

artículo original

Oxygen-ozone therapy is at a cross-road

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Abstract

Oxygen-ozone therapy is slowly coming into age and it is being used in many Countries. However, while the treatment of hernial disk by simply injecting a little volume of gaseous O₂-O₃ inside the nucleus pulposus is a great success, the use of the so-called major ozonated-autohemotherapy (M-O₃-AHT), consisting of 200 mL of autologous blood treated with gaseous O₂-O₃ mixture and rapidly infused into the donor, lags behind due to a number of reasons among which lack of standardization, need of numerous treatment, lack of knowledge, skepticism and even denial. Skepticism, which is fundamental to the scientific method, can be overcome only by showing the validity of M-O₃AHT in controlled and randomized clinical trials on specific diseases, as correctly requested by the official Medicine. This paper aims to emphasize all the problems plaguing this therapeutic modality and to suggest remedies to enhance its progress and development.

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INTRODUCTION

During the last two decades the considerable progress in understanding the biological mechanism of action of ozone has not been paralleled by a congruent progress in clinical results with the exception of a breakthrough in Orthopedics. Herniated disc (frequently L4-L5) is frequent and painful and can be often cured by the administration of a single volume of oxygen (O₂)-ozone (O₃) mixture (O₃ about 150-180 µg in 4-6 mL of O₂) injected into the affected nucleus pulposus [1]. By spreading the word, many thousands of patients undergo this treatment every year in all Continents. On the other hand, by using the classical and safe major ozonated autohemotherapy (M-O₃-AHT), no real progress has been made for the treatment of vascular diseases (stroke, chronic heart disease, CHD) and peripheral obstructive arterial disease (POAD) [2]. Although they represent the first cause of death and in spite of a possibly valid ozone therapy, these diseases remain in the realm of orthodox medicine with meager results for several reasons. The first problem is that randomized and well controlled clinical studies are still lacking because ozone therapy has no sponsors and is actually either objected or unknown to clinical scientists. A second problem is that the M-O₃-AHT must be performed for several months thus being expensive and cumbersome. Practitioners can do that but, by being carried out with different, often unsuitable procedures and schedules, results are not scientifically acceptable. The negligence and disinterest of most Health Authorities and the overpower of pharmaceuticals, which can profusely support clinical researches as well as clinicians, represent one of the main obstacle for the progress of ozone therapy. Moreover, a crucial question is if ozone therapy intended as a real scientific endeavor is correctly pursued or, by remaining in practitioners' hands, is and will remain an obscure complementary approach. Therefore, there is an urgent need to improve the efficacy of M-O₃-AHT. This is not a naïve question and indeed regarding M-O₃-AHT, which theoretically has the power to really improve some human diseases [2], an objective evaluation of the state of the art is necessary and is the aim of this paper. The following aspects will be examined:

- 1) Who practices the M-O₃-AHT and in what Countries?
- 2) What kind of ozone generators are in use? Are all of these generators safe and precise?
- 3) Are the present guidelines correct, effective and safe? Can they be improved?
- 4) A few of the present methodologies are of doubtful value and may be dangerous or ineffective.
- 5) The constant need to teach the most correct procedure to the ozonetherapists.
- 6) Unless randomized and well controlled clinical trials in vascular diseases and age-related macular degeneration (ARMD), dry form, are performed, ozone therapy will remain in limbo.

1) Who practices the M-O₃-AHT and in what Countries?

With rare exception, ozone therapy is in private practitioners' hands. University professor and distinguished clinical scientists either do not know the existence of ozone therapy or they completely disregard it. One of us (V.B.), during several University's congresses, has discussed ozone therapy and had conversation with prof. H. Sies, prof. M.U. Dianzani, Dr. H. Forman, who invariably thought that ozone therapy was an empirical and useless approach. Prof. W. Pryor, as other chemists, have been dead against this medical practice because for them ozone is "always toxic and should never be used in medicine". It is regretful that chemists do not understand the subtlety of biology where a calibrated but potentially dangerous stress is able to provoke a beneficial response. The only one who did not scorn the approach has been prof. L. Packer, a biochemist expert in antioxidant compounds, who actually wrote a letter saying that he firmly believed in the correctness of the concept.

In some Countries as Canada and England, some practitioners of naturopathy and technical engineers practice ozone therapy but hopefully avoid to perform M-O3-AHT. In theory only physicians should carry out this procedure but it remains uncertain their knowledge of the problem. Often they practice without having followed an appropriate course and simply follow the instructions, not necessarily correct or adequate for different diseases, of the ozone generator's producer. Another disturbing fact is that the physician, aiming to do the best for the patient, most often associates ozone therapy with phytotherapy, or homeopathy, or even magnetotherapy thus making any therapeutic results incomprehensible and useless for evaluation.

Table I shows that M-O3-AHT is performed in many Countries and, while in some there are two or three societies of ozone therapy most often in conflict of interest, in others ozone therapy is still in the initial phase. Germany has the higher number of private practitioners but to our knowledge M-O3-AHT is not practiced in University hospitals. The same happens in Italy and practically in all Countries with the exception of the Russian Federation, probably Ukraine, Cuba, India and China where the Health Authorities allow ozone therapy performed in public hospitals. This is so because they have to satisfy too many patients and whenever possible, ozone therapy helps to keep medical expenses very low. This behavior is laudable although, as it will be discussed in 4), the quality of the service is not always valid.

2) What kind of ozone generators are in use? Are all of these generators safe and precise?

This is one of the crucial points because a good ozone therapist performs a bad treatment by using a defective generator. Until 1990 they were unreliable but today a valid generator is built with the best ozone-resistant materials such as AISI316L stainless steel, pure titanium grade 2, Pyrex® glass, Teflon®, Viton®, avoiding any other material such as Al that could be released due to O₃ oxidation. Moreover, a precise photometer reading O₃ concentration at 253.7 nm, within the Hartley band, is essential for checking on a digital display the O₃ concentration in the gas mixture (medical O₂ ≥ 95% + O₃ ≤ 5%) flowing into the syringe during withdrawal. Owing to the decay of the Hg vapour lamp, the photometer must be checked every 6-12 months depending on its utilization. Another photometer measures O₃ concentration at 600 nm (Chappuis band) but it is 2500-fold less sensitive. The generator must be checked once a year and, if necessary, adjusted on the basis of O₃ concentration measured by the iodometric method considered the gold standard. When O₃ reacts with the KI solution, iodine is generated and the solution acquires an amber colour which, upon reduction with a titrated solution of Na₂S₂O₃ and a starch indicator allows the determination of the O₃ concentration with a reproducibility of about 2% with the measured O₃ concentration. Owing to O₃ rapid decay, what is very important is firstly the immediate use of the gas mixture and not so much small changes (± 1%) of concentration. Secondly, polypropylene, silicon-coated syringes must be used only once. Most of the medical generators can deliver the O₂-O₃ mixture from 1 to up to 110 µg/mL, at a slightly higher atmospheric pressure and these are safe. However, there is at least one German generator working with the so-called "hyperbaric method". This instrument delivers from 0 to 70 µg/mL of O₃ with a gas constant flow of 0.9 L/min. The term "hyperbaric" appears misleading because the O₂-O₃ gas mixture has a maximal pressure of 1.05 bar, hence as all other generators. The only difference is that the infusion of the ozonated blood into the patient occurs at a more elevated pressure (~ 200 mbar) similar to the arterial pressure of the patient. That is why this method is denominated "hyperbaric". It is important to emphasize that the O₃ concentration does not increase as if O₃ was under hyperbaric pressure and therefore the advantage is only a faster infusion that obviously must be well regulated to avoid embolism. How relevant is this faster infusion remains to be ascertained.

In reality there are too many different ozone generators, some of which do not have the photometer or do not have the CE symbol. Some generators are less expensive but of poor quality and, when properly checked, do not deliver the presumed O₃ concentration. This is a serious pitfall that makes impossible

to accept reliable data from practitioners. Almost needless to say that any type of generator must be provided with an ozone destructor, i.e. an effective catalyzer system to insure the conversion of excess or unused O₃ back into O₂. O₃ must never pollutes the air and must not be inhaled at anytime. Finally, the gas mixture to mix with blood must be always filtered (0.2 µm pore diameter) to prevent bacterial contamination from medical O₂, which is not sterile.

3) Are the present guidelines correct, effective and safe? Can they be improved?

Several guidelines have been presented at different times and will be compared [3-5]. The older one and still partly used in Germany is the most conservative and regarding M-O₃-AHT has been devised by Ozonosan-Hansler and based on the use of 250 mL glass bottles with a microporous device (the so-called Mikro-Perl system), which is used for insufflating the gas mixture directly into the 50 or at most 100 mL of blood anticoagulated with sodium citrate. The sudden gas inflow causes an intensive foaming that almost fills the bottle. After a few seconds mixing, the bottle is turned upside-down and the ozonated blood infused into the donor, via the same tubing used for blood withdrawal.

The general rule is to avoid the gas bubbling into blood but, in this case, it has been claimed that O₃ instantaneously mixes with blood. The examination of the ozonated blood showed that the PaO₂ have barely reached 95 mmHg and hemolysis was 3-5%. This is not surprising because the intensive foaming causes a sudden modification and breakdown of the erythrocyte membrane. Several years ago, a German ozonetherapist told us that the rapid infusion insured a further activation of the circulating blood like the spreading of wildfire under a gale. This sounds romantic, but it is not supported by experimental data. Useful O₃ concentrations are said to be in the range of 5-25 µg/mL of gas per mL of blood. These concentrations are said to be very active but clinical results in different diseases have never been published in peer-reviewed journals. Moreover, no comparative evaluation between this method and the one using 500 mL glass bottles (25 mL sodium citrate 3.8% + 225 mL of blood has been performed; in the latter case, O₃ concentrations ranging from 10 up to 80 µg/mL gas per mL of blood, 0.21-1.6 mM) has been determined as the useful "therapeutic window" [6]. On the belief that the German guideline has not been influenced by the homeopathic concept, our experimental work has aimed to clarify the biochemical modifications of human blood and to understand how ozone acts. In line with a previous evaluation [7], it was found that O₃, ten-fold more soluble than O₂, at normal atmospheric pressure dissolves very rapidly in the water of plasma and immediately reacts, partly with hydrosoluble antioxidant of plasma (ascorbic acid, uric acid, GSH, free cysteine) and with unsaturated fatty acids transported by albumin [6].

After 5 min of gentle mixing the blood with the gas mixture, O₃ is totally exhausted and acts as a pro-drug. The multiple reactions are now well known: Aa is partially oxidized to dehydroascorbate, uric acid to allantoin and GSH to GSSG. The generated H₂O₂ as a ROS creates a very transitory gradient between plasma and blood cell intracellular water and is essential for activating a number of biochemical reactions [6]. The generated alkenals (lipid oxidation products, LOPs), mainly 4-HNE, form an adduct either with Cys34 or with nine nucleophilic groups (Lys and His) available in albumin [8,9] which is the most important antioxidant preventing any damage on cell membranes [10]. With this procedure, the PaO₂ raises up to 400 mmHg and hemolysis remains negligible if the total antioxidant capacity is in the range of 1.4-1.8 mM [11]. Our recent metabonomic study has shown that an O₃ concentration of 10 µg/mL per mL of normal human blood (0.21 µmol) is practically ineffective because O₃ reactivity is totally quenched by antioxidants [12]. By increasing the O₃ concentration up to 80 µg/mL per mL of blood (1.6 mM) the antioxidant capacity is transiently reduced of about 30% and the hemolysis is still less than 1% [13]. Only by significantly increasing the O₃ concentration and exhausting the free antioxidants in plasma, the

hemolysis goes up (e.g. >5% at 800 µg/mL) [12]. Needless to say that a progressive increase of O₃ concentration (from 20 to 160 µg/mL per mL of blood, 0.42-3.2 mM) is also paralleled by an increased generation of H₂O₂, alkenals and hemolysis. These data indicate that our therapeutic window (0.42-1.6 mM) is quite safe. Actually, the slight increased hemolysis is favourable because free Hb, bound to haptoglobin, after being taken up by reticulo-endothelial system, enhances the release of heme-oxygenase-I (HO-I) which is one of the most protective enzymes [14]. Indeed, once patients have become ozone-tolerant, it would be interesting to evaluate the therapeutic activity of the highest range of O₃ concentration (1.6-3.2 mM) in ozone-resistant diseases. It is very unfortunate that old experiments unphysiologically performed with 3-fold washed human erythrocytes showed their damage when tested even with low O₃ concentrations [15,16]. These results have negatively and erroneously influenced the search of appropriate O₃ dosages and have conditioned the present guidelines.

While it is true that the recommended amount of sodium citrate is a good anticoagulant without any risk, it appears worthwhile to evaluate the relevance of Ca-heparin. In this case the physiological Ca²⁺ level (about 1.0 mM) remains available and the production of some cytokine is significantly enhanced in comparison to ozone stimulation of the same blood samples treated with citrate [17]. This is a relevant finding when an immune response needs to be improved. Furthermore, the experimental addition to blood of 5 up to 25 mM Ca²⁺ prior to ozonation, further increased pro-inflammatory cytokine synthesis. Apparently, the acute oxidative stress, by enhancing the plasmatic Ca²⁺ entrance into the cytoplasm, potentiates the synthesis of cytokines. Therefore, it is felt that, when necessary, the option to use Ca-heparin plus an extra 5 mM Ca²⁺ could be useful in immune-suppressed patients during an infection or after chemo-radiotherapy. Almost needless to say that before using heparin, one MUST evaluate if the patient uses already anticoagulants and does not present dyscoagulation. If not, it is suggested not to use more than 20 IU heparin per mL of blood and O₃ concentrations above 40 µg/mL gas per mL of blood because Ca-heparin enhances platelet aggregation [18].

After this methodological digression, the question is: how many ozonetherapists perform the correct M-O₃-AHT in 500 mL glass bottles? In Italy and likely in other Countries the real situation is worrisome because until recently some ozonetherapists were still using PVC bags idoneous only for the blood bank. PVC bags should never come in contact with O₃ because, as it was demonstrated [13] they release phthalates and plastic microparticles (2-25 µm size) into the ozonated blood. In fact, their use for M-O₃-AHT has been prohibited by the Italian Ministry of Health since 2002. As a bad example, they are still used in Turkey by some physicians but it is hoped that they will be substituted by glass or polypropylene bottles. Unfortunately, there is an anarchical trend because every ozonetherapist decides on his own irrespective of guidelines. Moreover, since Russians have publicized the use of ozonated saline in substitution of M-O₃-AHT, this approach is being used in other Countries because it is very cheap, quick to perform and as it will be discussed (Section 4) dangerous or scarcely effective.

The most recent guidelines are those published by ISCO₃ [5]. On the basis of different advices, O₃ concentration, volume of gas and schedules have been reviewed even though there was not 100% agreement. On the whole, O₃ concentrations have been slightly increased in comparison with the Hansler Ozonosan guidelines which, in some cases, use extremely low O₃ concentrations. It must be said that this line of thought is not supported by the results of controlled and extensive clinical trials but rather by ozonetherapists' practical experience. Even the present guidelines are not fool-proof and, on the basis of new data, they will need to be revised. Owing to the complexity of the biochemical events elicited by ozone firstly on blood "ex vivo" and then after the infusion by the organism, the use of a fixed dosage is a nonsense because different diseases, different disease's stages and different patients need

a personalized approach. The most sensible advice is “to start low and go slow” especially when the practitioner cannot measure the initial total antioxidant capacity of each patient [5]. If the patients at the next session reports a feeling of wellness, the O₃ dosage can be slowly and progressively increased. On the other hand, if he reports to have felt tired and sleepy, it is advisable to use a lower O₃ dose. The reason is that it is impossible to foresee how different patients will respond against the acute, although calibrated, oxidative stress imposed by the ozone action. This depends entirely by the disease stage, the age and gender and obviously every patient has a different capacity to react towards an acute homeostatic change. In some patients, this way of thinking allows to increase the O₃ dose up to maximum of the therapeutic window (20-80 µg/mL of gas per mL of blood, 0,42-1,68 mM) because it means that the patient is able to continuously upregulate his antioxidant system. Moreover, to date it is not yet known which may be the optimal, if any, O₃ concentration to elicit T-lymphocyte regulatory cells in autoimmune diseases. It has been supposed, but not yet discovered, if high concentrations, hence a plus of alkenals reaching the bone marrow, may stimulate the release of staminal cells, which would be useful to regenerate infarcted tissues.

4) A few of the present methodologies are of doubtful value and may be dangerous or ineffective.

There is no doubt that the most critical disadvantages of the M-O₃-AHT are twofold: the first is that, in most cases, the patient must go to the ozonetherapist's clinic and the second is that each session will last about 30 min. Unfortunately, so far, as it happens for most of the orthodox drugs, a valid substitute to be simply used at the patient's home has not been provided. As a consequence in the Russian Federation and at Cuba hospitals (their Health Authorities allow to use ozone therapy in public hospitals), where every day they have to treat thousands of patients, it appears impossible to perform M-O₃-AHT for lack of time and money. Coincidentally, in 1994 was found that the simple physiological saline (0.9% NaCl) could be ozonated by bubbling O₃ (70 µg/mL) in it for 10-15 min [13]. It was tested in ourselves but it procured an initial phlebitis. At that high O₃ concentration O₃ dissolves in the saline, generates H₂O₂ and partly remains in solution. There is also a transient production of some HClO which, although it rapidly decays [19,20], is very irritating. Even if this approach appeared dangerous, Russian chemists, in 1995, decided to use it in patients by ozonating the saline with ONLY 2-3 µg/mL for 20 min. Moreover, early morning they ozonize many bottles, which will be infused into patients with some delay. They claim to have good results in ALL diseases but a comparative analysis with M-O₃-AHT has never appeared in international peer-reviewed journals. In reality, Maslennikov and Gribkova have reported that a 50% decrease of symptoms can be regarded as a reliable improvement [21]. Therefore, there is no real proof that ozonated saline is therapeutically useful although it could exert a placebo effect. The lack of toxicity with 2-3 µg/mL of gaseous O₃ mixture is understandable because, at the infusion time, 250 mL of saline may contain only 0.5 mg of residual O₃ and H₂O₂ probably at a concentration as low as 0.01% . Thus both oxidants during the IV infusion will be promptly neutralized by the plasma antioxidants with a lack of biochemical effects. On the contrary, M-O₃-AHT on average receives about 8-10 mg O₃ (20-fold higher) and is pharmacologically active. As the infusion of ozonated saline is very cheap, minimal time-consuming than M-O₃-AHT and quite remunerative for the unscrupulous practitioners, physicians have started to use it also in several Western Countries and it is foreseeable that it will be extensively used in poor Countries, possibly at a dangerous, higher O₃ concentrations. Indeed, Ikonomidis et al. have reported that they maintain the saline solution under a constant flow of O₃ during i.v. infusion but they warned that the maximum amount of O₃ daily administered is usually 4-5 mg and should never exceed 8-10 mg [22]. In their publication they have stated “if we exceed these rates, the over coagulation syndrome starts” and they strongly recommended to perform coagulation tests before starting therapy. These warnings reinforce our preliminary objection to this approach. Moreover Foksinski et al. have

detected 8-oxodeoxyguanosine, a typical oxidative DNA damage in lymphocytes of atherosclerotic patients after the infusion of ozonated saline, that is a worrisome result never detected after M-O3-AHT [23]. It is clear that ozonation of saline is an unstable process because, if it is not promptly infused, O3 totally decomposes in 60 min and therefore this preparation does not comply with the pharmacotherapeutic principle, which requires the stability and exact knowledge of the constituents. Another problem is that commercial ozone generators differ and often they have a variable gas output, i.e. from an output of 1 up to 3-5 L per min and consequently the total amount of O3 delivered can vary from 1 up to 5-fold. Therefore some ozonetherapists, without a suitable preparation, may risk to intoxicate the patient! Another disturbing factor is that blood flow in the cubital vein varies especially in women with the consequence of an uncertain blood/H2O2-O3 relationship implying a variable bio-oxidation. In conclusion a further diffusion on the use of ozonated saline, although less dangerous than the direct i.v. infusion of O2-O3 that some practitioners of naturopathy still perform, does not represent an improvement and in any case it will be never accepted by the FDA or by EU Health Authorities.

Another route of administration of O3 is the insufflations of O2-O3 gaseous mixture into the rectum for treating chronic colitis and fistulae. In 1936, when Dr. Aubourg proposed the procedure [24], this was a reasonable application which it has been now extended to treat all diseases. The insufflations of a volume of 200-300 mL of gas into the rectum-colon at O3 concentration ranging from 5 to no more than 35 µg/mL can be precisely done but it remains unpredictable the effective O3 dose because of a possible flatulence and the presence of a more or less abundant luminal content. Thus, it is obvious to predict that a significant fraction of the dose will be neutralized by fecal material. The residual O3 dose will dissolve and be neutralized into the layer composed of glycocalix and mucoproteins covering the mucosa. O3 will instantly and fully reacts with these compounds but only a LOPs fraction will be absorbed with O2 by the mucosa. Indeed, in well-controlled rabbit experiments a transient presence of LOPs was shown in the portal vein [25], but obviously the real therapeutic effect on vascular diseases, diabetes and cancer remains uncertain.

Once again, while in Western Countries many patients object this route, at Cuba they have thousands of patients to be treated every day and they have adopted this rapid, inexpensive procedure in all patients always administering 200 mL O2-O3 mixture with the excessive O3 concentration of 50 µg/mL (O3 dose: 10 mg). Firstly, the O3 concentration is too high and during prolonged use may be mutagenic; secondly, this route, being so uncertain, should not be used in controlled clinical trials. Indeed, it seems that in only twenty days that dosage could cure (?) the diabetic foot in a number of patients treated with rectal ozone and topical ozonated oil [26].

5) The constant need to teach the most correct procedures to the ozonetherapists

This is absolutely essential and if Western Health Authorities one day will start to consider ozone therapy as a valid approach, the first requirement for a physician is to have profitably followed a theoretical and practical course of at least three days at the University level. Today, at least in Italy, conscientious physicians are keen to learn all the basic information about ozone and its mechanisms of action. This is the only way to fully understand the chemical and biological problems and become able to calibrate the therapy for different diseases and patients. Regretfully, there are also a few unscrupulous physicians, who practice ozone therapy just pushing the setting knobs of an ozone generator. They will use ozonated saline mostly for performing the illicit doping in naïve athletes or they may harm patients and further discredit this approach. Annual congresses, or refreshing brief courses would be equally useful. Obviously, even expert practitioners can make useful information but they remain anecdotal and untrustworthy because it remains uncertain the reliability of their ozone generators, hence real O3 dosages. Often they frequently

combine ozone therapy with other therapies and therefore they cannot perform a clinical trial.

6) Unless randomized and well controlled clinical trials in vascular diseases and age-related macular degeneration (ARMD) dry form are performed, ozone therapy will remain in limbo.

Official medicine correctly requires that any new medical procedure undergoes a rigorous control versus the gold standard according to the Helsinki regulations. During the last decade, in spite of having prepared several protocols, we could perform only one trial in PAD patients (III and IV stages) by using the extravascular blood circulation against O₂-O₃ (EBOO) because the expensive gas-exchange devices had been donated [27]. In such a case, the methodology is far more complex than M-O₃-AHT and only a small number of patients could be evaluated for a too short time. Nonetheless clinical results showed a clear improvement of patients treated with EBOO in comparison to the gold standard (Iloprost infusion).

The basic concept is to have two arms: one is the control patients, who are treated with the optimized orthodox therapy (not always curative), while the other arm (including an equal and comparable number of patients) treated with the same orthodox therapy plus M-O₃-AHT should markedly improve the outcome. On the basis of preliminary data, a minimal number of 30 + 30 patients should be enough to yield a statistically significant result if ozone therapy is truly effective. The problem are two-fold: one is to obtain the permission of the Ethical Committee and the second is to find support for paying insurance and other expensive costs. Priority must be given to diseases most suitable to be treated with ozone therapy.

- a) Vascular diseases such as stroke, chronic heart failure (CHF) and peripheral obstructive arterial disease (POAD). All together they represent the first cause of death;
- b) Degenerative-ischemic disease as the atrophic (dry) form of age-related macular degeneration (dry-ARMD). This is far more frequent than the exudative form and it is very debilitating;
- c) Chronic obstructive pulmonary disease (COPD), which is the fourth cause of death. Please note that b) and c) represent a serious social-economic problem;
- d) Diabetes with its various manifestations [28]. The present "epidemic" diabetes with so many awful complications is a disease requiring great attention and certainly cannot be cured in 20 days [26];
- e) Muscular-skeletal pathology. Within this broad area, it is felt that an appropriate metanalysis study showing the brilliant results achieved in hernial disc is most likely to already demonstrate an excellent results able to boost up the ozone as a therapeutic agent.

Finally there are several other pathologies such as chronic infectious diseases (HIV-AIDS, chronic hepatitis, TBC), cancer, degenerative diseases where ozonotherapy may be useful as a supportive therapy but unable on its own to cure the pathology. This aspect will be considered later on.

The "stroke" protocol, in our mind, is the most urgent and it has great possibilities to be successful. Moreover about 80% of patients with stroke, who arrive later than 4.5 hours at the stroke unit since the initial symptoms are NOT eligible for trombolysis and therefore can only be treated with traditional medications. The result is poor because mortality, morbidity and disability increase very much. Thus this 80% of patients, almost abandoned by official medicine, could also be treated with M-O₃-AHT, twice daily for at least ten days and from preliminary results they are likely to markedly improve beyond the best imagination. If this result can be demonstrated and published in one of the best medical journals, ozone therapy will receive great

attention. A multicenter trial would be very useful because, by using the same protocol, the value of the results can be enhanced.

After two decades of work in ozone therapy, in spite of the fact that the basic mechanisms of action of ozone in biological fluids have been clarified, orthodox medicine remains skeptical about its medical value and, with the exception of Cubans and Russian Health Authorities, it tends to ostracize this approach in public hospitals. The FDA, the USA regulatory agent, continues to predicate that ozone should not be used as a therapeutic agent. To date, the European Medicines Agency (EMA) and the Therapeutic Goods Administration of Australia have also not approved any form of ozone therapy.

CONCLUDING REMARKS

This paper may be found provocative but the aim is to underscore the problems discussed in the previous sections plaguing ozone therapy. Some are intrinsic in the methods but several others have been created by unscrupulous ozonetherapists, not to mention that nobody acts as a sponsor of our research and all the time both official medicine and Health Authorities ostracize our efforts. Consequently, it appeared worthwhile to call the attention of all ozonetherapists in the attempt to correct our mistakes and in order to improve and above all to show the value of ozone therapy in some diseases. This is difficult because it requires knowledge, fundings and good will but in our opinion it is the only solution. At the moment we are still at a cross-road and is up to us decide if we will enter the main road or we will prefer to remain in a blind alley. Our recent review entitled "The ozone paradox: ozone is a strong oxidant as well as a medical drug" has emphasized the dualistic effect of this gas [6]. Everyone knows that ozone is a toxic gas but it remains to us to show its validity and safety as a therapeutic agent.

Table 1- Countries where ozone therapy is performed, list of relative National Associations, and estimated number of ozone therapists.

Note. The compilation of the table has been possible thanks to the precious assistance of ISCO3 Legal Advisor, Roberto Quintero, based on his own research and information provided directly by national associations, international federations and/or ozonetherapists. It is to emphasize that the presented statistic is necessarily incomplete because the national associations do not have up to date figures of all physicians who practice ozonotherapy. Moreover for Countries not included in the list it does not automatically mean that ozonotherapy is not practiced in any of them. Indeed, at the recent "III World Congress of Oxygen-Ozone Therapy" (Brescia, Italy, 14-16 April 2011) we learnt that ozone therapy is already practiced in Bolivia, Costa Rica and Thailand. The readers are invited to perfect the table content.

Countries	National Associations of Ozone Therapy	Physicians' presumed number^b
Argentina	(1) Argentine Association of Ozone (ADELO). (2) Argentine Medical Association of Oxygen Ozone Therapy (AMAOO) (3) Inter American Society of Oxygen Ozone	167
Austria	Ozone Therapists Interest Group	30
Belarus	Belarus Association of Ozone Therapists	
Brazil	Brazilian Association of Ozonotherapy (ABOZ)	200
Canada		50
China		2.000
Cuba	Ozone Research Center ^a	500
Dominican Republic	Dominican Association of Ozone Therapy	42
Ecuador	Ecuadorian Society of Ozone Therapy	20
Egypt	Egyptian Medical Society for Ozonotherapy and Complementary Medicine Development	200
Finland	No association	6
France		15
Georgia	Georgian Association of Ozone Therapists	
Germany	German Medical Society for the Use of Ozone in Prevention and Therapy	11.000
Greece	Greek Scientific Association of Oxygene - Ozone Therapy	100
Israel		2
Italy	(1) Italian Federation of Oxygen Ozone Therapy (FIO). (2) Scientific Society of Oxygen Ozone Therapy (SIOOT).	3.000
India		100
Japan	(1) Japan Research Association for Medical & Hygienic Use of Ozone. (2) Japanese Society of Oxidative Medicine.	50
Lithuania	Lithuanian Association of Ozone Therapists	
Moldavia	Moldavian Association of Ozone Therapists	
Mexico	Mexican Association of Ozone Therapy	650
Romania	Scientific Romanian Association of Ozone Therapy	20
Russia	Russian Association of Ozone Therapy	3.500
South Africa		20
Spain	(1) Spanish Association of Medical Professionals in Ozone Therapy (AEPROMO). (2) Spanish Scientific Association of Application of Oxygen Ozone Therapy (ACEOOT). (3) Spanish Ozonotherapy Association (SEOT)	500
Switzerland	Swiss Medical Society for Ozone and Oxygen Therapies	67
Turkey		200
United Kingdom		15
Ukraine	Ukrainian Association Ozone Therapists	400
United States of America	American Academy of Ozonotherapy	200
Venezuela	Venezuelan Association of Ozone Therapy	25
		23.079

a) It is not an association but a government research center.

b) Such a number as a total of both association members and non members.

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