effect after those practice (e.g. in Table 1 and Fig. 1).

The most representative of the emerging therapies are: direct intra venous application (DIV), Robins method of direct intravenous ozone therapySM (RMDIVSM), Hyperbaric (HBO3) multi passes method, (3-10, or more passes) with 200 mL blood + 200 mL O₃ at 70 µg/mL at 1 bar pressure, and (2000- 25 000) IU heparin per pass, high dose ozone therapy (HDO), and Intraperitoneal Ozone (IPO3).

Direct intra venous application (DIV)

DIV has not any scientific evidence. A simulation of the effect of DIV in a preclinical study using mouse and rabbit models, get 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 lead, so its use is not justified in humans"³⁰ 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 15 papers.³¹⁻⁴⁵ However, the reading of the "materials and methods" section, evidences that the authors have called "intravenous ozone" to the classic major autohemotherapy (MAHT) or to the administration of ozonated saline solution (O3SS). In any case they do not use the ozone gas directly into the vein. In clinic, the only report claims the benefit of the use of DIV appeared in 2016⁴⁶, this article report the use of DIV and ozone by other ways in 3 patients suffering from Ebola Virus Disease (EVD) during the epidemic in Sierra Leona.

Gaseous embolism symptoms are evident in patients undergoing DIV. Despite the theoretical discussion, on whether oxygen (the main component of O₂/O₃) may be embolic gas or not, the fact is that there are reports of deaths by the application of this method.²⁵⁻²⁷

Hyperbaric (HBO3) multi passes method

Hyperbaric multi passes method uses extra-doses of ozone and extra-doses of heparin. As DIV, HBO3 has not any pre-clinical or scientific clinical evidence. According to anecdotal evidences from patients (Table 1) or practitioners the main side effects are: loss of vision, lung disturbances, colored urine (red, brown). Is well know, that the association of heparin with ozone increases the activations of platelets.^{47,48} This is the reason why the MAHT uses a citrate based anticoagulant. In a typical 10 passes (200 mL blood + 200 mL O₃ at 70 µg/mL + 2000 U of heparin) the patient receives a total dose of 140 mg of ozone (In MAHT 100 mL of O₃ at 40 µg/mL, the patients receive 4 mg) and 20 000 U of heparin. The dose of heparin is too high for a patient without coagulation disorder, and can exacerbate the main side effect of heparin: thrombocytopenia, mild pain, hematoma, hemorrhage, local irritation, erythema, increased liver aminotransferase, anaphylaxis and immune hypersensitivity reaction.⁴⁹ The observed side effects during de HBO3 multi pass are indicative of the toxicity of high ozone.

Intraperitoneal (i.p.) Ozone

It is being said that in case of mesothelioma, peritoneal carcinomatosis or peritonitis, endoperitoneal or endopleural injection of up to 2.5 L of gaseous mixture with an ozone concentration of 10-20 µg/mL can be performed.⁴ This modality is rarely used and must be performed by a specialist.¹ There is not any clinical trial documented its benefits. However, the use of this therapy in cancer is supported by a pre-clinical study.⁵⁰⁻⁵² The administration of drug in pre-clinical study by intraperitoneal (i.p.) way is usual, because the difficult approach to the animal's veins. I.p. is mainly considered an experimental way. The experimental model of cancer in rabbits, is done by the implantation of the tumor in the rabbit ear, as consequence the marginal ear vein cannot be used for drug administration. It is mean that result observed in preclinical trial probably does not depend of the administration way.

The use of i.p. in humans is not frequent, and as it involves a very invasive method needs quirophan condition. Therefore, the benefits of ozone as adjuvant in cancer should be reached using other way as the MAHT,⁵³ with low side effects, low cost and low invasiveness compared with the i.p. (Intraperitoneal hemorrhage, pain, etc.). Any intervention on cancer should be approved by the patient and consulted with an oncologist. The only fact available today about the role of ozone in cancer, is it role as adjuvant,⁵⁴ not as a cure. Promising or creating expectations of healing a patient with cancer is a serious lack of medical ethics.

Comparative analysis of various methods

A comparative table shows the differences between well-established methods of application of ozone and “the emergent methods” (Table 1). As shown the evidence, there are an important differences between the number of scientific evidence between regular ways of ozone applications and the “emergent methods”.

Table 1. Evidence basis comparison of some regular and “emergent” methods of applications of ozone therapy.

Method	Support by preclinic research	Support by clinical trials	Side effects
MAHT	Biochemical, Molecular and preclinical ⁵⁵	>11 000 treatments. ⁵⁶ > 577 patients. ⁵⁶	* Hepatitis C virus infections. ⁵⁷ Release of plasticizers when use PVC bag. ⁵⁸ Death. ⁵⁹
RIO3	Biochemical, Molecular and preclinical. ⁶⁰	>47 000 treatments. ⁵⁶ > 716 patients. ⁵⁶	** Minor side effects. ⁶⁰
O3SS	Biochemical, Molecular and pre-Clinical. ⁶¹⁻⁶³	>500 Russian doctoral theses. ⁶³ 70 children’s (appendicitis). ⁶⁴ 35 patients of brain trauma. ⁶⁵ 20 patients diabetic foot. ⁶⁶ 36 patients with icterus. ⁶⁷ 75 patients vulva disorders. ⁶⁸ 24 patients with delayed growth of the fetus. ⁶⁹ 37 patients with lymphoid failure. ⁷⁰	No side effect when Russian method is applied. ⁷¹ Phlebitis when high O ₃ concentration were used. ²³
DIV	1 Toxicological report, result in fatal death in mouse and rabbits. ³⁰	1 Clinical trials in 3 patients of EBOLA. ⁴⁶ >140 000 treatments. ^{***} > 2000 patients ^{***}	5 fatal deaths documented. ^{25,26,63} Symptoms of gas embolism: axillary bubbling sensation, cough, a feeling of retrosternal weight, dizziness, changes in vision (ambliopia), hypotensive crisis, signs of cerebral ischemia (paresis of the members), and death.
HBO3	-	0 Clinical trials. Number of treatment and patients unknown	Loss of vision, lung disturbances, colored urine (red, brown).
IPO3	3 Pharmacology in Cancer. ⁵⁰⁻⁵²	0 Clinical trials. Number of treatment and patients unknown	Intraperitoneal hemorrhage, pain.

Legend: MAHT, Major autohemotherapy; RIO3, Rectal insufflation; O3SS, Ozonized saline solution; DIV, Direct intra venous application; HBO3, Hyperbaric multi passes; IPO3, intraperitoneal Ozone.

*Described MAHT side effects are very discrete taste of metal at start of reinfusion, tiredness on next day, need to adjust the antidiabetic medication to lower doses, need to adjust the anti-hyperthyroidism medication to lower doses, need to adjust the Digitalis heart medication to lower doses, need to adjust anti-hypertensive medication.⁵⁵ Side effects describe in Table 1 for MAHT were consequence of *mala praxis*.

**When ozone was administered by rectal insufflation, cases of bloating and constipation were reported.^{54,72} Is also reported slight irritation and transitory flatulence⁷³ and mild, short-term irritation.⁷⁴ In two case was described slight transient flatulence immediately after rectal ozone insufflation.⁵⁶

*** Number of treatment and patients was referred by Howard F. Robins, D.P.M. in a letter to ISCO3 on 2014, entitle: The Safety and Benefits of Direct Intravenous Ozone Therapy (DIV). But not supported by any bibliography or relevant clinical study.

Conclusion

The new (emergent) methods in ozone therapy should be validated according to the scientific methods and following the ethical principles promoted by the World Medical Association.³ A rational clinical protocol fits to the good clinical practice and supported by and ethic committee is needed. The current emergent methods in ozone therapy are characterized by lack of scientific support and represent a serious risk for the human health. The ISCO3 does not recommend the clinical application of any of the emergent methods (except patients enrolled in a clinical trial) until new scientific evidence of the benefit/risk ratio is provided.⁴

References

1. Bocci V, Zanardi I, Travagli V. Oxygen/ozone as a medical gas mixture. A critical evaluation of the various methods clarifies positive and negative aspects. *Med Gas Res*. Apr 28 2011;1(1):6.
2. Schwartz-Tapia A, Martínez-Sánchez G, Sabah F, et al. Madrid Declaration on Ozone Therapy. *ISCO3*. 2015:50.
3. World Medical A. World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. *JAMA*. Nov 27 2013;310(20):2191-2194.
4. ISCO3. ISCO3/LEG/00/10 Not recommended application routes. www.isco3.org 2017.
5. ISCO3. Learning Methodology Instructions and Perfection in Ozone Therapy for Medical Doctors. www.isco3.org. 2015.
6. ISCO3. Guidelines and Recommendations for Medical Professionals Planning to Acquire a Medical Ozone Generator. <http://www.isco3.org/offdocs.html>. 2014. <http://www.isco3.org/offdocs.html>.
7. Bocci VA, Zanardi I, Travagli V. Ozone acting on human blood yields a hormetic dose-response relationship. *J Transl Med*. May 17 2011;9(1):66.
8. Re L. Therapy with Oxygen-Ozone or Ozohormesis: Recent Clinical Advances. *Medici & Medici*. 2008;16:19-21.
9. Pecorelli A, Bocci V, Acquaviva A, et al. NRF2 activation is involved in ozonated human serum upregulation of HO-1 in endothelial cells. *Toxicol Appl Pharmacol*. Feb 15 2013;267(1):30-40.
10. Re L, Martinez-Sanchez G, Bordicchia M, et al. Is ozone pre-conditioning effect linked to Nrf2/EpRE activation pathway in vivo? A preliminary result. *Eur J Pharmacol*. Nov 5 2014;742:158-162.
11. Delgado-Roche L, Riera-Romo M, Mesta F, et al. Medical ozone promotes Nrf2 phosphorylation reducing oxidative stress and pro-inflammatory cytokines in multiple sclerosis patients. *Eur J Pharmacol*. Sep 15 2017;811:148-154.
12. Diaz-Llera S, González-Hernández Y, Mesa JEG, Martínez-Sánchez G, Re L. Induction of DNA primary damage in peripheral blood leukocytes and exfoliated colorectal epithelial cells in rats treated with O3/O2 mix. *International Journal of Ozone Therapy*. 2009;8:217-221.
13. Liviac D, Creus A, Marcos R. Genotoxicity analysis of two halonitromethanes, a novel group of disinfection by-products (DBPs), in human cells treated in vitro. *Environ Res*. Apr 2009;109(3):232-238.
14. Díaz-Llera S, González-Mesa JE, González-Hernández Y, Wong R. Study of O3-O2 therapy in patients by single cell gel electrophoresis assay. On submission. 2011.
15. Diaz-Llera S, Podlutzky A, Osterholm AM, Hou SM, Lambert B. Hydrogen peroxide induced mutations at the HPRT locus in primary human T-lymphocytes. *Mutat Res*. Aug 21 2000;469(1):51-61.
16. Vanni D, Galzio R, Kazakova A, et al. Intraforaminal ozone therapy and particular side effects: preliminary results and early warning. *Acta Neurochir (Wien)*. Mar 2016;158(3):491-496.
17. Di Paolo N, Bocci V, Cappelletti F, Petrini G, Gaggiotti E. Necrotizing fasciitis successfully treated with extracorporeal blood oxygenation and ozonization (EBOO). *Int J Artif Organs*. Dec 2002;25(12):1194-1198.
18. Foksinski M, Bialkowski K, Skiba M, Ponikowska I, Szmurlo W, Olinski R. Evaluation of 8-oxodeoxyguanosine, typical oxidative DNA damage, in lymphocytes of ozone-treated arteriosclerotic patients. *Mutat Res*. Jan 2 1999;438(1):23-27.
19. Bocci V. Is it true that ozone is always toxic? The end of a dogma. *Toxicol Appl Pharmacol*. Nov 1 2006;216(3):493-504.

20. ISCO3. FIRST AIDS IN OZONE THERAPY. (Inhalatory exposition and accidental over dose). 2015:16.
21. Petrov GM, Kudriavtsev BP, Akulich, II. [The efficacy of using ozone preparations in the combined treatment of paranasal sinusitis]. *Voen Med Zh.* Dec 1996;317(12):26-28, 80.
22. Muminov AI, Khushvakova N. [Ozone therapy in patients with chronic purulent rhinosinusitis]. *Vestn Otorinolaringol.* 2001(6):48-49.
23. Bocci V, ed *Ozone: A new medical drug.* ISBN-10: 9048168058. ISBN-13: 978-9048168057. Netherlands: Springer; 2010.
24. Gao M, Singh A, Macri K, et al. Antioxidant components of naturally-occurring oils exhibit marked anti-inflammatory activity in epithelial cells of the human upper respiratory system. *Respir Res.* Jul 13 2011;12:92.
25. Zambello A, Bianchi M, Bruno F. Sicurezza in ozonoterapia. *Rev Ital Ossigeno Ozono Ter.* 2004;1:25-30.
26. Bocci V. Biological and clinical effects of ozone. Has ozone therapy a future in medicine? *Br J Biomed Sci.* 1999;56(4):270-279.
27. Di Paolo N, Bocci V, Gaggiotti E. Ozone therapy. *Int J Artif Organs.* Mar 2004;27(3):168-175.
28. FOXTV5. 2 accused of death during alternative medicine procedure. <http://www.fox5vegas.com/story/28995673/2-accused-of-death-during-alternative-med-procedure>. Posted: May 06, 2015 9:58 PM 2015. Accessed 4/9, 2017.
29. Schmidt H. Oxyvenierungstherapie nach Regelsberger eine Interpretation von Praxisergebnissen aus biochemischer und physiologischer Sicht. *Forsch Komplementärmed Klass Naturheilkd.* 2002;9:7-18.
30. Janet CC, Colom Loo Y, Cruz Piñón M, et al. Preclinical Toxicity Study of Direct Intravenous Ozone Therapy. *Rev Esp Ozonoterapia.* 2015;5(2 Suplement 1):47-48.
31. Takatori T, Shimasaki H. Metabolism of labeled fatty ozonides administered intravenously in rats. *Hokkaido Igaku Zasshi.* May 1977;52(3):261-264.
32. Belianin, II, Nikolaeva GM, Martynova LP. [Action of dissolved ozone on mycobacterium tuberculosis and alveolar macrophages in experimental tuberculosis]. *Probl Tuberk.* 1997(1):56-59.
33. Belianin, II, Shmelev EI. [Blood ozonation in the treatment of patients with progressive pulmonary tuberculosis concurrent with diabetes mellitus]. *Probl Tuberk.* 1998(1):30-33.
34. Belianin, II, Abdullaev R. [Use of soluble ozone in combined treatment of pulmonary tuberculosis: lipid peroxidation and blood antioxidative defense systems]. *Probl Tuberk.* 2000(3):41-44.
35. Belianin, II, Titiukhina MV. [Enhancing the impact of chemotherapy of tuberculosis with parenteral administration of dissolved ozone]. *Probl Tuberk.* 2000(6):57-61.
36. Belianin, II, Martynova LP, Shmelev EI. [Dissolved ozone treatment-induced change in the resistance of multi-resistant mycobacterial strain to isoniazid and rifampicin]. *Probl Tuberk.* 2002(1):46-49.
37. Tafil-Klawe M, Wozniak A, Drewa T, et al. Ozone therapy and the activity of selected lysosomal enzymes in blood serum of patients with lower limb ischaemia associated with obliterative atheromatosis. *Med Sci Monit.* Jul 2002;8(7):CR520-525.
38. Belianin, II, Shmelev EI. [Changes in drug resistance of Mycobacteria in the simultaneous use of chemotherapy and intravenous infusions of dissolved ozone]. *Probl Tuberk Bolezn Legk.* 2004(7):32-35.
39. Mandzhgaladze NR, Kharebava ER, Didia Ts G, Ardzhevanishvili MD, Gudzhabidze MV, Chigiashvili Ts N. [Influence of intravenous ozone treatment on the level of different specificity antibodies]. *Georgian Med News.* Sep 2006(138):93-95.
40. Artiukhin AA. [Conservative methods in the therapy of male sterility. Critical analysis]. *Vestn Ross Akad Med Nauk.* 2008(4):27-34.

41. Struchkov PV, Selitskii GV, Korobeinikova MV, Zubkova AV. [Use of ozonotherapy (OT) in combined treatment of patients with discirculatory encephalopathy (DEP)]. *Vopr Kurortol Fizioter Lech Fiz Kult.* Mar-Apr 2008(2):10-13.
42. Gimaev R, Drapova DP, Skvortsov D, Olezov NV. [The influence of intravenous ozone therapy on the electrophysiological properties of myocardium during combined treatment of the patients presenting with arterial hypertension]. *Vopr Kurortol Fizioter Lech Fiz Kult.* Nov-Dec 2013(6):48-51.
43. Zhakiev BS, Zhumabaeva AN, Kaliev AA, Kazbekova GA. [Application of direct electric current and intravenous ozone therapy in the complex treatment of destructive forms of acute pancreatitis in experiment]. *Eksp Klin Gastroenterol.* 2013(7):32-37.
44. Kaliev AA, Zhakiev BS, Eleulov GA, Konakbaeva NK. [Concomitant use of intravenous ozone therapy and small doses of direct current in the integrated treatment of patient with sterile pancreatonecrosis]. *Vestn Ross Akad Med Nauk.* 2014(1-2):16-18.
45. Hernandez F, Menendez S, Wong R. Decrease of blood cholesterol and stimulation of antioxidative response in cardiopathy patients treated with endovenous ozone therapy. *Free Radic Biol Med.* Jul 1995;19(1):115-119.
46. Rowen RJ, Robins H, Carew K, Kamara MM, Jalloh MI. Rapid resolution of haemorrhagic fever (EBOLA) in Sierra Leone with ozone therapy. *Afr. J. Infect. Dis.* 2016;10(1):49-54.
47. Bocci V, Valacchi G, Rossi R, et al. Studies on the biological effects of ozone: 9. Effects of ozone on human platelets. *Platelets.* 1999;10(2-3):110-116.
48. Valacchi G, Bocci V. Studies on the biological effects of ozone: 10. Release of factors from ozonated human platelets. *Mediators Inflamm.* 1999;8(4-5):205-209.
49. Bick RL, Frenkel EP. Clinical aspects of heparin-induced thrombocytopenia and thrombosis and other side effects of heparin therapy. *Clin Appl Thromb Hemost.* Oct 1999;5 Suppl 1:S7-15.
50. Schulz S, Haussler U, Mandic R, et al. Treatment with ozone/oxygen-pneumoperitoneum results in complete remission of rabbit squamous cell carcinomas. *Int J Cancer.* May 15 2008;122(10):2360-2367.
51. Häußler U. Effect of Ozone/Oxygen-Pneumoperitoneum on Tumour Growth and Metastatic Spread of the Rabbit VX2 Head and Neck Cancer Model. Marburg, Germany, Philipps Univ; 2009.
52. Rossmann A, Mandic R, Heinis J, et al. Intraperitoneal oxidative stress in rabbits with papillomavirus-associated head and neck cancer induces tumoricidal immune response that is adoptively transferable. *Clin Cancer Res.* Aug 15 2014;20(16):4289-4301.
53. Velez A M. Terapia adyuvante con ozono en paciente con cáncer de páncreas metastásico. *Revista Española de Ozonoterapia.* 2015 2015;5(1):21 - 31.
54. Clavo B, Santana-Rodriguez N, Llontop P, et al. Ozone Therapy in the Management of Persistent Radiation-Induced Rectal Bleeding in Prostate Cancer Patients. *Evid Based Complement Alternat Med.* 2015;2015:480369.
55. ISCO3. Major Autohemotherapy. www.isco3.org2016:13.
56. Renate VH, Sonia LFO, Fahmy Z. Ozone in Medicine: Clinical Evaluation and Evidence Classification of the Systemic Ozone Applications, Major Autohemotherapy and Rectal Insufflation, According to the Requirements for Evidence-Based Medicine. *Ozone: Science & Engineering.* 2016:25.
57. Faustini A, Capobianchi MR, Martinelli M, Abbate I, Cappiello G, Perucci CA. A cluster of hepatitis C virus infections associated with ozone-enriched transfusion of autologous blood in Rome, Italy. *Infect Control Hosp Epidemiol.* Sep 2005;26(9):762-767.
58. Ciborowski M, Lipska A, Godzien J, et al. Combination of LC-MS- and GC-MS-based metabolomics to study the effect of ozonated autohemotherapy on human blood. *J Proteome Res.* Dec 07 2012;11(12):6231-6241.

59. Marchetti D, La Monaca G. An unexpected death during oxygen-ozone therapy. *Am J Forensic Med Pathol.* Jun 2000;21(2):144-147.
60. ISCO3. Rectal Insufflation www.isco3.org. Vol ISCO3 MET 00 232017:9.
61. Korolev BA, Boiarinov GA, Monakhov AN, Shvets NA, Peretiagin SP. [Metabolism and ultrastructure of the myocardium in protection of the heart against ischemia using an ozonized cardioplegic solution]. *Grudn Khir.* Nov-Dec 1983(6):27-31.
62. Qu DD, Peng FJ, Liu L, Yang SL, Guo YB. [Effect of ozonized saline on signaling passway of Keap1-Nrf2-ARE in rat hepatocytes]. *Zhonghua Gan Zang Bing Za Zhi.* May 2011;19(5):367-371.
63. Schwartz A. Solución Salina Ozonizada (SSO3): Fundamentos Científicos. *Revista Española de Ozonoterapia.* 2016;6(1):121-129.
64. Azov NA, Azova EA, Chekalova SA. Ozone, EHF-therapy in complex treatment of children. *Rev. Española de Ozono Terapia.* 2016;6(2 Suppl. 1):5.
65. Barkalov SV, Y.V.Daniel. Evaluating the effectiveness of the use of ozone therapy in the treatment of combat trauma with brain damage. *Rev. Española de Ozono Terapia.* 2016;6(2 Suppl. 1):6.
66. Belyaev A, Rodin A, Zakhvatov A. Effects of ozone therapy on the course of diabetic wound process. *Rev. Española de Ozono Terapia.* 2016;6(2):11.
67. Belyaev AN, Kozlov SA, Belyaev SA, Kostin SV. Influence of ozone therapy on postoperative hemostasis system dynamics in patients with obstructive jaundice. *Rev. Española de Ozono Terapia.* 2016;6(2 Suppl. 1):13.
68. E.L. B, Malyshkina AI. The use of ozone therapy in the treatment of leukoplakia vulva in women. *Rev. Española de Ozono Terapia.* 2016;6(2 Suppl. 1):17.
69. Boyko EL, Mileeva PL. The use of medical ozone in complex treatment women with fetal growth retardation. *Rev. Española de Ozono Terapia.* 2016;6(2 Suppl. 1):18.
70. Knyazev VN, Fattyakhudinova ES. Phlebotropic adjuvant regional ozone therapy of lower limb lymphovenous failure. *Rev. Española de Ozono Terapia.* 2016;6(2 Suppl. 1):34.
71. Schwartz A. *Manual de Ozonoterapia Clínica.* Madrid, España: Medizeus Soluciones Medicas S.L.; 2017.
72. Clavo B, Ceballos D, Gutierrez D, et al. Long-term control of refractory hemorrhagic radiation proctitis with ozone therapy. *J Pain Symptom Manage.* Jul 2013;46(1):106-112.
73. Hidalgo-Tallon J, Menendez-Cepero S, Vilchez JS, Rodriguez-Lopez CM, Calandre EP. Ozone therapy as add-on treatment in fibromyalgia management by rectal insufflation: an open-label pilot study. *J Altern Complement Med.* Mar 2013;19(3):238-242.
74. Carpendale MT, Freeberg J, Griffiss JM. Does ozone alleviate AIDS diarrhea? *J Clin Gastroenterol.* Sep 1993;17(2):142-145.