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Special Issue on Ozone Therapy: Italian experiences in an International Context

CONTENTS

Safety, pitfalls, and misunderstandings about the use of ozone therapy as a regenerative medicine tool. A narrative review.

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Despite various opinions and healthy controversy on Ozone Therapy (OT), the practices of this therapy have increased worldwide. Main areas of study with consistent scientific outcomes are the topical treatment of both disk herniation and periodontal disease. On the other hand, there is a net dissociation of the scientific resonance concerning systemic oxygen/ozone treatments. It is our intention to discuss in logical terms the numerous papers that commendably reported adverse reactions attributable to OT, focusing our attention mainly to the techniques of administration and not to the simple contact of ozone with biological material. The case reports on OT treatments safety concerns discussed on international journals, make it possible to state that most safety issues are secondary to infections or traumatic reactions due to malpractice. Commonly, the molecule of ozone itself is not responsible of severe reactions at the therapeutic modalities. The millions of patients treated so far from the thousands of physicians correctly practicing OT world widely in the last 40 years demonstrate the safety of this simple and cost-effective regenerative medicine tool. The promising therapeutic implications also for the current COVID-19 emergency are a further stimulus to the standardization of this therapeutic resource with multiple application specificities.

Regenerative medicine regenerates human cells, tissue or organs, to restore normal function, among other functions. Ozone therapy is fully traceable to this context. Despite the use of ozone in the medical field dates to the beginning of the last century (1, 2), as well as its physiological beneficial effects were known earlier (3), hitherto it remains a riddle within the scientific community.

The paradox that a strong oxidant could be considered as a powerful tool to counteract the excess of oxidative stress when used at adequate doses is likely far to be completely understood (4). Among all the improper news regarding the use of ozone in the medical field, most of them derived by misunderstandings concerning its molecule that, since its discovery, was mainly joined to its action as a pollutant (5). Being ozone a strong oxidant, its negative action on breathing and lung represented the main topic of the toxicological studies since the beginning of the industrial era, when its artificial production in the big cities started to be prominent.

Despite the above, the medical use of ozone in web-based forums is mainly defined as a therapy "fully" free from side effects and thus creating more

Key words: oxidative stress; ozone therapy; side effects; drug safety; drug management; regenerative medicine

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and more confusion. Where is the truth? In our opinion, a more serious debate on the field of drug safety must be introduced, regardless of the accurate knowledge of its mechanism of action. For example, also if we can partially agree that the use of ozone in the medical field at the proposed concentrations is usually free from any side effects, the whole method of its production and administration must be clearly stated and evaluated in order to warrant a global safety.

The preparation of the mixture, its exact concentration, its microbiological requirements and other variables concerning its production before its administration must be submitted to the best technology in term of Good Clinical Practices (GCP). In other terms, it is the responsibility of healthcare professionals who thoughtfully choose to use ozone as a medicinal product to ensure all Quality Standards. In fact, the ozone therapist is at the same time the manufacturer and the administrator of the therapeutic agent. This is also the reason why, as usually occurs for the drugs accepted for marketing after their authorization, a list of possible side effects must be always clearly indicated and submitted to the attention of the consumer, thus means, of the patient.

This review is aimed at summarizing safety reports regarding the therapeutic use of ozone and putting them into a broader context. As for data sources, typical electronic databases were searched for such reports. No restrictions in terms of language were imposed. Study selection, case reports, case series and other types of articles reporting safety concerns were considered. As regards the period taken into consideration this ranges from 2000 to today. In fact, the latest works presented in systematic reviews concerning the safety of OT date back to that year (6). The purpose is that to give suggestions to those physicians or scientists who really want to do OT in only the interest of the patients, justifying it in terms of effectiveness, safety, purity, cheapness, provided that ozone dosage will be precise, accurate, adjustable, stable, reliable. All to eliminate the preconception that it is still considered a "quake" therapy. In this context, the numerous unscientific and apparently amazing advertisements on the disclosure of the OT benefits throughout Internet cannot be kept silent (7, 8). Most of this fake news put further discredit against OT and its possible role on medicine, generally confusing the powerful, disinfectant activity of ozone, with even more complex pharmacological activity induced by graded ozone amounts.

Before discussing most of the reports regarding several supposed side effects, we will point out some definition regarding the surveillance of drugs after marketing. Pharmacovigilance is formally defined as: "…the science and activities relating to the detection, assessment, understanding and prevention of adverse effects or any other possible drug-related problems…" (9). The last sentence "…any other possible drug-related problems" refers to issues with accuracy of prescribing and dispensing, dose, quality, interactions, adherence and many other aspects that become prominent in the case of OT. Modern-day pharmacovigilance is also concerned with the impact of medicines on patients' quality of life – not only with the purely physical effects. Medicines heal, but as any other therapeutic approach, either orthodox or not, can also harm.

Safer use of modern and traditional medicines must be the most ambitious goal for all physicians. It depends on patients and health professionals, health ministries, regulators, and manufacturers working actively together. The priority is to identify when patients suffer any kind of harm from their therapy and to reduce the risk of this happening in the future, with the aim of making the motto "primum non nocere" ever more current (10).

Frame

Looking at the articles concerning the ozone side effects, a paramount attention is mainly addressed to the gas itself instead of to the way and the modality with which is prepared and administered to the patient. This represents the most important mistake explaining the possible pitfalls in making ozone and OT the scapegoat of any adverse reaction occurring either during or after the procedure.

To our opinion, for a more comprehensive strategy in the aim to clearly define the inevitable side effects, either severe or moderate, that every medical approach could induce, a more accurate

knowledge of ozone chemistry and of its modality of preparation and administration must be carefully kept in mind from the Authors of such reports. This also to avoid the numerous pitfalls regarding its uses also when not appropriated or overestimated in the possible therapeutic activity.

In this context, the proposal of the usefulness of OT as a method of Flumequine (FQ) degradation in the body (11) represents one of the best examples. In our opinion, the proposal for using OT in these cases could not simply derive by the fact that ozonation has been described to be an effective method for removing FQ from the liquid water (12). The risk is that using OT without knowing completely its action at the molecular level and its possible outcomes in the total body in vivo, could induce a not correct result and improper effects in terms of safety.

One more fact that we want to point out is the active participation of patients to the evaluation regarding safety and side effects. Indeed, modern patients must be vocal partners in decisions about their own care, as such be provided with a clear, understandable informed consent. Models of such documents are reported in Fig. 1 and 2.

They follow the directives of the WFOT, which represents to date almost 31 Countries through 23 National Associations, 9 National Sections and 2 Individual Members. Nowadays, it is the largest federation and recognized as the biggest international World society for OT. In Fig. 3, an informed consent module for dental treatment is also shown.

In addition, there are commonly available technologies that permit reliable collection of information from them. Optimizing tactics for collecting these data is especially important in terms of both efficacy and adverse symptom events. The reporting of such information should become a standard component of OT medical treatment evaluation from a regulatory perspective. It is in this context that the current clinician-based approach to adverse symptom about the therapeutic use of ozone must be driven (13).

The Best Clinical Practices in Ozone Therapy is constantly followed by a prestigious Scientific Board which constantly suggest news on the best characteristics of Ozone Generators and all the best Ozone-resistant material with the maximum respect of all the manufacturers and avoiding any possible conflict of interest being always over the parts only for the best values of Ozone Therapy in terms of Accuracy, Science and Clinical Validation.

Ozone Therapy may be administered with different techniques either locally or systemically.

Among the local administration we include subcutaneous, intramuscular or intradiscal. These treatments are executed with sterile needles of various diameter (Gauge) and length (from 6 mm till 50 or 70 mm).

The infiltrative therapy with a balanced mixture of oxygen-ozone is indicated in various diseases mainly related to pain evoked by skeletal muscle component. Among these, the sciatica from disk disease cervical, dorsal and lumbar in osteo-articular pathologies including osteoarthritis, gonarthrosis, arthrosynovitis of the knee, shoulder pain (rotator cuff tendonitis), carpal tunnel syndrome, trigger finger, fingers hand arthropathy, hallux valgus, pathology painful foot (tarsal tunnel, capsulitis, bursitis, Morton's neuroma, plantar fasciitis), tendonitis (lateral epicondylitis, medial epicondylitis, Achilles tendon pathology), tender points (painful points) and temporo-mandibular joint pain syndrome.

Nevertheless, most of the painful diseases, of any nature either traumatic or degenerative not mentioned above, may be susceptible to good results.

The main routes of administration of the gaseous mixture are: muscle bundles injection (disc-radicular conflict, myofascial syndrome) by percutaneous symmetrical paravertebral injections at the space affected by herniated disc or disc disease or at the level of the roots involved, peri-neuronal injection (carpal tunnel, tunnel tarsal), injected intra-peri-articular (knee arthritis, osteoarthritis, shoulder pain), peritendinous, intradermal and/or subcutaneous injections in painful points. The infiltration of the gaseous mixture is performed after thorough disinfection, inserting very thin needles, disposable sterile, in a safe and accurate way, using special syringes and antibacterial filters, ensuring adequate asepsis conditions.

Infiltrations are generally performed twice a week, at different times. A few sessions of maintenance are also recommended as well after time. It has been speculated that the mechanism of action of infiltration with oxygen-ozone action is realized through local metabolic effect resulting in a trophic relaxant action, with reduction of pain and inflammation, with a good therapeutic efficacy (75 ÷ 80%).

The procedure is generally well tolerated by the patient, sometimes may occur experience of a temporary feeling of heaviness or burning pain, there is also the possibility of a temporary awakening, usually for a few minutes of pain ("pain awakened "). The risks associated with this procedure are those related to the puncture (hematoma at the injection site) or to a vagal reactions (sweating, bradycardia, hypotension with cardiac rhythm disturbances).

Absolute clinical contraindications are: severe heart attack or severe heart disease, uncontrolled hyperthyroidism, major G6PD-deficiency, acute alcoholic psychosis. Relative contraindications are represented by: pregnancy, convulsion, severe cardiopathy. It is suggested to pay attention and take care of patients in therapy with antidepressant, neuroleptics or other psychoactive drugs due to the relative low initial response to ozone. A strong psychological support it is suggested to acquire the compliance of the patients.

Ozone Therapies have never been reported to cause allergy. There are occasional Herxheimer reactions including self-limiting, mild to moderate flu like symptoms: fever, chills, pain, aching, headache, fatigue, lethargy, rashes and phlegm.

Any procedure involving needles can have a risk of infection and bleeding.

Ozone Therapies have yet not been approved by the Health Care Authorities and are therefore considered experimental treatments. This is the reason why we must suggest this procedure except on the condition to receive from the patient the release of an informed consent confirming any legal responsibility for harm resulting from its treatment for the patient status.

The signature on this agreement will constitute a full and final release of our legal responsibility resulting from the administration of Ozone Therapies and/or any other medical treatment, which may be necessary as a result thereof.

Read and understand the above statement. I hereby place myself under care for Ozone Therapy, and agree to the above release. I understand the possible risks of Ozone Therapy, understand that any results are not guaranteed and give informed consent to receive treatment.

I confirm that Dr. _____ has provided me with ample opportunity to ask questions and have received answers that are to my complete satisfaction.

Date:_____

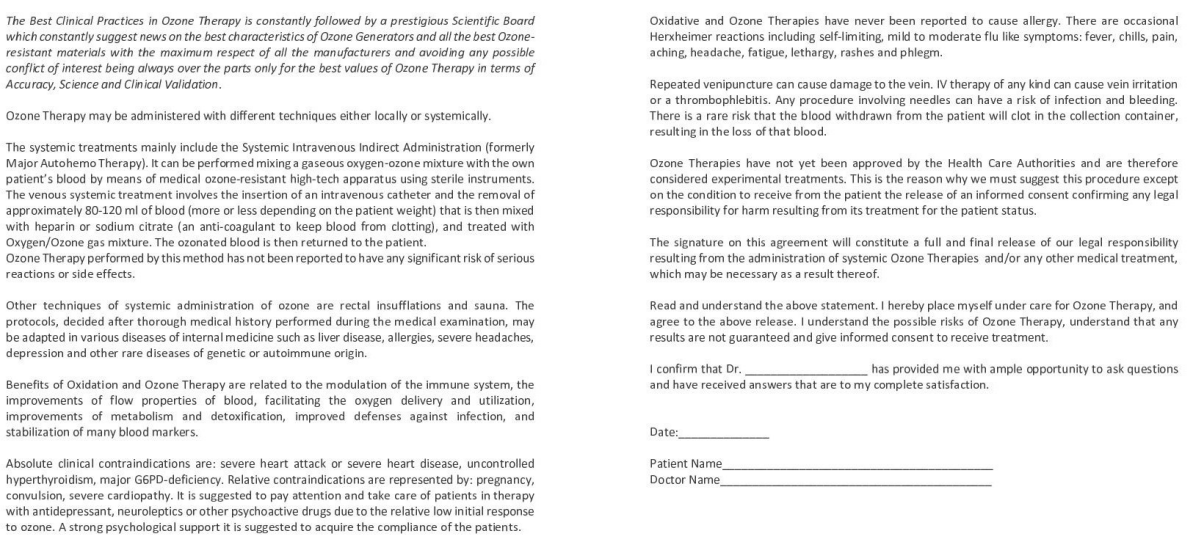
Patient Name_____

Doctor Name_____

Fig. 1. *Informed consent form to receive infiltrative OT.*

On the other hand, observational studies permit the evaluation of medical treatment safety in many patients in a real-world setting, where practice patterns can be assessed. Unfortunately, such studies have limited ability to determine causation, also if they can detect signals that may suggest a safety concern (14).

Despite debates over its credibility, Wikipedia is reportedly the most frequently consulted online health care resource globally. Wikipedia pages typically appear among the top few Google search results and are among the references most likely to be checked by Internet users. We therefore also evaluated Google searches and Wikipedia page views



The Best Clinical Practices in Ozone Therapy is constantly followed by a prestigious Scientific Board which constantly suggest news on the best characteristics of Ozone Generators and all the best Ozone-resistant materials with the maximum respect of all the manufacturers and avoiding any possible conflict of interest being always over the parts only for the best values of Ozone Therapy in terms of Accuracy, Science and Clinical Validation.

Ozone Therapy may be administered with different techniques either locally or systemically.

The systemic treatments mainly include the Systemic Intravenous Indirect Administration (formerly Major Autohemo Therapy). It can be performed mixing a gaseous oxygen-ozone mixture with the own patient's blood by means of medical ozone-resistant high-tech apparatus using sterile instruments. The venous systemic treatment involves the insertion of an intravenous catheter and the removal of approximately 80-120 ml of blood (more or less depending on the patient weight) that is then mixed with heparin or sodium citrate (an anti-coagulant to keep blood from clotting), and treated with Oxygen/Ozone gas mixture. The ozonated blood is then returned to the patient.
Ozone Therapy performed by this method has not been reported to have any significant risk of serious reactions or side effects.

Other techniques of systemic administration of ozone are rectal insufflations and sauna. The protocols, decided after thorough medical history performed during the medical examination, may be adapted in various diseases of internal medicine such as liver disease, allergies, severe headaches, depression and other rare diseases of genetic or autoimmune origin.

Benefits of Oxidation and Ozone Therapy are related to the modulation of the immune system, the improvements of flow properties of blood, facilitating the oxygen delivery and utilization, improvements of metabolism and detoxification, improved defenses against infection, and stabilization of many blood markers.

Absolute clinical contraindications are: severe heart attack or severe heart disease, uncontrolled hyperthyroidism, major G6PD-deficiency. Relative contraindications are represented by: pregnancy, convulsion, severe cardiopathy. It is suggested to pay attention and take care of patients in therapy with antidepressant, neuroleptics or other psychoactive drugs due to the relative low initial response to ozone. A strong psychological support it is suggested to acquire the compliance of the patients.

Oxidative and Ozone Therapies have never been reported to cause allergy. There are occasional Herxheimer reactions including self-limiting, mild to moderate flu like symptoms: fever, chills, pain, aching, headache, fatigue, lethargy, rashes and phlegm.

Repeated venipuncture can cause damage to the vein. IV therapy of any kind can cause vein irritation or a thrombophlebitis. Any procedure involving needles can have a risk of infection and bleeding. There is a rare risk that the blood withdrawn from the patient will clot in the collection container, resulting in the loss of that blood.

Ozone Therapies have not yet been approved by the Health Care Authorities and are therefore considered experimental treatments. This is the reason why we must suggest this procedure except on the condition to receive from the patient the release of an informed consent confirming any legal responsibility for harm resulting from its treatment for the patient status.

The signature on this agreement will constitute a full and final release of our legal responsibility resulting from the administration of systemic Ozone Therapies and/or any other medical treatment, which may be necessary as a result thereof.

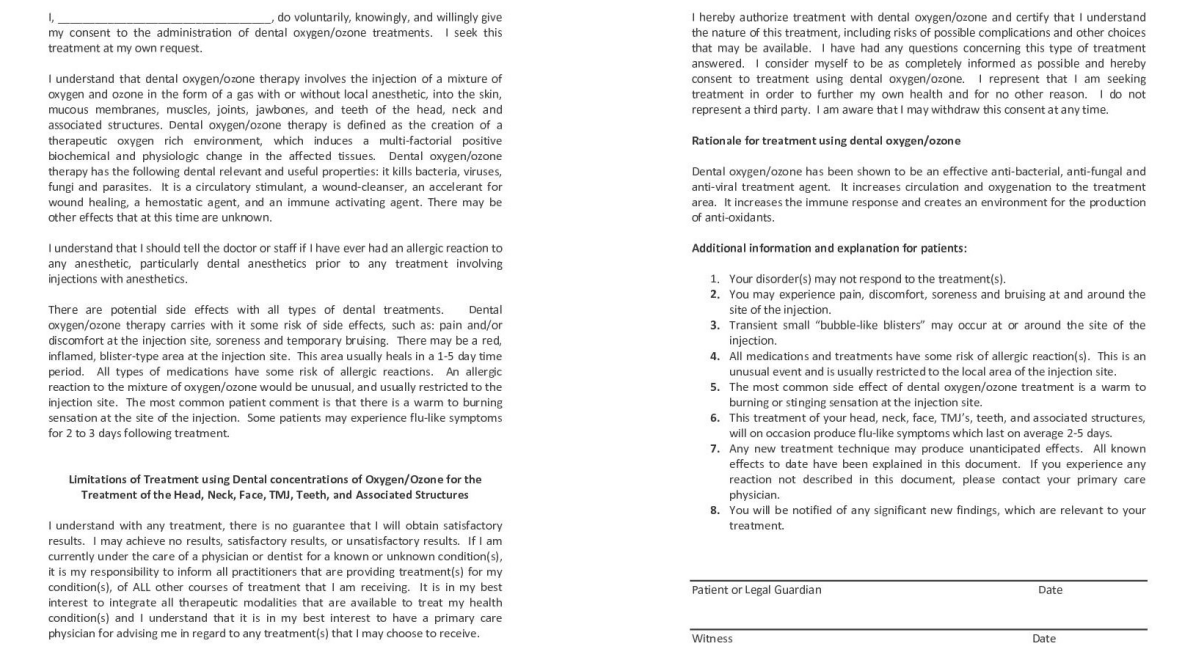
Read and understand the above statement. I hereby place myself under care for Ozone Therapy, and agree to the above release. I understand the possible risks of Ozone Therapy, understand that any results are not guaranteed and give informed consent to receive treatment.

I confirm that Dr. _____ has provided me with ample opportunity to ask questions and have received answers that are to my complete satisfaction.

Date:_____

Patient Name_____
Doctor Name_____

Fig. 2. *Informed consent form to receive systemic OT.*



I, _____, do voluntarily, knowingly, and willingly give my consent to the administration of dental oxygen/ozone treatments. I seek this treatment at my own request.

I understand that dental oxygen/ozone therapy involves the injection of a mixture of oxygen and ozone in the form of a gas with or without local anesthetic, into the skin, mucous membranes, muscles, joints, jawbones, and teeth of the head, neck and associated structures. Dental oxygen/ozone therapy is defined as the creation of a therapeutic oxygen rich environment, which induces a multi-factorial positive biochemical and physiologic change in the affected tissues. Dental oxygen/ozone therapy has the following dental relevant and useful properties: it kills bacteria, viruses, fungi and parasites. It is a circulatory stimulant, a wound-cleanser, an accelerant for wound healing, a hemostatic agent, and an immune activating agent. There may be other effects that at this time are unknown.

I understand that I should tell the doctor or staff if I have ever had an allergic reaction to any anesthetic, particularly dental anesthetics prior to any treatment involving injections with anesthetics.

There are potential side effects with all types of dental treatments. Dental oxygen/ozone therapy carries with it some risk of side effects, such as: pain and/or discomfort at the injection site, soreness and temporary bruising. There may be a red, inflamed, blister-type area at the injection site. This area usually heals in a 1-5 day time period. All types of medications have some risk of allergic reactions. An allergic reaction to the mixture of oxygen/ozone would be unusual, and usually restricted to the injection site. The most common patient comment is that there is a warm to burning sensation at the site of the injection. Some patients may experience flu-like symptoms for 2 to 3 days following treatment.

Limitations of Treatment using Dental concentrations of Oxygen/Ozone for the Treatment of the Head, Neck, Face, TMJ, Teeth, and Associated Structures

I understand with any treatment, there is no guarantee that I will obtain satisfactory results. I may achieve no results, satisfactory results, or unsatisfactory results. If I am currently under the care of a physician or dentist for a known or unknown condition(s), it is my responsibility to inform all practitioners that are providing treatment(s) for my condition(s), of ALL other courses of treatment that I am receiving. It is in my best interest to integrate all therapeutic modalities that are available to treat my health condition(s) and I understand that it is in my best interest to have a primary care physician for advising me in regard to any treatment(s) that I may choose to receive.

I hereby authorize treatment with dental oxygen/ozone and certify that I understand the nature of this treatment, including risks of possible complications and other choices that may be available. I have had any questions concerning this type of treatment answered. I consider myself to be as completely informed as possible and hereby consent to treatment using dental oxygen/ozone. I represent that I am seeking treatment in order to further my own health and for no other reason. I do not represent a third party. I am aware that I may withdraw this consent at any time.

Rationale for treatment using dental oxygen/ozone

Dental oxygen/ozone has been shown to be an effective anti-bacterial, anti-fungal and anti-viral treatment agent. It increases circulation and oxygenation to the treatment area. It increases the immune response and creates an environment for the production of anti-oxidants.

Additional information and explanation for patients:

1. Your disorder(s) may not respond to the treatment(s).
2. You may experience pain, discomfort, soreness and bruising at and around the site of the injection.
3. Transient small "bubble-like blisters" may occur at or around the site of the injection.
4. All medications and treatments have some risk of allergic reaction(s). This is an unusual event and is usually restricted to the local area of the injection site.
5. The most common side effect of dental oxygen/ozone treatment is a warm to burning or stinging sensation at the injection site.
6. This treatment of your head, neck, face, TMJ's, teeth, and associated structures, will on occasion produce flu-like symptoms which last on average 2-5 days.
7. Any new treatment technique may produce unanticipated effects. All known effects to date have been explained in this document. If you experience any reaction not described in this document, please contact your primary care physician.
8. You will be notified of any significant new findings, which are relevant to your treatment.

Patient or Legal Guardian Date

Witness Date

Fig. 3. *Informed consent form for dental OT.*

for OT looking specifically for references to safety warnings (15), agreeing with previous studies (16). Finally, according to the routes of administration, effectiveness and safety, we think interesting to cite the official positions described by the two Malaysia Technology Review Reports (17, 18).

In the report, dated 2017, the authors described the results in terms of both efficacy/effectiveness and safety regarding the technique of the "autologous blood transfusion", which it is previously reported as major autohemotherapy (MAH) (17). For an international homogeneity requirement, we will continue to use the term "autohemotherapy and its acronym MAH, although the definition of systemic *ex vivo* intravenous ozone therapy would be the most accurate and appropriate. It is worthy to note that in the conclusion of the same document some statements need to be carefully discussed. Indeed, regarding the possible effectiveness they concluded, "There was no new high level of evidence retrieved to determine the effectiveness and safety of autohemotherapy …" thus certifying that also if a not "high level" a certain effectiveness has been scientifically proven.

Furthermore, the authors followed "In terms of safety, a study reported high incidence of hepatitis infection in autohemotherapy. The autohemotherapy (autologous blood transfusion) ozone therapy exposed patients to blood-borne diseases. Hence, more high-quality evidence is needed to proof the safety and effectiveness of autohemotherapy (autologous blood transfusion) ozone therapy for treatment of medical conditions". It is almost strange to note that the concerns regarding ozone safety derived from only one study where probably the side effect, i.e. the infection, went from the outside environment who could have contaminated the blood of the patients of the study and not from the ozone itself.

In the body of the present review we will avoid in evaluating the possible risks or safety concerns regarding either the ozone treatments by rectal insufflation widely used in Cuba (19-21), or in Russia with the infusion of ozonated saline solution (22, 23). Moreover, experimental intralesional ozone injection in diabetic foot ulcers is considered outside the objectives of this paper (24).

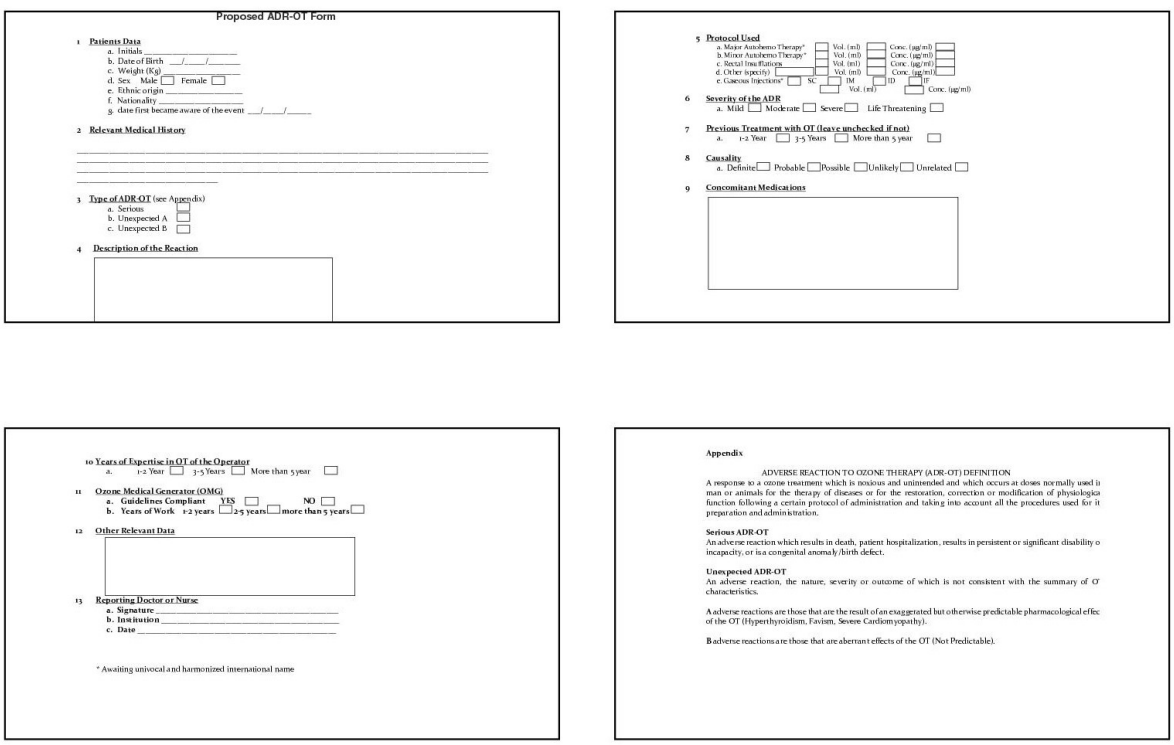


Fig.4. *Adverse reaction sheet either during or following OT.*

Before the discussion on the single papers published on the safety of OT in the last two decades, we must remember those conditions that are considered contraindication to the treatment with ozone. Firstly, sentences like "ozone therapy is forbidden for individual ozone intolerance" or similar do not apply because ozone itself does not give rise to allergic reactions. Secondly, the contraindications will be different in terms of the administration modalities. The guidelines of the most important ozone Societies worldwide and the same World Federation of Ozone Therapy (WFOT) (www.wfoot.org) clearly define as contraindications the following conditions:

In the case of infiltrative treatment:

- convulsive syndrome, that is, sudden frequent involuntary contractions of the muscles caused by pathological impulses of the central nervous system (CNS).

On the other hand, absolute contraindications for the use of systemic OT, term within which today it is preferred to include the MAH, are:

- a decrease in the ability of blood to clot (eg thrombocytopenia; major G6PD deficiency).
- acute form of myocardial infarction.
- uncontrolled thyroid disease, in which the level of thyroid hormones increases in the blood.
- acute alcoholic psychosis.

In terms of relative contraindications, pregnancy, severe cardiopathy.convulsions should be considered. Different is the case that will be discussed later of patent foramen ovale, where its frequency in about 25 percent of patients is in contrast with a fatal casualty (25). In such a case, a well-designed recording of adverse reactions can be a fundamental aid (Fig 4).

Cases

Below, the most important cases conferred on international journals on probable side effects or adverse reaction occurred either during or after OT regarding either spinal treatment for disk herniation or systemic treatment trough MAH (ADR-OT) are reported. Firstly, it can be said that most of the OT side effects reported in the literatures following disk herniation treatment are secondary to infections or traumatic reactions due to malpractice.

Commonly, the molecule of ozone itself is not responsible of severe reactions at the therapeutic concentrations and only mild redness could be seen in the treatment area. Among these, the accidental inhalation of the gas may lead to eyes burning, coughing, nausea or vomiting, together with mild headache in very sensitive individual. It may worsen respiratory diseases and asthma. Such a condition is the only harmful due to the complete lack of antioxidants in the thin fluid film of alveoli.

In the case of MAH sometimes a patient may have a mild redness in the face mostly because of citrate as anticoagulant especially in the case of rapid infusion. Iron taste in the tongue and euphoria could be described by some patients either during MAH or subcutaneous injections of large doses of oxygen/ozone mixtures.

Direct intravenous (DIV) delivery of ozone is not recommended by the most recognized Ozone Societies. Indeed, also if not severe reactions were reported, it can cause respiratory discomfort in patients with reactive airway as well as the very common side effect of phlebitis. None of these side effects occur with MAH. At least one documented case of acute respiratory failure has been reported by AAOT also if not properly indexed (26). To our opinion, in the aim to finally validate this technique (27), clear and deep clinical studies must be performed.

Moreover, it is good to emphasize immediately that OT must be practiced only by persons qualified for this medical act with a specific preparation. In fact, previous backgrounds about the naturopathic use of OT could create confusion on the practitioners that could safely administer these techniques, as reported in a specific blog (28).

Specific case reports in occasion of loco-regional applications

In 2004 was described a case of acute bilateral intraocular hemorrhages occurring after injection of oxygen/ozone mixture probably due to an uncommon but significant complication of intradiscal (ID) infiltration or any other technique that could increase intra cranial pressure (ICP) (29).

In the same year, Corea et al. reported a severe reaction following lumbar (L4/L5) ID ozone injection.

It is almost strange to note that, while in the title the authors claim that OT was responsible of a stroke, they conclude that "We are fairly confident that a direct toxic effect of the ozone can be excluded" (30).

Ginanneschi et al. discussed a case of ventral and dorsal root injury occurring after transcutaneous ID infiltration of oxygen/ozone for L4-L5 disk herniation (31). The Authors stated, "Until randomized controlled trials on efficacy and short-term safety have been carried out, we think that physicians should be informed about the risk of potential complications when recommending this procedure". We wondered if they referred to OT or to the ID procedure!

In 2007 we found a further case regarding intravertebral treatment for disk herniation describing a fulminating septicemia (32). As the same authors stated, the risk of such complication is a rare event that can occurs also in other methodologies like myelography, lumbar puncture, paravertebral injections, epidural anesthesia, acupuncture, and ID therapy with chymopapain. The fact explains itself reasoning that such a reaction could only be ascribed to the lack of adequate asepsis and not to the ozone molecule.

Here and in the following discussion of cases, we want to point out again that it is not our intention to stand up to paladins of the absolute harmlessness of such methodology, but simply give a more in-depth explanation in order to help get a clear evaluation of the risks that OT could really have in terms of harm for patients.

In 2007, a strange case of thunderclap and headache appeared after minimally invasive medical procedures (33). In the first case the cause was an inadvertent intrathecal puncture during OT for lumbar disk herniation while the second was due to the penetration of the needle in the subarachnoid space.

In the light of these emerging reports on OT risks, Pellicanò et al. decided to better define the characteristics of the technique based on ozone administration (34). In their study, the authors concluded: "When the guidelines of the Italian Oxygen-Ozone Therapy Federation are followed, intradiscal and periganglionic injection of oxygen-ozone mixture is considered safe, without any complications or collateral effects making intradiscal-intraforaminal oxygen-ozone injection under CT guidance the method of choice in the percutaneous treatment of herniated disc. Our study covered about 12,000 patients, and all the procedures were performed following the guidelines of the Italian Oxygen-Ozone Therapy Federation (FIO) with no complications".

Other severe events of purulent discitis emerged in 2009 after the treatment with OT for a cervical disc herniation (35). In their conclusions, the authors pointed out the needing to pay attention to the sterility procedures for this kind of intervention, and we can only agree and suggest to all the authors to be more cautious when writing the title of their report.

On the same line, the paper of Balan et al. where, confusing data regarding severe infectious pathologies after OT without indicating the possible carrier of the infectious noxae, have been reported (36). Indeed, if the side effect is described as an infectious problem, we wonder how they can scientifically demonstrate that the ozone molecule itself could really be the carrier of some parasites. Furthermore, following the citation of Magalhaes et al. they forgot to cite other important articles that contribute to the assessment of the Cochrane libraries attesting the usefulness and the validity of the OT as a medical treatment for disk herniation (37).

Indeed, different authors proposed interesting reviews including several studies with meta-analysis approaches demonstrating that the treatment of herniated discs with oxygen/ozone is an effective and extremely safe procedure. In fact, the main adverse effects are rare and passing like mild transient headaches (38-40). In our opinion, most of the reported data do not clarify exactly if the procedure or the used agent is the cause of the event. Being certain that at any extent the molecule ozone could be considered toxic at the used concentrations, other articles participate in creating even more confusion with no real help for the operator, in terms of increasing the quality of the delivered procedure (41-43).

Similar reports must be deeply revised before being considered real and clear evidences of OT side effect (44-46). Other case reports were produced in the last few years. The most recent one, describes the potential role of paradoxical embolism in spinal

cord infarction and myocardial infarction after ID oxygen–ozone injection for disc herniation (47). The authors' character is collected and scientifically correct. However, surprisingly, they do not indicate the concentration of ozone. Furthermore, 8 mL is not part of some guideline. Unfortunately, a similar clear discussion on the methods and on the techniques used for the ozone administration to the patients is rarely encountered (48-53).

To note in the last years the paper of Beyaz et al. that, also if not so aggressive in its analysis, seems to lack in the minimum knowledge regarding OT (54). Following are the points that, according to us, must be clarified:

i) The generators and the production of mixtures. Usually, the ozone therapists do not pay enough attention to this very delicate point being sure that the machine is good, the syringe sterile and so on. In their paper there is no mention of the apparatus characteristic. We do not mean the name of the producer, but the most important data regarding the presence or not of the photometer. The authors do not consider at all these aspects.

ii) The fact that inadvertently administered 20 μg of ozone is not important to our opinion. More important is the administration of a 20 ml volume.

iii) A significant description of the collocation of the Racz catheter that, they said, could have injured the dura, thus again not an ozone problem (55). Furthermore, the fact that the mixtures reached the cranium, i.e. an area very far from the L5/S1 segment, means that the problem must likely be ascribed to a technique error and not to the ozone itself or to any other substance that would be used for the treatment and injection, directly in the central nervous system.

iv) Most of the cited literature agree with point iii. Stroke (30), spinal nerve injury (31), intrathecal puncture (33) are relevant examples. Eventually, some Editors have recently agreed to publish detailed comments on the matter (56-59).

Specific case reports in occasion of MAH:

The first MAH case that we can find in the literature is a fatal case described in 2000 by Marchetti and La Monaca (25). However, it could not be considered a true side effect to ozone due to the presence of the congenital heart abnormality patent foramen ovale (47). The conclusion of the same Authors, which stated that the case could be due to "incorrect medical ozone administration", de facto remits the ozone molecule as a possible cause.

More recently, other supposed severe reactions to systemic ozone using the technique of MAH appeared in some journals. Unfortunately, attention of the authors is usually focused to the MAH techniques with the reports of severe reactions after OT (60, 61), not always supported by strong scientific evidence of its supposed mechanisms (62).

Tang et al. pointed out that OT could be responsible of severe damage mainly in patients suffering of chronic kidney disease (CKD) (63). Our opinion is that the use of medical ozone, being safe, is often indicated by many conditions like CKD with excellent clinical results as a complementary or integrative procedure, associated with other orthodox therapies. Indeed, recent studies reported the discovery of interesting metabolic pathways activated by the administration of correct ozone dosages (64), as recommended by the international guidelines (65). We have also found it imperative to note how scarce and poor is the literature reported in the article of Tang and we are even more astounded reading the case presentation and the discussion, as most of the statements are both unscientific and meaningless, demonstrating little knowledge of the subject matter and lack of responsibility on behalf of the Authors. Firstly, we have no information concerning the method used to inject ozone into the patient. No report or paper may be worthy of being published based on what was stated by the same Authors "the dose was unknown". How could a side effect be defined without knowing the administered dose?

Furthermore, the MAH technique is one of the safest methods when compared to other therapeutic approaches. In our clinics over the last 30 years, we have administered approximately 50 thousand MAHs to humans without relevant side effects. Only in very few cases mild reactions were observed, like facial redness and tingling. No elevation of serum potassium was ever reported, even when administered to CKD patients.

Thousands of physicians around the world

have been performing MAHs since 1960, and we can only wonder how many procedures have been administered so far. Finally, we were really surprised to notice that the authors confused MAH with "input of red blood cells in vitro". MAH cannot be defined as a blood transfusion procedure, or even be compared to it since it lacks, by definition and by practical means, the fundamental characteristic of a transfusion: withdrawal, deposit and subsequent re-infusion of heterologous blood from a donor to a receiver. The release of potassium from lysed red blood cells is related to the storage of the blood. However, there is no scientific evidence that could explain a possible increase of serum potassium secondary to re-injection of the autologous ozonated blood according to the standard MAH procedure, including in patients with CKD.

We remember again that the importance of the generators used to produce the mixtures, in terms of declared and real ozone concentrations, is a fundamental aspect. We are increasingly convinced that the time has come to prove that OT cannot be considered a scapegoat of all the damage occurred for scarce attitude, low knowledge, or negligence. This section may be divided by subheadings. It should provide a concise and precise description of the experimental results, their interpretation as well as the experimental conclusions that can be drawn.

Gold standard reproducible research would support clarification regarding its physio-pharmacological actions. Unfortunately, OT is currently not consistently supported by strong, peer reviewed, literature in clinical studies. Coherent quality educational programs that can explain the basic science and clinical application, will further expand integration of this therapy into medicine and dentistry. To successfully integrate this supportive therapy into the mainstream of medicine, research should be modeled in a fashion understandable and acceptable to the majority audience.

In light of the literatures discussed above, we think that simple and clear indications must be furnished to better define any possible side effect induced by OT intended not only as simple chemical agent but also as complete methodology of medical administration (66). We propose an adverse reaction sheet either during or following OT that could be suitable at the purpose of collect and signaling valid ADR-OT's in the aim to schedule clear and evident side effects deriving from the use of OT in medicine.

In regards to DIV injection of oxygen/ozone mixtures, the position of AAOT (26) is that the method is strictly forbidden and that all members of the AAO must avoid using it in humans. Our opinion, never forgetting the concept that every new medical approach must always be considered following the Hippocratic Oath in the aim to avoid damage or further sufferance to the patients, is that we must always be respectful to all the colleagues proposing new methodologies, encouraging them to set up serious clinical evaluations, concerning the benefit/risk ratio.

The use of OT in humans or animals, must be performed by Medical Doctor, Dentistry or Veterinary Doctors with a specific preparation from medical, dentistry or veterinary schools delivered at University level. The degree in one of the courses above is a fundamental and essential element for practicing the OT profession.

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In view of the emergency COVID-19 and the prospective use of ozone therapy among the candidate clinical interventions as an adjuvant therapy at international level (67-71), it is important to have forms for reporting any adverse reactions. Univocal standardization and documentation criteria represent the only tools for the correct recognition of this therapeutic modality with multiple application specificities. In addiction ozone therapy is useful in dentistry (72-78).

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MEGS, and PM assisted with the development and revision of the paper, gathered references.

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Clinical and imaging selection for CT-guided fluoroscopy 0203 disk treatment

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Low Back Pain (LBP) is the most common spine disease and it is the most common cause of absence from work in developed countries. At lumbar level, the natural history of herniated disc is characterized by a disappearance of clinical symptoms in up to 60% with conservative treatment through simple rest for about 6 weeks and reduction of the disk heniation revealed by CT or MR scans within eight to nine months after the onset of back pain. Surgery is considered the treatment of choice for extruded, migrated and free fragment herniated disk associated to clinical symptomatology of cono-cauda syndrome, progressive foot droop and hyperalgic radiculopathy. patients with a small or contained herniated disk, without any benefit from conservative medical treatment, can be candidates for one of minimally invasive percutaneous techniques, whose outcome, though, depends on the characteristics of hernia itself and on the chosen technique. The aim of this paper is to discuss about O2-O3 treatment for symptomatic not extruded herniated disk at lumbar level, highlighting about indication inclusion exclusion criteria and our results.

Low Back Pain (LBP) is the most common spine disease and it is the most common cause of absence from work in developed countries. Around 80% of adults suffer (LBP) during their lifetime and the most common cause of LBP with or without classical irradiation along the nerve root is the disk herniation (1). At lumbar level, the natural history of herniated disc is characterized by a disappearance of clinical symptoms in up to 60% with conservative treatment through simple rest for about 6 weeks (2-5) and reduction of the disk herniation revealed by CT or MR scans within eight to nine months after the onset of back pain.(6-8).

Surgery is considered the treatment of choice for extruded, migrated and free fragment herniated disk associated to clinical symptomatology of cono-cauda syndrome, progressive foot droop and hyperalgic radiculopathy. Currently, the incidence of recurrence of hernia in patients already underwent to surgery is approximately 2-6% (13). In fact, patients with a small or contained herniated disk, without any benefit from conservative medical treatment, can be candidates for one of minimally invasive percutaneous techniques, whose outcome, though, depends on the characteristics of hernia itself and on the chosen technique (14).

Since Oxygen-ozone therapy was first used to treat herniated discs in 1985, many studies have reported positive outcomes ranging (75 to 90% in patient cohorts treated for LBP with or without sciatica caused by a herniated intervertebral disc (1, 3-8, 12, 14-26, 28-34, 36-39). Its mechanism of action on the disc is mechanical (volume reduction) and anti-inflammatory; this combination gives pain relief and it is a cost/effective procedure that presents a very low complication rate (0.1%) for the patients.

Ozone is an highly unstable and colorless gas; it's prepared and used in real time, transforming

Key words: Low Back Pain, O2-O3 treatment, radiculopathy, herniated disk, of minimally invasive percutaneous techniques

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a small percentage of O2 in O2-O3 mixture by special generators and it lasts in this form about 5 minutes after which it returns to its original O2 form producing a mixture.The aim of this paper is to discuss about O2-O3 treatment for symptomatic not extruded herniated disk at lumbar level, highlighting about indication inclusion exclusion criteria and our results.

MATERIALS AND METHODS

LBP and/or sciatica treatment requires an accurate diagnosis based on thorough history-taking and physical examination followed by appropriate imaging tests, such as computed tomography (CT) and/or magnetic resonance (MR) scans in addition often to standard X-rays of the spine. From January 2017 to December 2019 we treated by O2-O3 technique 2450 patients (1455 Male,1210 Female, average age 62) affected by LBP and/or sciatica with clinical follow-up to 1 year. Our strategy follows a therapeutic protocol that provides:

- Visit and evaluation of the patient's inclusion and exclusion criteria.
- Treatment to time zero.
- Follow-up at 4 weeks and first patient's clinical evaluation after treatment; if satisfactory clinical and pain recovery has not been achieved (it means that the patient did not have a clinical improvement in ODI of 30% and in VAS scale of 3 score), a second and last treatment is performed at same time.
- Final follow-up at 12 months with clinical evaluation and ODI and VAS scale.

To date we were able to check up to 1860 patients with a clinical follow-up at 12 months.

After 1 year from the treatment the patients underwent clinical examination with ODI and VAS scale.

Inclusion criteria

The patient selection starts by clinical evaluation of lumbalgia with or without sciatalgia (Lasegue tests), CT or MRI imaging and VAS scale. We included patients with lumbalgia or sciatalgia for 3 months without response to medical conservative treatment in association with disk protrusion or hernia compatible with clinical symptoms. Clinical evaluation includes:

- refereed pain in a well-discriminated and constant cutaneous dermatome with positive Lasegue test
- pain score at least 5 or more on the visual analogue scale (VAS) at least 3 month no responder to classic conservative treatment (physical therapy, FANS, FAS)
- No major motor impairment with falling foot patients.

Exclusion criteria

- severe canal stenosis
- cono/cauda syndrome,
- calcified or extruted hernia,
- facet joint syndrome and facet joint arthrosic degeneration.
- LBP without radiculopathy associated

General absolute contra-indication to the procedure is referred allergy to proposed drugs and pregnancy bleeding disorder, G6PD deficiency, severe anaemia, recent myocardial infarction and history of mental disease.

CT or MRI (1.5T) imaging criteria

- presence of disc protrusion or small/medium-sized hernia in the median or paramedian/preforaminal position.
- free fragment herniated disc but only in case of no other contra-indication to treatment (see exclusion criteria).

Usually we use both TC and MRI to characterize the hernia and rule out, that it has calcifications. Calcifications in fact is a prognostically unfavorable factor for successful treatment.

Technical notes

After receiving written informed consent from the patients, the treatment is performed placing the patient in a prone position under CT or fluoroscopy guidance and once centering the level of treatment on the basis of the previous clinical and instrumental exam, the skin was disinfected using a polyvinylpyrrolidone iodine solution and the puncture was then performed omolateral to the seat of the symptomatology, using a needle with a caliber between 18G and 22G at a 45° angle from the skin plane, progressively under CT or fluoroscopy guidance, the needle is pushed up to the center of the disk. An aseptic technique was used to fill a 15 ml polyethylene syringe with a gas mixture of oxygen-ozone at a concentration 30 µg / ml, of which about 4 ml were injected intradiscally, then the needle retracted inject 11-12 ml in periradicular

and paravertebral soft tissues. Then, if necessary, using the same needle, an injection of corticosteroid (1mL, 40mg of metilprednisolone) and local anesthetic (1mL, 2% mepivacaine) in the intraforaminal space was performed. Patients were then kept under observation for around 30 min and subsequently discharged.

RESULTS

Of these patients at one-year follow-up:

- 1295 (69%) patients have benefited from the treatment and did not show clinical impairment and reported a quality of life improvement; they refer moreover a score in VAS less then 5.
- 408 (21%) patients have not benefited from treatment and did not report a quality of life improvement and the score in VAS is still above 5.
- 52 (2%) patients did not show up at follow-up because they underwent surgery.
- 95 (5%) patients did not show up at follow-up for no reason.

DISCUSSION

LBP is one of the most common and important health problems affecting human population in developed country, in high rate of the cases there are associated sciatic symptoms which, in about 90% of the time, are generated by disk herniation. The mechanism of LBP is very complex and multifactorial; it's likely due to direct nerve root compression or even just a compression of the epidural vassels afferent to the nervous structures; another possible mechanism of genesis of pain is the inflammatory factor that onset when there is a lesion of the disc or more generally an alteration of vertebral biomechanics.

The natural history of disc herniation or protrusion tends to be favorable. In fact, most patients enjoy satisfactory outcomes after conservative and symptomatic treatment because a herniated disc often undergoes spontaneous resorption, so the first medical approach is almost always conservative. The conservative treatment is therefore considered the best chose, in fact through simple rest for about 6 weeks we can see the reduction of the disk herniation by CT or MR scans within eight to nine months after the start of back pain.

Following these considerations, our study addressed the use one of the least mini-invasive technique currently available according to a conservative approach that is an intradiscal and peri foraminal injection of oxygen-ozone mixture. In oxygen-ozone therapy, ozone is administered in the form of an oxygen-ozone gas mixture, at nontoxic concentrations varying from 1 to 40 μg of ozone per milliliter of oxygen; it is colorless and odorless with a characteristic pungent smell.

Research has not yet defined with certainty the real mechanism of action of the O3-O3 mixture but, has formulated hypotheses on its mechanism of action and any biologic effects have been attributed to ozone: increased glycolysis; effects on red blood cells; effects on rheology; bactericidal, fungicide, and virustatic; immunomodulating action; and analgesic and anti-inflammatory effects. This broad spectrum of action explains the many indications for medical ozone administration. Regarding its use in LBP conservative treatment, it could act according to several combined mechanism of action:

- improve tissue oxygenation and reduction tissue inflammation by direct a pain mediator.
- direct action of O2-O3 mixture on the mucopolysaccadyres of nucleus polposus of the disc and volume reduction.
- microcirculation improvement.

A reduction in herniated disk volume is one of the therapeutic motives for intradiscal administration of medical ozone, as a reduction in disk size may reduce nerve root compression. Then another reason for using medical ozone to treat disk herniation is its analgesic and anti-inflammatory effects, which may counteract disk-induced pain. There are various techniques of Oxygen-Ozono therapy for the treatment of herniated discs; intradiscal, intraformational and paravertebral.

As mentioned previously, the technique we use is a mix of the intraformational and intradiscal one and it's based on the infiltration of O2-O3 inside the disc (about 3-4 ml) and at the level conjugation foramen (about 10ml), an anatomical narrowing through which nerves and vassels normally run. Then our

study protocol provides clinical follow-up at 1 month and 12 months; the clinical follow-up of our patients is usually obtained without imaging control. There were many imaging studies that reported no strong correlation between patient response and morphological changes on imaging; the patients may show significant clinical response with little or no changes in imaging.

There were no relevant complications during our treatment and our follow-up period (studies report adverse effects under about 0,1%), however the complications reported in literature are: paresthesia on the anterolateral portion of the left leg and foot, suggesting nerve irritation; thunderclap headache after O2O3 therapy related to pneumocephalus as a consequence of inadvertent intrathecal puncture; a few temporary episodes of impaired sensitivity; discitis (probably these complications were due to inadequate asepsis and iatrogenic inoculation of the bacteria during the injection). In addition to ozone therapy it has been demonstrated very efficiency in dentistry (25-31).

Our study shows an excellent response to the minimally invasive treatment with O2O3 of protrusions and herniated discs that fall within the selection criteria. In fact about 70% of the patients treated report a resolution of the symptoms; only 23% of patients did not report a clinical improvement with the achievement of the well-being threshold and only 2% of all patients decided with a surgical intervention while falling within the criteria for being treated with O2O3.

In conclusion the minimally invasive treatment with O2O3 is consolidating itself as an excellent therapeutic approach for patients who respect the inclusion and exclusion criteria. Our high success rate is largely attributable to strict patient selections; indeed being the genesis of LBP very complex and difficult to identify , there is a risk of treating patients with inappropriate mini-invasive techniques that will most likely not benefit from the treatment.

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Non-discogenic low back pain treated with oxygen-ozone: outcome in selected applications

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Low back pain and sciatica are highly debilitating conditions affecting all socioeconomic groups at an increasingly early age. They are caused by different often concomitant spinal disorders: disc or facet joint disease, spondylolysis (with or without listhesis), vertebral body and interapophyseal arthrosis, spinal stenosis, radicular and synovial cysts and, more rarely, infections and primary or metastatic cancer. Treatment of low back pain and/or sciatica requires an accurate diagnosis based on thorough history-taking and physical examination followed by appropriate imaging tests, namely computed tomography, and/or magnetic resonance scans in addition to standard and morphodynamics X-rays of the spine. In recent years, several reports have demonstrated the utility of oxygen-ozone therapy in reducing the size of herniated discs. The present study reports on the outcome of oxygen-ozone treatment in 576 patients with non-discogenic low back pain caused by degenerative disease of the posterior vertebral compartment (facet synovitis, Baastrup syndrome, spondylolysis and spondylolisthesis, facet degeneration).

Oxygen-ozone therapy was first used to treat herniated discs in 1985 (1-25). Since then, many literature studies have reported positive outcomes ranging from 75% to almost 90% in patient cohorts treated for low back pain with or without sciatica due to nerve root compression caused by a herniated intervertebral disc (1-34).

Low back pain and sciatica are highly debilitating conditions affecting all socioeconomic groups at an increasingly early age. Onset may be acute following injury or an unusual movement, or slowly progressive with gradually worsening pain. They are caused by different often concomitant spinal disorders: disc or facet joint disease, spondylolysis (with or without listhesis), vertebral body and interapophyseal arthrosis, spinal stenosis, radicular and synovial cysts, and, more rarely, infections and primary or metastic cancer (35, 36).

Treatment of low back pain and/or sciatica requires an accurate diagnosis based on thorough history-taking and physical examination followed by appropriate imaging tests, namely computed tomography (CT) and/or magnetic resonance (MR) scans in addition to standard X-rays of the spine).

The present study reports on the outcome of oxygen-ozone treatment in 576 selected patients with low back pain and/or sciatica due to causes other than disc herniation or protrusion. We focused on degenerative disease of the posterior vertebral compartment as a possible source of pain.

Facet synovitis

Facet synovitis is an inflammatory disease of the synovial membrane lining the spinal interapophyseal joints, usually caused by injury or recurrent microtraumas over time. The disease may also arise

Key words: oxygen-ozone, ozone therapy, facet synovitis, Baastrup syndrome, Spondylolysis, Spondylolisthesis, facet degeneration

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in young adults following excessive stress on the spine, typically induced by extreme sports.
In most cases, onset consists of acute lumbar pain with monolateral symptoms if only one joint is affected or bilateral low back pain in a band-like distribution if both facet joints are involved (6). Our cohort included only patients with post-traumatic conditions, excluding infectious synovitis and possible SAPHO syndrome (synovitis, acne, pustulosis, hyperostosis, osteitis) (31, 37). Facet synovitis diagnosis is often challenging and spine MR scans with contrast administration are helpful to confirm the diagnosis (6, 38, 39).

Baastrup syndrome

Baastrup syndrome or "kissing spines" was first described by the Danish radiologist Christian Baastrup in 1933. It is characterized by degenerative changes to the spinous processes of adjoining lumbar vertebrae that form new joints between them, often causing low back pain refractory to conservative anti-inflammatory and analgesic treatment. Women are mostly affected with an F:M ratio of 4:1 and the syndrome is usually diagnosed in the third decade of life.

Diagnosis is radiological: spinal X-ray will depict extreme hyperlordosis of the lumbar spine with degenerative changes and contact between adjacent spinous processes. The disease has a progressive course and worsening low back pain is an indication for MR study of the lumber spine. Scans should include fat saturation (Fat/Sat) sequences and possibly i.v. gadolinium administration to disclose any signs of acute interspinous inflammation (38, 39).

Spondylolysis and spondylolisthesis

Spondylolysis is a bony defect or fracture (stress fracture or trauma fracture) in the pars interarticularis of vertebral arch. The condition can be associated with forward shift of one vertebral body on another and this is called spondylolisthesis (a term coined by Kilian in 1854). The severity of spondylolysthesis is determined by the Meyerding classification in four grades depending on the degree of vertebral body slippage over the vertebra beneath it (Grade I 0-33%, Grade II 34-66%, Grade III 67-99%, Grade IV 100%).

Grade I spondylolysthesis is often an occasional finding and most patients are asymptomatic. However, a small percentage present low back pain with or without sciatica. These cases do not require surgery and treatment mainly involves physiotherapy and exercise. However, symptoms refractory to conservative therapy may warrant spinal stabilization surgery.

Facet degeneration

Between 25 and 45% of cases presenting chronic low back pain are due to facet joint syndrome that may be flanked by other conditions, thereby exacerbating the patient's pain and disability. Facet joint syndrome is commonly encountered in elderly patients. It has multiple causes, the most frequent being articular degenerative changes. The condition leads to a reduction or loss of joint cartilage, erosion of the adjacent bone margin, abnormal bone growth of the facet joints and articular processes, and lastly joint instability that may result in degenerative spinal subluxation (pseudospondylolisthesis). Back pain is caused by irritation to the sensory nerve endings in the facet joints and surrounding tissues innervated by the medial ramus of the posterior branch of the spinal nerve.

Candidates for interventional treatment are selected based on history-taking and clinical findings supported by diagnostic imaging. Standard X-rays are accompanied by CT scans to disclose the joint relations, growth and deformation of the articular bones and narrowed joint spaces, an indirect index of cartilage rearrangement. MR scans, namely T1 and T2-weighted Fat/Sat sequences with paramagnetic contrast administration, are used to depict the active inflammatory process involving the facet joint and surrounding tissues (6, 38, 39). The different treatments proposed to relieve pain caused by facet joint syndrome include intra-articular drug injections, nerve block with anesthetic and radiofrequency ablation.

MATERIALS AND METHODS

We selected 576 patients with different conditions involving the posterior vertebral compartment resulting

in low back pain with or without sciatica: 14 had facet synovitis, 47 had Baastrup syndrome, 92 had spondylolisthesis and 423 had facet degeneration. Diagnosis had been confirmed in all cases by MR scans on a Siemens Magnetom AERA 1.5 T system with SYNGO MR D13 software using standard sequences followed by Fat/Sat sequences without and with contrast administration (Table I).

All patients underwent CT-guided targeted injection of an oxygen-ozone gas mixture using the deep paravertebral infiltration technique. For the production of the oxygen-ozone mixture, a "Maxi Ozon Active International produced by Medica S.r.l." generator device was used, equipped with a digital photometer for the regulation of ozone concentrations, with check valves for the collection of the gaseous mixture in absolute sterility.

Infiltration technique

After receiving written informed consent from the patients, the injection level was established based on neuroradiological findings and clinical symptoms. The level was confirmed by preliminary CT scans with patients in a prone position to determine the point of needle entry. The skin was disinfected using a polyvinylpyrrolidone iodine solution after local anesthetic with ethyl chloride spray. CT-guided puncture was then performed using needles with a caliber between 22 G and 25 G. CT guidance also served to check the correct position of the needle in the joint in cases of facet synovitis and degeneration, in the interspinous space in patients with Baastrup syndrome and in the root canal and isthmic lysis, points to treat spondylolysis.

An aseptic technique was used to fill a 10 ml polyethylene syringe with a gas mixture of oxygen-ozone at a concentration of 25 µg/ml and between 3 ml and 5 ml was injected depending on the condition to treat, using a microporous filter to minimize the risk of contamination. Further CT scans were done after infiltration to confirm the correct distribution of the gas mixture in the treatment site. Patients were then kept under observation for around 30 min and subsequently discharged.

Facet synovitis

Most of the 14 patients treated with oxygen-ozone therapy for facet synovitis were relatively young (75% were under 40) aged between 17 and 56 years. In all cases, the onset of acute lumbar pain was linked to injury and the side of facet synovitis correlated with the side

Table I. *MRI scan protocol*

T2 SAG	(Thickness 3 mm, Gap 20%, TR 3500, TE 100, Fov 300 mm, Matrix 384 Pd HF)
T1 SAG	(Thickness 3 mm, Gap 20%, TR 550, TE 9.7, Fov 300 mm, Matrix 384 Pd HF)
T2 AX	(Thickness 3 mm, Gap 10%, TR 4280, TE 100, Fov 220 mm, Matrix 384 Pd AP)
T2 SAG pair	(Thickness 3 mm, Gap 20%, TR 3900, TE 100, Fov 300 mm, Matrix 384 Pd HF)
T1 COR	(Thickness 3 mm, Gap 15%, TR 420, TE 9.1, Fov 300 mm, Matrix 384 Pd RL)
T1 FS SAG	(Thickness 3 mm, Gap 20%, TR 2500, TE 39, Fov 300 mm, Matrix 384 Pd HF, Fat/Sat)
T1 FS AX	(Thickness 3 mm, Gap 20%, TR 3500, TE 39, Fov 220 mm, Matrix 384 Pd AP, Fat/Sat)

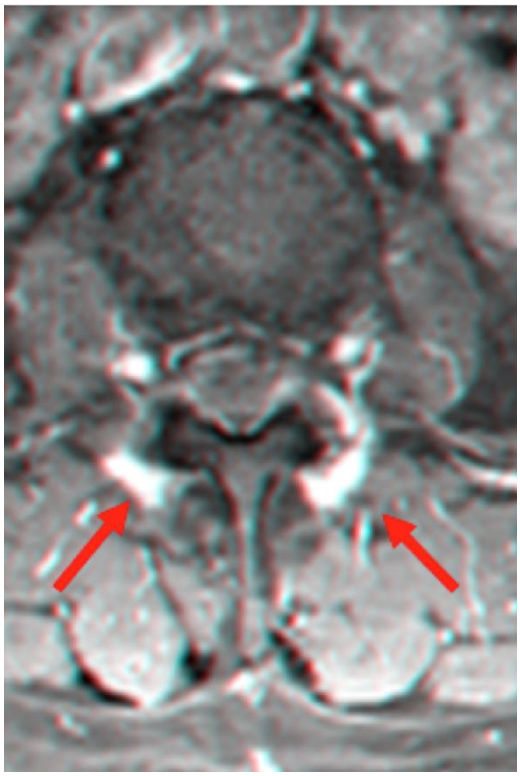


Fig. 1. *T1 Fat/Sat axial MR scan after gadolinium administration: bilateral post-traumatic facet synovitis (arrows).*

of the patient's clinical symptoms. No signs of ongoing infection were present. MR scans with Fat/Sat sequences after paramagnetic contrast (gadolinium) administration confirmed the diagnosis (Fig.1).

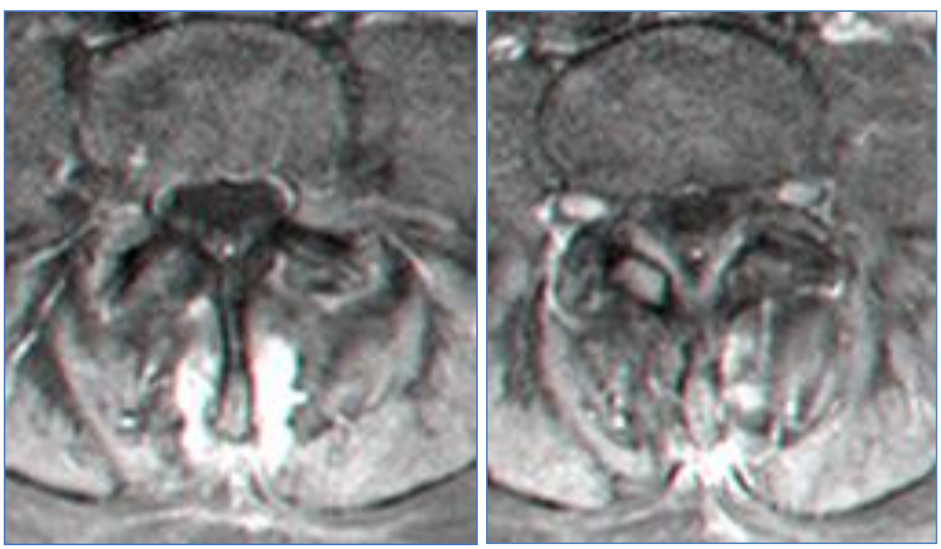
Baastrup syndrome

The 47 patients with Baastrup syndrome (28F-19M, aged range 51/83 years) were treated. In all cases, MR scans depicted inflammation in the interspinous ligament and perispinal soft tissues (Fig. 2A, B).

Patients presented pain in the medio-inferior dorsal and lumbosacral spine where degenerative changes were most evident, and all had an associated antalgic contracture of the paraspinal muscles. Four cc of the oxygen-ozone gas mixture were injected into the inflamed interspinous ligaments depicted on the MR scan using 23G needles followed by infiltrations into the paraspinal muscles affected by antalgic contracture.

Spondylolysis and spondylolisthesis

Given the widely acknowledged success of oxygen-ozone therapy for nerve root compression caused by herniated disc, we administered oxygen-ozone to 92 patients (39F-53M, aged ranging 21/63) with Grade I



a. **b.**

Fig. 2. *T1 Fat/Sat axial MR scan after gadolinium administration.* ***A)****: Before treatment contrast uptake is visible in the interspinous ligament and paraspinal soft tissues;* ***B)****: After treatment there is a marked reduction of inflammatory contrast uptake in the paraspinal soft tissues.*

spondylolysthesis (64 with L5 over S1 and 28 with L4 over L5) with bilateral isthmic lysis and associated disc disease (65 bilateral median-paramedian protrusions and 27 contained lateral disc herniations) and low back pain with or without sciatica. Thirty-seven patients also presented associated disc herniation. Diagnosis was confirmed in all cases by standard X-ray with morphodynamic (flexion-extension) tests and CT scan of the lumbosacral spine. Treatment was administered by CT-guided bilateral periganglionic ozone infiltration and injection of the gas mixture in close proximity to the lysis points in the neural arch (Fig. 3, 4).

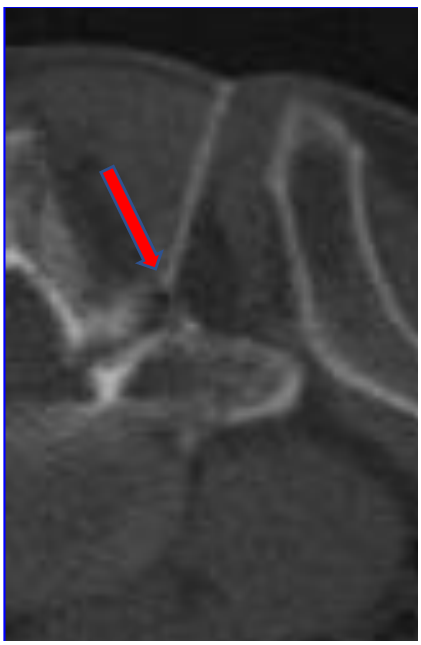


Fig. 3. *Intraprocedural CT scan with a bone algorithm confirms the correct positioning of the needle tip in close proximity to the isthmic lysis (arrow).*

Facet degeneration

We selected 423 patients with facet joint syndrome (262 M-161F, aged between 58 and 91 years, average age 73.2 years) for oxygen-ozone therapy. The clinical diagnosis was based on patient reports of pain during daily activities and following certain passive and active movements designed to mobilize the facet joints. The main symptoms are listed in Table II.

All patients were treated by CT-guided injection of 2-3cc of the oxygen-ozone gas mixture into or around the facet joint using a 22G needle (Fig. 5, 6). One level was treated in 264 patients (62.4%) of whom 159 (37.6%) only on the side of pain. Different levels were treated in the remaining 159 patients.

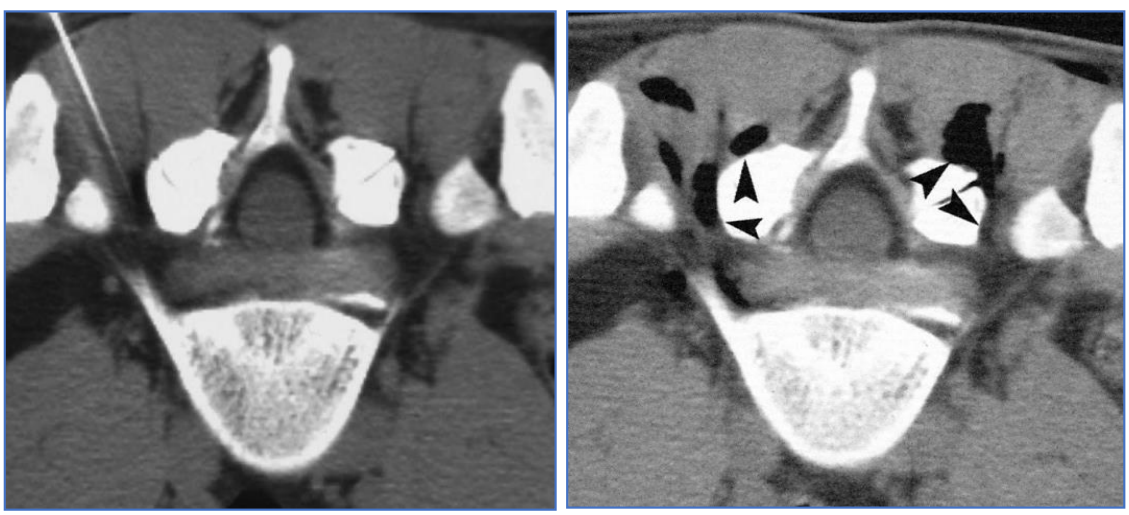


Fig. 4. *Intraprocedural spinal CT scan. **A**): Correct positioning of the needle in the periganglionic region; **B**): CT-guided control of the oxygen-ozone gas mixture injected into the foraminal region and facet joints (**arrowheads**).*

Table II. *Main symptoms in facet degeneration*

• Deep-seated low back pain, usually prevailing on one side
• Pain in the groin, thigh, buttock and iliac crest
• Pain applying finger pressure over the facet joints
• Worsening of pain induced by extension of the spine (back arching)
• Pain on rotating the trunk towards the affected side
• Worsening of pain after prolonged standing (++) and sitting (+)
• Improvement with bed rest
• Spinal rigidity
• Absence of lower extremity motor-sensory neurological deficits
• Typical features of facet degeneration disclosed by standard X-rays, CT and MR scans.

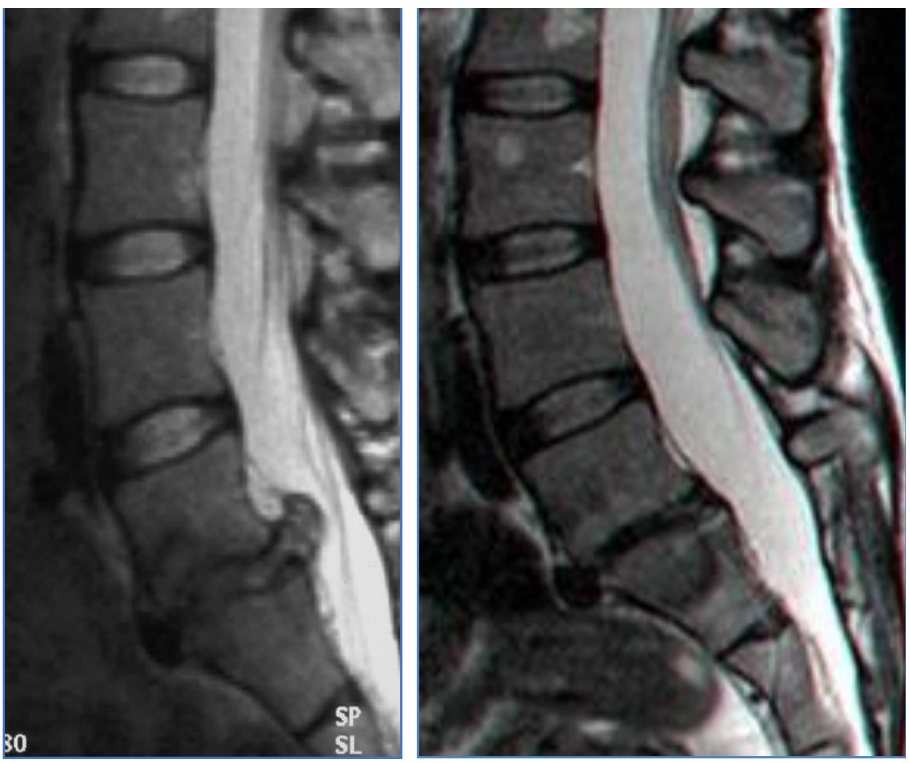


Fig. 5. *Intraprocedural CT scan in a patient with facet degeneration: control of the correct needle position in the facet joint interline and regular distribution of the gas oxygen-ozone gas mixture in and around the joint* ***(arrows)***

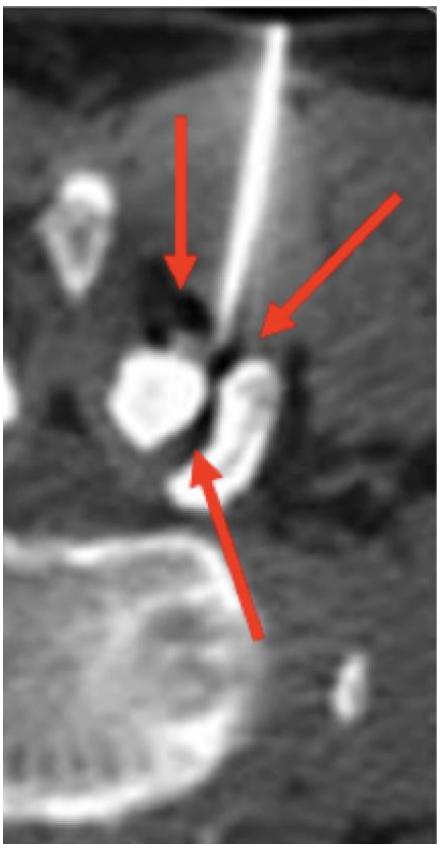


Fig. 6. *Intraprocedural CT scan in a patient with facet degeneration. **A**): Control of the correct needle position in the facet joint interline; **B**): Regular distribution of the gas oxygen-ozone gas mixture in and around the joint.*

RESULTS

Facet synovitis

Nine (64.3%) of the 14 patients treated had an almost complete resolution of pain after just one oxygen-ozone infiltration so no further injections were administered. The remaining five patients (35.7%) underwent a second infiltration ten days after the first treatment.

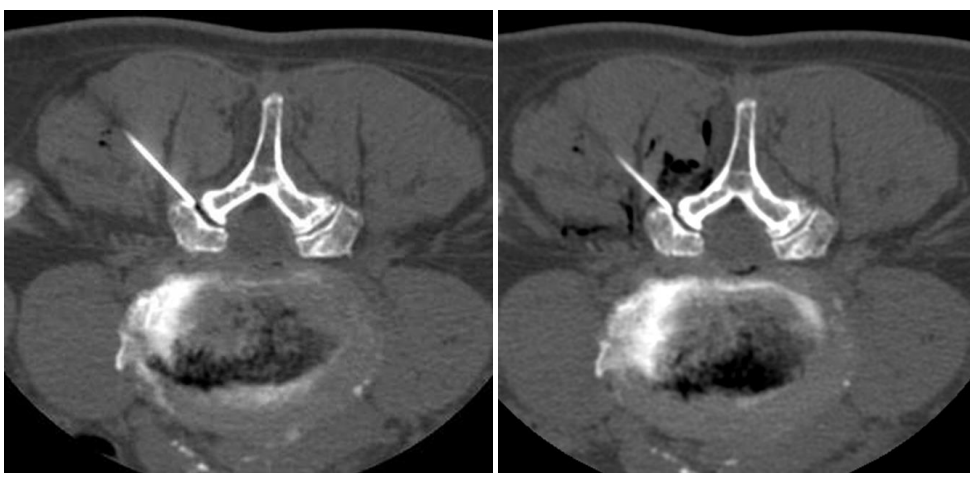
A multidisciplinary approach was adopted for this set of patients and a physiatrist devised a physiotherapy program for each case based on postural re-education. Six patients (42.9%) also underwent associated Tecar therapy.

Baastrup syndrome

All 47 patients treated reported a clear-cut improvement in pain one week after oxygen-ozone therapy, but all had a partial return of pain six months later. A second infiltration was performed in 28 cases (59.6%) again with a marked reduction of pain and a decrease in perispinal contrast uptake at MR follow-up (Fig. 2A, B). All patients underwent a physiotherapy and exercise program to prevent relapse (in five cases the physiatrist prescribed cycles of mud balneotherapy every 6-12 months).

Spondylolysis and spondylolisthesis

All 92 patients treated by oxygen-ozone therapy



a. **b.**

Fig. 7. *T2 sagittal MR scan before and after oxygen-ozone therapy in a patient with Meyerding Grade I spondylolysis and spondylolisthesis. **A**): Herniated disc in L5-S1 before treatment; **B**): Complete dehydration of the herniated disc at long-term follow-up after treatment.*

were followed up at one, three and six months after treatment using a modified MacNab scale. A complete resolution of pain was obtained in 58 patients (63%) after the first infiltration and clinical wellbeing persisted throughout the follow-up. The remaining 34 patients (37%) had only a partial remission of pain and the treatment was repeated after the first follow-up. These patients had a partial benefit from the second infiltration at the three-month follow-up after which eleven patients (32.3%) underwent a third oxygen-ozone injection.

The 34 patients with partial pain remission at six-month follow-up were referred for neurosurgical assessment and seven (7.6%) underwent spinal stabilization surgery, while the remaining 27 received only rehabilitation with a physiotherapy and exercise program.

Among the 37 patients (40.2%) with herniated disc associated with spondylolisthesis follow-up CT scan demonstrated complete dehydration of the herniated disc in 18 cases (48,6%), obviously with no change in the listhesis grade (Fig. 7).

Facet degeneration

At clinical follow-up ten days after oxygen-ozone infiltration in the 423 treated patients, 311 (73.5%) reported a major reduction of pain. The remaining 112 patients (26.5%) had an unsatisfactory therapeutic outcome. They declined further oxygen-ozone therapy and were referred to a physiatrist.

Of the 311 with an excellent or good initial outcome, 216 underwent clinical follow-up after three months. Of these, 143 (66.2%) reported a persistent good outcome, whereas the remaining 73 (33.8%) complained of symptom recurrence and underwent a second infiltration; 56 of these cases (76.7%) again had an excellent therapeutic outcome with an almost total resolution of pain.

DISCUSSION

The well-known mechanisms of action of oxygen-ozone infiltration are responsible for the excellent therapeutic outcome obtained in our cohort of patients with facet synovitis, Baastrup syndrome or facet degeneration and spondiylolysis/ spondylolysthesis.

An oxygen-ozone gas mixture injected into the site of inflammation exerts a strong anti-inflammatory and analgesic effect. This is ascribed to the capacity of ozone to normalize the cytokine and prostaglandin levels, increase superoxide dismutase, minimize reactive oxidant species and improve local periganglionic circulation with a eutrophic effect.

In patients with post-traumatic facet synovitis the effect of oxygen-ozone infiltration is almost immediate, and a single injection may be sufficient to resolve the condition. Instead, in patients with Baastrup syndrome the treatment proved effective in resolving pain with a secondary effect on muscle tone, reducing the antalgic contracture of the paraspinal muscles. This group of patients obtained a clinical benefit after just one targeted oxygen-ozone infiltration.

In line with the scientific literature, facet joint syndrome was the most common cause of non-discogenic back pain in our cohort of patients (73.4%). Facet degeneration is currently treated by infiltration of drugs or using highly effective radiofrequency techniques. We substituted targeted infiltration of steroids and anesthetic with injection of an oxygen-ozone gas mixture (Fig.6), obtaining a positive outcome in over 70% of cases, reserving radiofrequency ablation for patients failing to respond to ozone therapy.

Oxygen-ozone infiltration yielded pain relief in 58 of our patients (63%) with spondylolisthesis and spondylolysis. In the light of this, ozone therapy could be proposed to treat patients with Grade I listhesis, although a good outcome requires a multidisciplinary approach and the support of a physiatrist for postural re-education after oxygen-ozone infiltration to ensure long-term benefit. In addition to physiatry referral, patients failing to respond to ozone therapy should be assessed by a neurosurgeon for possible spinal stabilization surgery.

In recent years, the application of MR scans with fat saturation sequences and gadolinium administration in routine diagnostic imaging has helped clinicians establish and confirm the diagnosis in patients with a degenerative disease of the lumbar spine and low back pain, leading to targeted treatment strategies.

In patients with non-radicular low back pain, symptoms often stem from disease or degenerative changes in the posterior elements of the lumbar spine ("the posterior vertebral compartment"). Appropriate patient selection for oxygen-ozone therapy and the choice of the best targeted treatment yield good clinical results in most cases. This was demonstrated by the successful treatment outcomes in between 61% and 74.5% of our cohort.

The rapid resolution of pain with no complications, the ease of performing the technique and CT-guided control of infiltration suggest oxygen-ozone administration is a viable alternative to conservative therapies and probably the first choice treatment for disorders of the posterior vertebral compartment. In addition, oxygen-ozone therapy does not preclude subsequent infiltrations or surgical treatment.

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Efficacy ozone therapy in reducing oral infection of periodontal disease: a randomized clinical trial

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Periodontal diseases are among the most common infectious diseases in the world, caused by pathogenic bacteria that trigger innate, inflammatory, and adaptive immune responses, leading to the destruction of supporting periodontal tissues and, if untreated, tooth loss. The objective of this study was to explore the efficacy of medical device that produced ozonized water (Medica S.r.l. Bologna, Italy) in the treatment of chronic periodontitis of adult patients. A randomized controlled split-mouth study was carried out in ten patients (5 men and 5 women age 42-73 mean 55 ± 7) with a diagnosis of chronic periodontitis. None of these patients received any surgical or non-surgical periodontal therapy and demonstrated radiographic evidence of moderate bone loss. The mouth has been divided into upper right and left quadrants. The upper and lower right quadrants were treated with ultrasonic scaler, the left quadrants with ultrasonic scaler with ozonated water. Ten microbiological samples were collected from upper left quadrants and 10 from upper right quadrants from each patient. Microbiological samples were collected from the sites of the patients at baseline and at the 7th day. Twenty localized chronic periodontitis sites were selected (10 in left quadrants and 10 in right quadrants). After the treatment with ozonized water, a remarkable decrease in bacteria amount, both for some species and for the total count was observed in the left quadrants respect to right ones. Our study demonstrated the efficacy of the ozonized water in the management of moderate to severe chronic periodontitis.

Periodontal diseases are among the most common infectious diseases in the world, caused by pathogenic bacteria that trigger innate, inflammatory, and adaptive immune responses, leading to the destruction of supporting periodontal tissues and, if untreated, tooth loss. Dental plaque or biofilm is a sophisticated structure comprising of a large variety of inter-related oral species that develops on the surface of the teeth. Depending on several local and/or systemic modulator factors, these structures may acquire pathogenic features such as a cariogenic or a periodontal pathogenic profile (1). Accumulation and persistence of a biofilm comprising of high proportions of periodontal pathogens on teeth, lead to the development of gingivitis, an inflammation of the marginal gingiva characterized by oedema, redness and gingival bleeding (2).

Although the main stages of biofilm formation and gingival inflammation are observed in most people, the rate of microbial remodelling, host response and tissue destruction may vary a lot. For instance, in the experimental gingivitis model in humans, the onset of gingivitis varied among individuals, indicating that biofilm composition and host-related

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factors may determine biofilm pathogenicity and disease progression. In individuals with periodontal disease, periodontal pockets constitute reservoirs of periodontal pathogens that may promptly colonize other sites of the oral cavity, including healthy sulci.

Although there is still controversy whether these species are merely contaminants or transient members, strong evidence has been showing that they may indeed colonize the oral microbiota. In normal conditions of oral health, one should not expect these microorganisms to surmount in quantitative proportions, to the very well adapted oral species. On the other hand, these pathogenic species may increase significantly in frequency and counts in individuals presenting oral infections such as periodontitis, poor hygiene and/ or immunosuppression. The same way oral pathogens have been implicated in extra-oral infections, high levels of medically important pathogens in the periodontitis-associated microbiota may pose a risk for systemic dissemination and development of infections at distant body sites, particularly in immunocompromised individuals (3, 4).

Good oral hygiene has the aim to control bacterial plaque, but when the patient's attention to oral hygiene decreases, it is possible to experience a recurrence of the disease. In addition, periodontal disease presents a characteristic trend with periods of remission and exacerbation of the disease. Scaling and root planning are efficacy, but a better microbiological control of oral microbiota can be reached by the administration of ozonated water in association. The aim of this study was to investigate the efficacy of ultrasonic scaling with ozonated water, acting as bactericidal in the management of moderate to severe chronic periodontitis.

Ozone therapy

The main use of ozone in dentistry is relays on its antimicrobial properties. It is proved to be effective against both Gram-positive and Gram-negative bacteria, viruses and fungi (5). Nagayoshi et al. (5) examined the effect of ozonated water on oral microorganisms and dental plaque. Dental plaque samples were treated with 4mL of ozonated water for 10 seconds. They observed that ozonated water was effective for killing gram -positive and -negative oral microorganisms and oral Candida albicans in pure culture as well as bacteria in plaque biofilm and therefore might be useful to control oral infectious microorganisms in dental plaque.

MATERIALS AND METHODS

Ten patients (5 men and 5 women) were randomly selected with a diagnosis of chronic periodontitis. Patients who qualified for the study were in the age group of age 42-73 (mean 55 ± 7) and did not receive any surgical or non-surgical periodontal therapy. The patients were excluded from the study if they meet any of the following criteria: pregnancy; a history of taking antibiotics or using anti-bacterial mouth rinses for the past 6 months; has teeth with furcation involvement; patients with a history of smoking, and drug or alcohol abuse. The subjects that volunteered to participate in the study, signed consent forms and followed a detailed verbal description of the procedure. This study was conducted in accordance with the Declaration of Helsinki.

Clinical methods

Twenty sites (distal pockets of the left and right first upper molar) were selected from the 10 patients. Each selected site was subjected to microbial analysis. At the first visit, after recording the clinical parameters at each site in selected patients at baseline, microbiological samples were collected from pre-selected sites. The mouth has been divided into upper right and left quadrants. The upper and lower right quadrants were treated with ultrasonic scaler, the left quadrants with ultrasonic scaler plus ozonated water (4 mg/ml in distilled water) (Medica s.r.l. Bologna, Italy). Microbiological samples were collected from the sites of the patients at baseline and at the 7th day. For the collection of subgingival samples, the sites were isolated using cotton rolls. Sterile absorbable paper points (size 60) were used for the collection of sub-gingival samples and were immediately transferred to microbiological laboratory for processing. The microorganisms processed were the three bacterial species more involved in periodontitis that constitute the red complex group: Porphyromonas gingivalis, Tannerella forsythia, and Treponema denticola.

Several methods have been used for microbiological testing in periodontitis (7). However, many techniques

have not been fully accepted due to low sensitivity or specificity, moreover sometimes they are slow, expensive and laborious. In this study we have used the (LAB-test®) developed by the LAB corporation (Ferrara, Italy), which is rapid and sensitive, allowing for it to detect and quantify the three bacterial species more involved in periodontitis that constituted the red complex group: Porphyromonas gingivalis, Tannerella forsythia, and Treponema denticola. Both P. gingivalis and T. denticola occur concomitantly with the clinical signs of periodontal destruction. They appear closely 'linked' topologically in the developing biofilm and are considered the first pathogens involved in the clinical destruction of periodontal tissues.

Real-Time Polymerase Chain Reaction

Primers and probes oligonucleotides have been designed based on 16S rRNA gene sequences of the Human Oral Microbiome Database (HOMD 16S rRNA RefSeq Version 10.1) counting 845 entries. All the sequences were aligned to find either consensus sequences. For each sample, two real-time polymerase chain reaction (PCR) runs were performed. The first reaction quantified the total amount of bacteria using two degenerate primers and a single probe, matching a highly conserved sequence of the 16S ribosomal RNA gene. The second reaction detected and quantified the three red complex bacteria in a multiplex PCR. The reaction included a total of six primers and three probes that were highly specific for each specie. Oligonucleotide concentrations and PCR conditions were optimized to ensure sensitivity, specificity and no inhibitions in case of unbalanced target amounts. Absolute quantification assays were performed by using the Applied Biosystems 7500 Sequence Detection System. The amplification profile was initiated by a 10min incubation period at 95°C to activate polymerase, followed by a two-step amplification of 15s at 95°C and

Table I. *Bacterial loadings of the twenty sites (distal pockets of the left and right first upper molar) selected from the 10 patients.*

Side	Bacteria	p-value
Right side (Ultrasonic scaler)	AA - AA1	.342
	PG - PG1	.221
	TF - TF1	.269
	TD - TD1	.203
	FN - FN1	.047
	CR - CR1	.568
	TBL - TBL1	.000
Left side (Ultrasonic scaler plus ozonated water)	AA - AA1	.343
	PG - PG1	.007
	TF - TF1	.018
	TD - TD1	.038
	FN - FN1	.002
	CR - CR1	.041
	TBL - TBL1	.000

The mouth has been divided into upper right and left quadrants. The upper and lower right quadrants were treated with ultrasonic scaler, the left quadrants with ultrasonic scaler plus ozonated water (4 mg/ml in distilled water) (Medica s.r.l. Bologna, Italy). ***AA:*** *Aggregatibacter Actinomycetemcomitans.* ***PG:*** *Porphyromonas gingivalis. TF: Tannerella forsythia* ***TD:*** *Treponema denticola.* ***FN:*** *Fusobacteriun Nucleatum.* ***CR:*** *Campylobacter rectus.* ***TBL:*** *Total bacterial. 1 – after treatment.*

60 s at 57°C for 40 cycles. All the experiments were performed including non-template controls to exclude reagents contamination.

Plasmids containing synthetic DNA target sequences (Eurofin MWG Operon, Ebersberg Germany) were used as a standard for the quantitative analysis. Standard curves for each target were constructed in a triplex reaction, by using a mix of the same amount of plasmids, in serial dilutions ranging from 101 to 107 copies. There was a linear relationship between the threshold cycle values plotted against the log of the copy number over the entire range of dilutions. The copy numbers for individual plasmid preparations was estimated using a Thermo NanoDrop spectrophotometer.

The absolute quantification of total bacterial genome copies in samples allowed for the calculation of the relative amount of red complex species. To prevent samples and polymerase chain reaction contamination, plasmid purification and handling were performed in a separate laboratory with dedicated pipettes.

Statistical analysis

Descriptive statistics was registered using Microsoft Excel spreadsheets. Paired T-test from Spss program was used to statistically evaluate the change in specific bacteria loading before and after treatment.

RESULTS

After the treatment with ultrasonic scaler (right side), a decrease in total bacteria amount was observed, but not in the individual microbic species. After the treatment with ultrasonic scaler plus ozonated water (Medica s.r.l. Bologna, Italy) (left side), a remarkable decrease in total bacteria amount and in the individual microbic species was observed (Table 1). Ozonated water (Medica s.r.l. Bologna, Italy) did not show any side effects, discomfort, or adverse reactions over time. No patient reported pain, burning, tingling sensation or numbness. Results demonstrated a reduction of microbial concentration after treatment with ozonized water.

DISCUSSION

The concept of periodontal disease has changed considerably over the years. The putative pathogens associated with periodontal diseases are susceptible to a variety of antiseptics and antibiotics. Non-surgical periodontal therapy has long been documented to preserve the natural dentition by achieving and maintaining a healthy periodontium. According to the current state of knowledge, species such as the red complex organisms, play a major role in the pathogenesis of periodontal diseases. The gold standard of periodontal therapy has been scaling and root planing; however, various adjunctives, like ozonized water, have been used in conjunction to this to improve the therapeutic results.

The use of ozonized water, along with mechano-therapy dramatically improves clinical results, and at the same time is free from its inherent adverse effects and disadvantages. Ozonized water into the pocket, demonstrates bactericidal property for most periopathogens, and at the same time, exhibits negligible impact on the microflora residing in other parts of the body.

The effects of mechanical instrumentation may be improved with the association of ozonized water, providing an additional benefit in controlling the progress of disease. Nagayoshi et al (5) tested the efficacy of three different concentrations of ozonized water (0.5, 2 and 4 mg/ml in distilled water) on the time-dependent inactivation of cariogenic, periodontopathogenic and endodontopathogenic microbes (Streptococcus, Porphyromonas gingivalis and endodontalis, Actinomyces actinomycetemcomitans, Candida albicans) in culture and in biofilms. They confirm that ozonated water was highly effective in killing of both gram positive and gram-negative micro-organisms. Depending on the dosage, the oral microbes were inactivated after 10 seconds. Gram negative anaerobes, such as Porphyromonas endodontalis and Porphyromonas gingivalis were substantially more sensitive to ozonated water than gram positive oral streptococci and Candida albicans in pure culture. Furthermore, ozonated water had strong bactericidal activity against bacteria in plaque biofilm. In addition, ozonated water inhibited the accumulation of experimental dental plaque in vitro.

Ramzy et al (6) irrigated the periodontal pockets

by ozonized water in 22 patients suffering from aggressive periodontitis (age range from 13 to 25 years). Periodontal pockets were irrigated with 150 ml of ozonated water over 5 to 10 minutes once weekly, for a clinical four weeks study, using a blunt tipped sterile plastic syringe. High significant improvement regarding pocket depth, plaque index, gingival index and bacterial count was recorded related to quadrants treated by scaling and root planning together with ozone application. They also reported significant reduction in bacterial count in sites treated with ozonized water.

In the study by Karapetian et al, (8) peri-implantitis treatment with conventional, surgical and ozone therapy methods was investigated, and it was found that the most effective bacteria reduction was in the ozone-treated patient group. The authors concluded that the main challenge seems to be the decontamination of the implant surface, its surrounding tissue and the prevention of recolonization with periodontal pathogenic bacteria.

Kshitish and Laxman (9) conducted a randomized, double-blind, crossover split-mouth study on 16 patients suffering from generalized chronic periodontitis. The study period of 18 days was divided into two time-intervals, i.e. baseline (0 days) to 7th day, with a washout period of 4 days followed by a second time interval of 7 days. Subgingival irrigation of each half of the mouth with either ozone or chlorhexidine was done at different time intervals. They observed a higher percentage of reduction in plaque index (12%), gingival index (29%) and bleeding index (26%) using ozone irrigation as compared to chlorhexidine. The percentile reduction of AA (25%) using ozone was appreciable as compared to no change in AA occurrence using chlorhexidine.

The antifungal effect of ozone from baseline (37%) to 7th day (12.5%) was pronounced during the study period, unlike chlorexidine did not demonstrate any antifungal effect. No antiviral property of ozone was observed. The antiviral efficacy of chlorhexidine was better than that of ozone. They concluded that despite the substantivity of chlorhexidine, the single irrigation of ozone is quite effective to inactivate microorganisms. This study allows to conclude that ozonated water is effective in dentistry (10-16). The results of this investigation demonstrated an overall decreasing in bacterial loading and in the specific microbic species after ozonized water treatment.

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Psycho-social characteristics in patient with disease treated with CT-guided oxygen ozone therapy: quality of life, coping strategy and mood state

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The pathologies of the musculoskeletal apparatus are the most common cause of chronic diseases, with a huge impact on people and society. Scientific literature has discovered how experiencing chronic pain directly affects peoples' well-being, lifestyle, social relationships and can also cause psychological distress. The present study aims to investigate pain experience in patients with hernias or protrusions of the cervical and lumbosacral tract on a sample of 120 patients, recruited from patients of Poliambulatorio Oberdan, medical centre in Brescia (Italy) specialized in physical rehabilitation and CT-guided oxygen ozone therapy. In a bio-psychosocial perspective, the research aimed to investigate how the perception of pain, the mood state associated with it, the coping strategies adopted and the quality of life differ according to each patient's gender and to the more or less prolonged use of pain medication. The data were collected by means of medical and psychological anamnestic interviews and self-report tests (WHOQOL-BREF, COPE-NVI, POMS). The quantitative analysis, carried out through SPSS 25 (2017) software, showed how functional impairment of one's autonomy (walking, driving) affects mood states. In particular, the female sample expressed a more deflected mood, despite the greater use of relational and/or transcendent support (coping strategies) compared to men. The study suggests that the greater impairment of the moods of women can be attributed both to the caregiving role they play, which often results in a greater fatigue and difficulties in redefining this role following the algic condition, and more general differences in the expression of suffering, which, on a cultural level, sees men emotionally coerced. The analysis also shows how taking pain medication for a long period of time has a negative impact on the quality of life. The results suggest that the patients treated with analgesic therapy tend to adopt avoidant coping styles, which usually escalate into postponement of the time when dealing with a stressful situation and, if used in the long run, may lead to worsening health condition.

The World Health Organisation (WHO) refers to good health as "dynamic state of complete physical, mental, social and spiritual well-being, not merely the absence of disease" (1). This perspective, which promotes a bio-psychosocial model (2), does not reduce the concept of health to biomedical standards, but supports the importance of taking into account both physical conditions and various conditions of psychological nature. Since the middle of the last century, many authors have questioned

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the relationships between biological, psychological and social variables and how they can determine wellness, health and quality of life. With reference to the state of physical pain, studies have found that experiencing chronic pain directly affects the well-being of patients, as well as their ability to maintain an autonomous lifestyle, their productivity and their social relationships (3-6). Moreover, it turned out that chronic pain is associated with expressions of psychological distress, such as mood disorders, pervasive anger and depression and debilitating anxiety conditions (7-10).

Since socio-demographic variables are conditions linked to the perception of pain, they need to be considered as central factors in the study of pain and of psychological suffering (8, 11). Among these variables, gender plays a key role. Within the population of patients with pain attributable to the musculoskeletal system, men and women differ, for example, in the respective emotional manifestations related to pain. In this regard, it seems that women have a more deflected mood than men and that depressive manifestations are twice more likely to occur in females (12-15). Men and women with algic symptomatology are also different in the adoption of coping strategies (16), namely methods used, to deal with situations perceived as difficult and stressful. Coping is particularly important in reference to physical pain since the use of passive strategies (entrusting the management of one's own pain to external sources, hoping but not acting proactively, praying or letting areas of life other than physical health be compromised by pain) increases the risk of developing disabling conditions (17, 18). Studies confirm how women tend to face chronic pain conditions related to musculoskeletal system with passive coping (16, 19). Bartley and Fillingim have conducted a review of the literature concerning the gender differences between patients who experience pain, concluding that epidemiological and clinical results clearly demonstrate that women are exposed to an increased risk of developing chronic pain and that some evidence suggests that women experience more disabling clinical pain (20).

It has been shown that chronic pain has a negative impact on the quality of life (4, 20, 21); this effect is particularly intense in the female population, especially regarding the quality of life related to physical dimensions (22). An additional factor that seems to affect in a decisive way the quality of life of people with algic symptomatology is intake of pain medication. The uses of pain medication was found to be associated with a greater self-perceived disability, conceived as the compromise of important daily functions (23).

In view of the explored literature, the current research sets itself the following objectives; firstly to analyse gender differences with regard to pain, to psychological characteristics associated with the latter, coping styles, mood and to perception of one's own quality of life, conceived in the sense of bio-psycho-social meaning. Then to verify the impact of the intake of pain medication on the quality of life, coping styles and mood. Lastly to study the possible relationship between the compromise of the different overall functioning areas of patients and their mood.

MATERIALS AND METHODS

Participants

The present study was conducted on a sample of 120 people recruited from the patients of Poliambulatorio Oberdan between the ages of 21 to 79. (M = 49.09; SD = 11.43). 62.50% of those who took part in the study were male, while the remaining 37.50% were female. As for the qualification obtained, 25% of the people interviewed had only a primary school leaving certificate, 21.40% had a lower middle school leaving certificate, 55% had a higher middle school leaving certificate and 20% had graduate or post-graduate specialization.

Seventy-five percent of participants claimed to be married or live together with a partner, while 12.50% of the sample declared to be single and 12.50% to be separated, divorced or a widower. Most of the people interviewed said they were entrepreneurs / craftsmen / traders (27.50%). The professions most frequently indicated by the sample include clerk (22.50%), worker (22.50%), teacher / care profession (3.33%). Of the sample 6.67% said they are currently retired and 5% stated they were students or housewives.

Instruments

Anamnestic interviews of a medical and psychological nature and self-report test batteries were used within this study.

Medical history

A specifically created checklist was used to collect anamnestic medical data. The data - shown below were collected during an interview conducted by a physician of the facility:

- Lumbar and/or cervical discopathy (hernia and/or protrusion) reason for access.
- Possible presence of paraesthesia.
- Possible prior treatments.
- Possible use of pain medication/anti-infiammatories/ cortisone for algic symptomatology.
- Type of pain experienced (acute-subacute, chronic in the acute phase).

Psychological anamnesis

Specifically created checklist was used to collect psychological medical data. The data - shown below - were collected during an interview conducted by a psychologist of the facility:

- Perception of pain at present time, measured by the Numeric Pain Rating Scale (10 point Likert scale).
- Areas possibly compromised by the symptomatology at present time: autonomy, work, relationship, sleeping.

Self-report scales

Quality of life

To investigate the quality of life, the WHOQOL-BREF (De Girolamo et al. 2000) has been used, a self-report questionnaire composed of 26 items which investigate 4 domains: physical health e.g. 'To what extend does physical pain stop you doing things you have to do?', psychological health e.g. 'Can you focus on the things you do', social relationships e.g. 'Are you satisfied with your personal relationships with other people?' and environment e.g. 'Is the environment in which you live safe for your health?' and single items related to general health and quality of life.

The items require participants to estimate the perception of the phenomena in question through the use of a 5-point Likert scale: 1 meaning 'Not at all', 2: 'A little', 3:'Average', 4: 'Much', 5: 'Very much'.

Coping strategies

COPE-NVI (Sica et al. 2008) has been administered to investigate the *coping* strategies predominantly used. The *self-report* questionnaire consists of 60 *items* which investigate 5 dimensions related to how people deal with difficulties: social support (e.g. 'I talk to someone about my feelings'), avoidance strategies (e.g. 'I devote myself to work or other activities so as not to think about what worries me'), positive attitude (e.g. 'I try to use this experience to grow as a person'), problem solving (e.g. 'I prepare an action plan'), transcendent orientation (e.g. 'I put my hope in God'). Items composing the test require to express the frequency with which the respondents implement the action in question through the use of a 4 point Likert scale (1: 'Usually I don't do it', 2: 'I do it sometimes', 3: 'I do it with a certain frequency', 4: 'I always do it').

Affective states

POMS (Farnè et al. 1991) has been proposed in order to measure the affective states experienced by the participants in recent times. This self-report tool, composed of 58 adjectives, implies that the participants express about a 5 point Likert scale (0: 'Not at all', 1: 'A little', 2: 'A middle way', 3: 'A lot', 4: 'Very much') of how they have felt in the last week.

The test investigates 6 factors: Tension-Anxiety (e.g. 'Nervous', 'Restless'), Depression-Dejection e.g. ('Unhappy', 'Worthy of contempt'), Anger-Hostility (e.g. 'Grumpy', 'Resentful'), Vigour-Activity e.g. ('Full of life', 'In a good mood'), Fatigue - Inertia (e.g. 'Exhausted', 'Lazy'), Confusion-Bewilderment (e.g. 'Unable to focus', 'Puzzled').

Within the questionnaire, after completion of privacy form, socio-demographical variables were also investigated: gender, age, qualification, marital status, job, residence geographical area.

Procedures

Participants in this research project were identified among the people who had set up an initial appointment to undertake a therapeutic path within Poliambulatorio Oberdan. These people have been contacted by phone; on that occasion, researchers have explained the aim of the project, investigating their will to participate in it. Once in the clinic, participants signed an informed consent form,

aimed at guaranteeing the anonymity of the collected data; when the adhesion to the project was ascertained, the questionnaire was administered to the participants. Subsequently, the procedure provided for subjects involved to meet different professional figures (physician and psychologist) for the collection of anamnestic data. The data collection process began on January 30th, 2018 and finished on January 30th, 2019.

The collected data were subsequently entered into a database and analyzed using SPSS 25 (2017) software through which the statistical analysis necessary to pursue the project's purposes were conducted. Specifically, descriptive analysis relating to data frequency, analysis of variance (ANOVA), correlations (Chi², Pearson correlation) and linear regressions were conducted.

RESULTS

To report the symptomatology of the patients who had access to the clinic, frequency analysis on the medical records of the sample has been carried out. In relation to these results, it can be said that 65% of the patients only had hernia or a lumbosacral protrusion, 17.50% only hernia or a cervical protrusion and as many people had hernias or protrusions in several areas of the spine (17.50%).

Furthermore, most of the sample declared the chronicity of their own symptomatology (existence of more than three months) (65.83%). 19.17% of the participants had a chronic condition in the acute phase and 15% complained a recent occurrence of the symptomatology, considering the latter in the acute-subacute phase (present for less than three months).

Of the participants 88% reported that they have subjected themselves to treatments before entering the department, to deal with their pathology. About pain management, just over half of the sample reported of being, now on pain medication 51.67%. 58.33% of the patients had declared the presence of paraesthesia in the limbs.

Compared to the Numeric Pain Rating Scale, the sample reported a moderate pain level equal to 5.29 (SD = 2.20). The picture that emerges shows a good level of variability: within the scale, there are no values which have not been chosen by at least one participant.

Compared to the functional limitations resulting from the clinical situation, the domain more frequently identified, as compromised by the sample 74.17% turned out to be personal autonomy. Of the participants 70% have noticed a limitation in work or study, 50% reported an alteration of sleep activity, 39.17% felt their own sports activity limited, whereas 30% reported an impairment in reference to their social relationships.

Regarding the self-report instruments filled in, the mean values of the scale which make up WHOQOL

Table. I. *WHOQOL BREF*

	Reference range	Mean	Standard deviation
Overall quality of life	1-5	3.38	0.83
General health status	1-5	2.88	0.89
Physical domain	7-35	21.99	4.35
Psychological domain	6-30	20.26	3.40
Social relationships	3-15	10.88	1.77
Environment	8-40	27.49	3.58

BREF (Table. I), POMS (Table. II) and COPE NVI (Table. III) are given below.

From the analysis carried out, no relationship between gender and the present pathology (cervical hernia/protrusion, lumbosacral hernia/protrusion, multiple hernias/protrusions) emerges. In fact, the combination was not found to be meaningful (p = 0.25). On the contrary, men and women differ when other variables are considered.

A series of analysis of the univariate variance was conducted to test the possible presence of significant differences towards some constructions measured through self-report instrument scales. With regard to welfare indicators, a significant impact of the gender on the perception of the physical domain (F(1.118) = 18.50, p<0,001, eta squared = 0.14; M_{men} = 23.23, M_{women} = 19.93), psychological domain (F(1.118) = 11.93, p<0.01, eta squared = 0.09; M_{men} = 21.05, M_{women} = 18.93), 'overall quality of life' (F(1.118) = 4.17, p<0.05, eta squared = 0.03; M_{men} = 3.49, M_{women} = 3.18) and 'overall health' (F(1.118) = 13.95, p<0.001, eta squared = 0.11; M_{men} = 3.11, M_{women} = 2.51) emerges. Regarding perceived well-being, therefore, the results seem to point out some gender differences such that men perceive higher levels of psychological and physical well-being compared to women, as it has been pointed out in literature.

With regard to coping strategies (that is the methods used by people to face situations perceived as difficult and stressful), the variance analysis points

Table. II. *POMS*

	Normative range	Mean	Standard deviation
Fatigue	2-10	6.89	4.81
Tension	4-15	10.69	6.74
Depression	0-17	9.62	10.14
Vigour	10-21	13.57	7.13
Confusion	4-12	7.46	4.41
Anger	8-22	9.87	9.55

Table. III. *COPE NVI*

	Reference range	Mean	Standard deviation
Social support	12-48	27.18	6.47
Avoidance strategies	16-64	21.98	4.29
Positive attitude	12-48	29.68	5.80
Problem solving	12.48	32.30	7.11
Turning to religion	8-32	19.87	4.99

out a significant impact of the gender on the social support ($F(1.118) = 19.75$, $p<0.001$, eta squared = 0.14; $M_{men} = 25.29$, $M_{women} = 30.33$) and turning to religion ($F(1.118) = 7.38$, $p<0.01$, eta squared = 0.06; $M_{men} = 18.93$, $M_{women} = 21.42$). It seems that women, compared to men, confronted with difficult situations, rely more on an external reference point.

In relation to the effectiveness felt in the last week, data pointed out a significant influence in gender for fatigue ($F(1.118) = 6.35$, $p<0.05$, eta squared = 0.05; $M_{men} = 6.05$, $M_{women} = 8.29$), depression ($F(1.118) = 6.73$, $p<0.05$, eta squared 0.05; $M_{men} = 7.80$, $M_{women} = 12.64$), and vigour ($F(1.118) = 17.06$, $p<0.001$, eta squared = 0.13; $M_{men} = 15.52$, $M_{women} = 10.31$). These results show mood characteristics, more deflected in women compared to men. On the other hand, there are no differences regarding tension, anger and confusion.

To examine the effect of intake of pain medication on the quality of life of the patients, a series of one-way analysis of variance has been carried out. The test of the effects between the individuals highlights a significant influence of the intake of pain medication on the perception of the overall quality of life ($F(1.118) = 7.66$, $p<0,01$, eta squared = 0.06; $M_{Intake} = 3.18$, $M_{Not\ intake} = 3.59$), on the physical domain ($F(1.118) = 8.o1$, $p<0.01$, eta squared = 0.06; $M_{Intake} = 20.94$, $M_{Not\ intake} = 23.12$) and on the psychological domain ($F(1.118) = 6.98$, $p<0.01$, eta squared = 0,06; $M_{Intake} = 19,48$, $M_{Not\ intake} = 21,09$). It appears, therefore, that the intake of pain medication, although giving relief to the symptomatology, negatively affects the perception of the quality of life. Moreover, this consumption leads to the adaption of avoiding strategies as a coping style to facing the difficulties of life ($F(1.118) = 4.12$, $p<0.05$, eta squared = 0.03; $M_{Intake} = 22,74$, $M_{Not\ intake} = 21.17$), while not directly influencing the mood states experienced the week before the filling in of the questionnaire.

To evaluate the impact of compromise in everyday life of the mood state of the patients, a series of one-way analysis of variance has been carried out. The effect test underlines a significant influence of the impairment of autonomy of fatigue ($F(1.118) = 7.53$, $p<0.01$, eta squared = 0.06; $M_{Comopromised\ Aut} = 7,58$, $M_{Not\ Compromised\ Aut} = 4,90$), tension ($F(1.118) = 6.49$, $p<0.05$, eta squared = 0.05; $M_{Compromised\ Aut} = 11.60$, $M_{Not\ Compromised\ Aut} = 8.10$), depression ($F(1.118) = 4.00$, $p<0.05$, eta squared = 0.03; $M_{Compromised\ Aut} = 10.70$, $M_{Not\ compromised\ Aut} = 6.52$) and anger ($F(1.118) = 6.23$, $p<0.05$, eta squared = 0.05; $M_{Compromised\ Aut} = 11.12$, $M_{Not\ Compromised\ Aut} = 6.26$). These results show that the loss of their own autonomy negatively affects the mood state of the patients, causing deflected mood, more tension, fatigue and anger. The remaining impairment domains were inconsistent to define mood variation of the patients.

DISCUSSION

Starting with the scientific literature, which underlines how the perception of pain affects the daily functioning of people, causing also psychological suffering, this study has set itself the goal of examining the areas of daily life compromised by pain in order to investigate to what extent the latter affects mood states.

Notably, it emerged that functional impairment of autonomy (walking, driving) influences mood states. In particular, the points for 'fatigue', 'depression', 'tension' and 'anger' are higher, while emotional distress was not significant for aspects such as work, sleeping, relationships and sport. It is possible to assume that the impairment of one's own physical autonomy, affecting the degree of independence in daily activities, impacts self-perception.

The data collected in this research also allows us to confirm the presence of a greater impairment reported by the female population regarding the quality of life (24). Faced with the same medical conditions, it appears that women, compared to the male population, tend to assess their physical, psychological and relational health as more affected.

The results of this study confirm that the female population with algic symptomatology tends to have a more deflected mood compared to men, reporting higher levels of fatigue, depression, and less vigour (11-15).

Consistently with the existing studies (25), women's greater suffering in living with this physical condition could be attributable to more general gender differences in emotional communicativeness

(emotional expression, emotional management, acknowledgement and emotional understanding).

The data of this research also allow us to confirm what is reported in the literature about coping strategies adopted by men and women with algic symptomatology. If, in fact, studies show that women tend to adopt passive coping styles (behaviours for which the individual entrusts responsibility for managing the difficulty to external sources or allows other areas of life to be adversely affected by pain) (17-19), the female sample of the current research faces their difficulties by using "social support" and "transcendental support" as coping strategies, therefore relying more on external sources of both relationship and spiritual support.

We can assume that difficulties and suffering are more shareable for women, while men tend not to share these negative experiences. In fact, the female role, in western culture, is fundamental in maintaining family relationships, supported by emotional competences which, even at the stereotypical level, are more recognized to women than to men (26). It could be assumed that the use of delegation of family care, contingent on the algic situation, places the woman in a condition that not only limits her autonomy, but that forces her to review the roles within the family, probably creating disorientation and "emptiness" regarding her role functions. This could explain the seemingly counterintuitive data that shows the mood of females more deflected compared to the males, despite the use of relational and/or transcendental support (which is instead expected to be able to guarantee greater support).

On the other side, we observe how the male sample resorts with significantly lesser extent to coping strategies as social support and transcendental support which could confirm the hypothesis whereby men express and share their feeling to a lesser extent, starting with a socio-cultural context which legitimizes them sharing suffering to a lower extent.

The recordings carried out show that taking pain medication for a long period, while providing a relief of the symptomatology, negatively affects the quality of life (whoever takes pain medication reports a lower score in "self-reported quality of life"). In fact, the use of medication would affect the self-perception as sick, fragile, addicted, passive and powerless with regard to pain condition. The result is consistent with the studies which show the perception of disability as the outcome of a continuous analgesic therapy (23).

Furthermore, the results suggest that patients treated with analgesic therapy tend to adopt avoidant coping styles (use of distractions from the problem, passive attitude, alcohol or substance use). The literature underlines the fact that avoidance coping, usually leads to postponing the moment to deal with a stressful situation, causing worsening if used in the long term. In this sense, the use of avoiding strategies may negatively impact the evaluation of the quality of life perceived in chronic disease conditions. In fact, these modalities seem to result in a strategy as "not to feel" and, at the same time, avoiding taking care of themselves in global terms.

This study is not free from limits. The recruitment of the participants has implied the creation of a "convenience" sample. For instance, one of the selection criteria for the participants was the exclusion of those who had intense and particularly disabling pain when accessing the medical center.

The study is relevant for healthcare professionals because the results provide useful food for thought for effective patient care. This could be enriched by integrating an evaluation of the patients' caregiving, to compare the perception and the experience of each patient. More thorough measures of pain and emotional states are also considered useful. The data suggests paying more attention to communication methods and emotional aspects, in the relationship with female patients, who seem to experience algic condition with greater suffering. Also, with regard to male patients, data suggest that it is necessary to discuss and develop men's coerced and hardly explicated emotional experiences, since they represent an important element in the experiences related to symptoms of chronic pain.

These measures are also necessary in relation to the patients subjected to continuous pain medication, as this condition has been associated with a qualitatively lower perception of domains which determine the quality of life, and with regard to those who perceive an impairment of their autonomy,

because it is a condition which affects the mood.

The collected data raise the question of what the most effective strategies would be to appropriately embrace the emotional experiences of patients, enhancing their specific differences and peculiarities. This research confirms the importance for healthcare professionals to use an integrative approach while taking care of patients with physical pain conditions.

Given the proven role of mood and emotional state, future research could deepen the importance and effect of every single emotion in a state of physical pain. It would also be interesting to evaluate how much the ability to read one's own emotional states affects dealing with an algic symptomatology condition.

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The use of oxygen ozone therapy in the treatment of cervicobrachial pain: case series study

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This retrospective, observational, uncontrolled case series study was carried out to evaluate the clinical efficacy and safety of intramuscular paravertebral injections of an oxygen-ozone (O2-O3) mixture in patients with cervicobrachial pain. One hundred and sixty-eight subjects affected by cervicobrachial pain, referred to Ozone Therapy Unit at San Pietro Fatebenefratelli Hospital in Rome (Italy), were enrolled in the study. All the subjects (n=168, 106 females and 62 males) completed the treatment and the follow-up visits. Subjects received 12 cervical intramuscular injections of an O2-O3 mixture (5 mL) with an O3 concentration of 16 µg/mL once a week. The overall reduction of pain was measured by the change in mean of Visual Analogue Scale (VAS) score from baseline to the end of treatment and from baseline to one, two, three, four and five years of follow-up. Patient satisfaction was assessed at the end of treatment, by modified MacNab Questionnaire. Possible adverse events related to the treatment were recorded. The mean (± standard deviation) VAS pain score at baseline, at the end of treatment, and during the follow-up at one, two, three, four and five years were 7.82 (±1), 1.6 (±1.5), 1.5 (±1.4), 1.4 (±1.3), 1.6 (±1.2), 1.5 (±1.3) and 1.60 (±1.2), respectively, showing a significant reduction in pain over time ($p<0.001$). Of 156 patients who responded to treatment, 128 (82.05%) were pain free at one year, 110 (70.51%) at second year, 103 (66.02%) at third year, 94 (60.25%) at fourth year and 86 (55.12%) at fifth year follow-up visit. According to pain distribution all subjects showed a significant reduction in pain over time in each group ($p<0.05$). No significant differences were observed between groups. No serious adverse events were observed during the entire study. We suggest the use of intramuscular paravertebral injections of an oxygen-ozone (O2-O3) mixture in patients with cervicobrachial pain as an effective and safe treatment option to consider before surgical intervention.

Cervicobrachial pain (CP) is a common cervical spine disease, characterized by neck pain radiating to the upper limb, which may be associated with strength reduction (muscular weakness or motor deficiency) and/or sensitivity alterations (paresthesia, alteration of thermal or pain sensitivity). The most common cause of CP is the presence of intervertebral disc protrusions or hernias that cause compression of one or more brachial plexus roots. Other causes of CP include facet joint diseases, spondilo discoarthrosis, shoulder fractures or dislocation, myelopathies, tumors (1).

Pain often causes limitation of active upper limb

Key words: cervicobrachial pain, oxygen-ozone, intramuscular paravertebral injections

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movement, reducing patient's quality of life and working ability (2). The treatment of CP caused by intervertebral disc disease may be surgical or conservative. However, surgery does not appear to provide long-term benefits to conservative therapy, and above all, it presents several complications (3-5). On the other hand, the conservative approach consists in the use of pharmacological and rehabilitative therapies (3, 6-10). In terms of pharmacological therapies, the most used are anti-inflammatory drugs, corticosteroids, analgesics, and muscle relaxers, which may or not be associated with neurotrophic drugs. Complementary therapies as osteopathy, chiropractic and acupuncture, are included in the conservative approach (11). Ozone therapy, also among the complementary therapies, is a medical practice involving the use of an Oxygen-Ozone (O2-O3) gaseous mixture at appropriate concentrations and is often used in the treatment of cervical discogenic pain. Ozone (O3) is a natural gas with typical pungent odor, and it is characterized by high chemical reactivity.

Ozone is a very unstable molecule with high oxidizing power, which in contact with biological tissues stimulates our body to respond to oxidative stress (12). Unlike conventional drugs that act with a receptor mechanism, ozone behaves as a molecule capable of inducing minimal and controlled oxidative stress. In fact, at the doses and with the appropriate techniques it activates a myriad of intra and extra-cellular reactions with the formation of second messengers activating the paradoxical antioxidant processes that works to ensure cell survival and improve the production of antioxidant enzyme.

In recent years, ozone biochemical mechanisms and biological effects, possible routes of administration and its therapeutics effects, have been clearly studied and understood (13-20). Ozone is a highly reactive molecule, which in contact with biological fluids immediately reacts with the plasmatic/interstitial water and polyunsaturated fatty acids (PUFAs) present in the tissue, leading to reactive oxygen species (ROS) and lipid peroxidation products (LOPs) production (17-23). Both ROS and LOPs could damage important cellular components, so their production needs to be carefully controlled to obtain an exclusive beneficial effect as ozone second messengers, without causing any tissue damage. Antioxidant systems (ascorbic acid, uric acid, reduced glutathione, albumin, etc.) present in almost all biological tissues, reacting with Ozone trying to neutralize it (17).

If excessive Ozone doses were used, greater tissue oxidation and harmful effects could be observed. Hence, a proper execution of the therapy - using appropriate gas volumes and concentrations, considering that each tissue has a certain antioxidant power - is of paramount importance. Ozone treatment should generate an acute but well-controlled oxidative stress. Using a correct dosage, Ozone is not harmful, on the contrary it is able to act stimulating a multitude of beneficial biological effects, such as anti-inflammatory, analgesic, muscle relaxant, immunostimulant and immunomodulant, neurotrophic and able to improves cellular oxygenation, favoring the release and use of oxygen, improving the antioxidant cellular power, and improving the microcirculation (13-20). On the contrary, for clarity's sake ozone does not present in the body districts in its conditions of use a direct germicidal (fungicidal, antibacterial and antiviral) action.

To date, recent studies show that O2-O3 therapy can be used to treat symptoms related to disc herniation that fails to respond to conservative management. Specifically, Oxygen-ozone (O2O3) therapy is a minimally invasive treatment currently available, based on the chemical properties of ozone. The Ozone treatment for disc herniation can be direct or indirect. The direct approach (intra-discal/intra-foraminal injection) is carried out by needle insertion in the herniated disc followed by a direct insufflation of the oxygen-ozone gas mixture (3-10 mL; ozone concentration about 30 μg/mL) at the level of the pathologic intervertebral disc under radiological control. The indirect approach (intramuscular/paravertebral injection) is another widely approach very popular in Italy, Germany, Spain, Cuba, Canada, and China. It consists in one or several injections of oxygen-ozone mixture (5–10 mL ozone, concentration range: 14-20 μg/mL) in the paravertebral muscle corresponding to the metamere of the herniated disc (20). We decided to present our experience regarding the clinical efficacy and safety of O2-O3 paravertebral muscular injection in CP.

MATERIALS AND METHODS

This study was designed as an uncontrolled, retrospective, observational case series, to assess the clinical efficacy and safety of intramuscular oxygen-ozone therapy in the management of CP, with a follow-up of five years. The study has obtained permission from the ethical committee (Oss-R-149, Comitato Etico Lazio1, c/o AO San Camillo-Forlanini, Rome, Italy). From January 2004 to December 2011, patients affected by CP, aged over 18 years, who referred to Ozone Therapy Unit at San Pietro Fatebenefratelli Hospital in Rome (Italy), were screened for eligibility. The cervicobrachial diagnosis was carried out with clinical examination and confirmed by instrumental investigations (MRI and, if necessary, electromyographic examination). All patients had been experiencing pain for over a month and had previously been treated with corticosteroids, NSAIDs or Physio Kinesio Therapy without benefit on pain symptoms. They also were drug free from four weeks.

Participants were free to interrupt or continue the treatment, depending on their impressions of improvement and satisfaction. The following inclusion criteria were used: cervicobrachial pain (CP) for at least one month (VAS pain >4), MRI evidence of single or multiple protruded/herniated disc, age over 18 years, previous pharmacological, exercise and physical therapy failure. The following exclusion criteria were used: history of G6PDH (glucose 6 phosphate dehydrogenase) deficiency, pregnancy, severe congestive heart and uncontrolled hypertension, uncontrolled hyperthyroidism, complete neurological damage considered as lesions requiring surgery, diabetic neuropathy, local cutaneous infection or infection in the site of injection, systemic rheumatological disease (Rheumatoid Arthritis).

After applying the inclusion criteria, 168 patients were enrolled in the study (106 females and 62 males; mean age: 52.7±12.19, range 24-77) and all patients completed the treatment and the follow-up visits. No subjects were eliminated from the study protocol for any adverse event related to treatment. All patients were informed about the treatment and provided written informed consent before it. During the baseline visit the demographic characteristics, clinical neurological evaluations, VAS pain, pain's period of onset, previous pharmacological therapies, MRI images and, if necessary, electromyographic examination, were recorded.

All patients received 12 paravertebral cervical injections of an O2-O3 mixture (5 mL) with an O3 concentration of 16 μg/mL, once a week. To produce the oxygen-ozone mixture, a "Maxi Ozon Active International produced by Medica Srl Bologna Italy". Intramuscular injections were administered in the site of protruded/herniated disc, at level of the paravertebral cervical muscles, on the left, on the right side of the spine or bilaterally, according with clinical manifestation (left cervicobrachial pain, right cervicobrachial pain or bilateral cervicobrachial pain). The injections were administered under sterile conditions, at 2-3 cm from the vertebral spinous process using an extraspinal lateral approach, by insertion of a 27-gauge 19 mm needle, in the site of protruded/herniated disc.

No premedication or anesthesia were given, and the procedure was performed in an outpatient clinic. The outcome measures were:

i) Pain reduction, measured by the change in mean of VAS score from baseline to the end of treatment, and from baseline to 1, 2, 3, 4 and 5- year follow-up. Pain intensity was assessed using a VAS scale, with values between 0 and 10, with 0 representing "no pain" and 10 representing "the most intense pain imaginable."

ii) Patient satisfaction at the end of treatment, measured by Modified Mac Nab questionnaire, composed of five response options: (1) very much improvement (complete resolution), (2) much improvement (clinical resolution, episodic cervical/cervicobrachial pain that spontaneously withdraws without drugs within 24 hours, no limitation of work activity), (3) little improvement (partial but insufficient symptoms improvement, continuous or cyclic drugs administration, limitation of daily physical activity), (4) no change, (5) worsening.

iii) Adverse Events, if any, were recorded.

Follow-up: At the end of treatment patient were evaluated in term of VAS pain, Patient Satisfaction and Adverse Events. At the end of treatment, all the 168 patients accept to contact the physicians in case of disease recurrence. Disease recurrence is defined as pain symptomatology reappearance for at least 5 consecutive days, with a VAS value >4. The patients were contacted by the physician at 1, 2, 3, 4 and 5 years after the end of treatment, to collect VAS value.

Statistical analysis

Statistical analysis was performed according to the

principle of intention-to-treat, with missing data imputed with "last observation carried forward" technique. We used parametric tests if data were normally distributed and homogeneous, instead we used non-parametric tests if these two conditions were not satisfied. These assumptions were assessed by Kolmogorov-Smirnov's test and Levene's test, respectively. Paired sample t-test was used to assess VAS score differences within subjects over time. According to pain distribution and diagnostic categories, one-way ANOVA (followed by the Bonferroni-Holm post hoc test) was performed to assess VAS score differences between subjects over time. The Statistical Package for Social Sciences (SPSS) version 18 was used for calculations. All data were analyzed by a single researcher. Computed P values were 2-sided, and $p<0.05$ was used to determine statistical significance. Quantitative data were expressed as mean ± standard deviation (SD). Qualitative data were expressed as frequency and percentage. A priori power calculation was performed according to the mean VAS score change between baseline and 1, 2, 3, 4 and 5-year follow up, assuming a two tailed α value of 0.05 (sensitivity 95 %), a β value 0.5 (study power: 95%) and an effect size of 0.40. We determined that at least 80 subjects were required (G Power3 power analysis program).

RESULTS

The patients (n=168) were classified in terms of both clinical and radiological examinations.

In terms of clinical classification, the patients were classified as right cervicobrachial pain, left cervicobrachial pain or bilateral cervicobrachial pain. In terms of radiological classification, the patients were classified using MRI as single or protrusion/ hernia. In terms of clinical classification 72 patients (42.85%) were affected by right cervicobrachial pain,

Table I. *Baseline characteristics of the sample (**CCS**: Corticosteroids, **NSAIDs**: Non-Steroidal Anti-Inflammatories Drugs, **FKT**: Physio Kinesio Therapy).*

Age	Male: n = 62 (53.17±11.55) Female: n = 106 (52.5±12.15)
Clinical Classification	Right cervicobrachial pain: n = 72 (42,85%) Left cervicobrachial pain: n = 40 (23,80%) Bilateral cervicobrachial pain: n=56 (33.33%)
Radiological Classification	Single protrusion/hernia: n=66 (39,28%) Multiple protrusions/hernias: n=102 (60.71%)
History of Disease (months)	> 6 months: n=90 (53,56%) > 4 months: n=12 (7,2%) > 3 months: n=36 (21,42%) > 2 months: n=18 (10,71%) > 1 month: n=12 (7,1)
Previous Treatments	CCS: n=56 (33,30%) NSAIDs: n=128 (76,19%) FKT: n=74 (44,04%) CCS+FKT+NSAIDs: n=38 (22,6%) FKT+NSAIDs: n=30 (17,8%) CCS+NSAIDs: n= 16 (9,5%)
VAS mean (1-10)	7,8 (SD ±1,007)

40 patients (23.8%) by left cervicobrachial pain, 56 patients (33.33%) by bilateral cervicobrachial pain. In term of radiological classification 66 patients (39.28%) showed at MRI a single protrusion/hernia while 102 patients (60.71%) multiple protrusions/hernias. In term of pain onset, 90 patients (53.56%) showed presence of pain for over 6 months, 12 patients (7.2%) for over four months, 36 patients (21.42%) for over 3 months, 18 patients (10.71%) for over two months and 12 patients (7.1%) for over one month.

All the subjects were previously treated with corticosteroids, NSAIDs and physiotherapy alone or in association without pain reduction. In term of previous therapies, 56 patients (33.30%) have been treated with corticosteroids (CCS), 128 patients (76.19%) with non-steroidal anti-inflammatory drugs (NSAIDs) and 74 patients (44,04%) with physiotherapy (FKT). The 22.6% of the patients (n=38) was treated with the association of CCS+NSAIDs+FKT, the 17.8% of patients (n=30) with NSAID+FKT and the 9.5% of patients (n=16) with CCS+NSAIDs without a complete pain relief. Table 1 shows the baseline characteristics of the sample.

The mean VAS pain at baseline was 7.8 (SD±1.0) and at the end of treatment was 1.6 (SD±1.5). A significant reduction in pain over time was found ($p<0.001$). Patient satisfaction at the end of treatment measured by Modified Mac Nab Questionnaire: 52 patients (31%) reported a very much improvement, 104 patients (62%) much improvement, 8 patients (5%) little improvement, 4 patients (2%) no change, and no patients reported a worsening. Of 168 patients, 156 were successfully treated (93%), the others 12 patients did not respond to the treatment (7%).

In terms of adverse events, two subjects reported an episode of dizziness and transient hypotension immediately after injection; one patient reported an episode of modest illness without lipothymia, nausea and hypotension spontaneously resolved in a few minutes, without therapy. No other adverse events were observed during the entire treatment cycle. Follow-up: the mean VAS score (±SD) of the sample (n=168) at baseline was 7.82±1.0. At the end of treatment, 156 patients respond to the treatment (92.85%) and the mean VAS score was 1.6 ±1.5. At the one-year follow-up, of the 156 responder patients, 28 patients (17.9%) showed a recurrence of symptoms, while 128 (82.1%) resulted pain free (mean VAS score 1.5 ±1.4). At the two-year follow-up, 46 patients (29.5%) showed a recurrence of

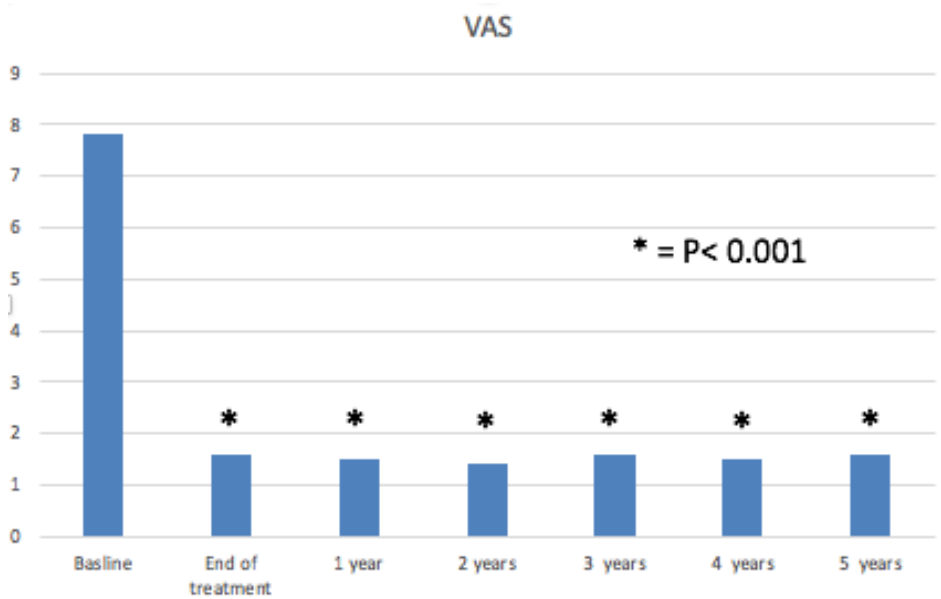

Fig. 1. *Mean VAS pain score at baseline and at each protocol-specific follow-up time point through 5 years.*

symptoms, while 110 (70.5%) resulted pain free (mean VAS score 1.4±1.3).

At the three-year follow-up, 53 patients (34%) showed a recurrence of symptoms, while 103 (66%) resulted pain free (mean VAS score 1.6±1.2). At the four-year follow-up, 62 patients (39.7%) showed a recurrence of symptoms, while 94 (60.3%) resulted pain free (mean VAS score 1.5±1.3). At the five-year follow-up, 70 patients (44.9%) showed a recurrence of symptoms, while 86 (55.1%) resulted pain free (mean VAS score 1.6±1.2) (Fig. 1).

According to pain distribution all subjects showed a significant reduction in pain over time in each group ($p<0.05$). No significant differences were observed between groups. At final evaluation of follow-up, of the 156 patients who responded to treatment, 86 (55.12%) were pain free at five years, while if we consider the totality of the enrolled patients (n=168) the percentage drops to 51.12%.

DISCUSSION

CP, as well as low back pain, is a very frequent cause of morbidity and disability in industrialized countries, (24) that cause the abuse of analgesic and anti-inflammatory drugs. Among the pathologies that cause CP, the most frequent is the presence of hernias or disc protrusions, but it is important to underline that the pain symptomatology often has a multifactorial origin (20-21).

Disc-radicular conflict pain mainly depends on two pathogenic components: mechanical and inflammatory. The mechanical components can be direct or indirect. The direct components: 1) spinal ganglio compression (intraforaminal/extraforaminal hernias), 2) deformation and stretching of the ligaments and annulus, where nociceptors are located, 3) deformation and stretching of nerve fibers with fragmentation of the myelin sheath and consequent possible onset of conduction anomalies.

The indirect components (vascular-mediated): 1) ischemic, with nerve trophic disorders for the compression on arterial afferents and on the microcirculation of the nerve bundle, and secondary anoxic demyelination of nerve fibers, 2) venous stasis, with edema and nerve trophic disorders due to partial or total blockage of venous reflux. Regarding the inflammatory component, it was demonstrated that neural and perineural inflammation acts in the pathogenesis of pain, and for this reason the use of corticosteroids often proves effective (25-26). The herniated disc contains high levels of Phospholipase A2 (PLA2), able to stimulate the production of pro-inflammatory chemical mediators such as prostaglandins and leukotrienes; is attacked by metalloproteinases whose production increases (MMPs), with an increase in the inflammatory reaction; directly stimulates the production of Prostaglandin E2 (PGE2) and Interleukin-6 (IL-6) (27-28).

The Ozone can act on all these factors (mechanical and inflammatory) and is able to inhibit inflammation, to correct ischemia and venous stasis, and inducing a reflex therapeutic effect by stimulation of the anti-nociceptive analgesic mechanisms. (20-21) Ozone reacting with interstitial and intradiscal aqueous environment, improves intra and trans-tissue oxygenation with consequent improvement of both hypoxia and venous and lymphatic stasis, inhibits the release of proteinases from macrophages and polymorphonuclear neutrophils, inhibits the synthesis of pro-inflammatory prostaglandins and the release of bradykinin or algogenic compounds, neutralizes endogenous ROS, stimulates the production of antioxidant enzymes (29-31).

Pain of the disco-radicular conflict is also due to the paravertebral muscle contracture. If injected locally, Ozone is a muscle relaxant capable of activating aerobic glycolysis and increasing the intake of O2. In the literature there are several works that demonstrate the efficacy of ozone in the treatment of herniated radiculopathy due to the lumbar and cervical spine (32-44). In the spine pathology the Ozone can be administered directly (intradiscal), (33, 35-43) or indirectly, by intramuscular paravertebral administration (32, 34, 44). The latter route of administration has been compared to a chemical acupuncture, since both the mechanical needle introduction and the gas administration give way to a complex reaction that lead to the disappearance of the pain in 70-80% of cases (45).

Regarding the intradiscal or paravertebral

approach, several studies have shown that the efficacy at six months and at one year, is similar both for the lumbar and the cervical pathology, with the only difference that the intradiscal technique determines a faster resolution of pain symptoms. In a Magalhaes article (42), a systematic review and meta-analysis of the literature on the efficacy of ozone therapy in low back pain caused by herniated disc based on the Cochrane Library criteria, has been demonstrated that both the intradiscal and the paravertebral procedure are effective therapeutic approaches, comparable to other minimally invasive procedures.

The results of our study provide further evidence that O2O3 therapy is an effective and safe minimally invasive treatment in the management of patients with cervicobrachial pain. The present study has some limitations, the absence of a control group, and the number of patients relatively small. The results of this study are promising, and it would be interesting to carry out further randomized controlled studies with a larger sample of patients, to confirm our data. In conclusion, paravertebral ozone therapy may be an effective procedure and, when performed by experienced doctors who have acquired proper training, is safe. This therapy could be considered in the conservative treatment to reduce pain in patients with cervicobrachial pain.

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Electromyographic analysis of the results given by lumbar discal herniation treatment with intradiscal oxygen-ozone gas mixture injection

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Among patients treated by intradiscal oxygen-ozone administration, in the period from January to June 18, because of disco radicular conflict, we randomly selected a group of 200 cases for this study. The classical instrument for studying nerve functioning alteration is EMGraphy. Repeated EMGraphic control during the treatment gives a valid parameter to quantify nerve root dysfunction: this is objective, repeatable and is precise data. The evolution of EMGraphic picture does not always correspond to the clinical situation. In several cases the normalization of the last radicular conflict will coexist with residual signs of EMGraphic dysfunction.

In the last years several statistical analyses have shown that 65% to 80% of adults have an episode of low back pain at some time in their lives, and although most cases resolve quickly, 40% recur and 5% result in a residual disability after 1 year. The extremely high frequency of the problem, and the discrepancy between pathological findings in neuro-imaging and clinical situations, both before and after open surgery, have recently brought a very ample revision of the pathophysiology and reconsideration of the possible treatments.

Mechanisms of disc degeneration and subsequent nerve dysfunction and pain.

Disc cells synthesize their matrix and break down existing matrix by producing and activating degradative enzymes, such as matrix metalloproteinases (MMPs) and a disintegrin and metalloproteinase (ADAMS) (1-7). Molecular markers of matrix turnover are naturally most plentiful during growth but usually decline thereafter (8). The proteoglycan content of the disc is maximal in the young adult and declines thereafter (8) presumably because of proteolysis. Disc cells appear to adapt the properties of their matrix to suit prevailing mechanical demands, although the low cell density and lack of a blood supply ensure that changes are not as rapid or pronounced as in adjacent vertebrae (9). Adaptive remodeling probably contributes to the large variation in compressive strength of adult discs, which ranges from 2.8 to 13.0 kN when they are tested in a manner that causes failure in the disc rather than the adjacent vertebra (10).

Injured discs show increased levels of catabolic cytokines, increased MMP activity (8, 11) and scar formation, (12) especially in the vicinity of annular tears (12, 13). They also show evidence of renewed matrix turnover (8, 14) and a more variable range of collagen fibril diameters (15). However, gross injuries to a disc never fully heal. Scalpel cuts in the outer anulus fill with granulation tissue, with only the outer few millimeters being bridged by scar tissue (16, 17). Annular tears are not remodeled as in bone, presumably because the sparse cell population

Key words: intradiscal oxygen-ozone administration, EMGraphy, disco radicular conflict, disc degeneration, nerve root dysfunction

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is unable to break down the large collagen fiber bundles of the anulus and replace them with new (18). Collagen turnover time in articular cartilage is approximately 100 years (19) and could be even longer in the disc. Proteoglycan turnover is faster, possibly 20 years, (18) and some regeneration of nucleus pulposus appears to be possible in young animals.(20) Injuries that affect the inner anulus or endplate decompress the nucleus (21), and healing processes are then overtaken by severe degenerative changes (15).

A series of biochemical and histologic changes have been described as typical of disc aging process. The blood supply to the vertebral endplate decreases during early childhood, and microstructural clefts and tears become common by the age of 15 years, especially in the nucleus and endplate (22). Cell density decreases throughout growth (23), and the nucleus pulposus tends to condense into several fibrous lumps, separated from each other and from the cartilage endplate by softer material (34).

Proteoglycan fragmentation starts during childhood (31), and with increasing age, the overall proteoglycan and water content of the disc decreases, especially in the nucleus (17) and the collagen content increases. Fine type II collagen fibrils in the inner anulus are replaced by type I fibers as the anulus encroaches on the nucleus, and type I fibers throughout the disc become coarser. If the proteoglycan fragments remain entrapped in the disc, they can fulfill a functional role like that of the intact proteoglycan. Reduced matrix turnover in older discs enables collagen molecules and fibrils to become increasingly cross-linked with each other, and existing cross-links become more stable (23).

In addition, reactions between collagen and glucose lead to nonenzymatic glycation (extra cross-links that give old discs their characteristic yellow-brown appearance) (24). Increased cross-linking inhibits matrix turnover and repair in old discs, encouraging the retention of damaged macromolecules (18) and probably leading to

Table I. *The distribution of signs of mono or pluri-radicular dysfunction in the series of 125 patients among 200, before treatment.*

125 patients with EMGraphic signs of dysfunction before treatment				
monoradicular	L3	in	6	patients (4.8%)
	L4		16	(12.8%)
	L5		53	(42.4%)
	S1		42	(33.6%)
pluriradicular	L4 and L5		4	(3.2%)
	L5 and S1		4	(3.2%)

Table II. *The distribution of signs of mono or pluri-radicular dysfunction in the series of 125 patients among 200, after treatment. An important modification of the percentages is observed.*

125 patients with EMGraphic signs of dysfunction after treatment				
monoradicular	L3	in	3	patients (2.4 %)
	L4		7	(5.6 %)
	L5		20	(16.0%)
	S1		19	(15.2%)
pluriradicular	L4 and L5		3	(2.4%)
	L5 and S1		1	(0.8%)

reduced tissue strength. Matrix synthesis decreases steadily throughout life but sometimes increases again in old and severely disrupted discs (8). Reduced synthesis is partly attributable to decreased cell density, although proteoglycan synthesis rates per cell also decrease (26).

All these characteristic aspects of disc degeneration are to be kept in mind when considering the nerve root disease which comes from disc degeneration that provokes both physical compression on the nerve root with consequent ischemia, and metabolic intoxication. Ingrowth of nerves and blood vessels has been shown to be an important feature of structurally disrupted discs, and appears to be directly, though variably, associated with pain (27). Ingrowth could be facilitated by the loss of hydrostatic pressure that characterizes internal regions of intact discs, and that would collapse hollow capillaries. Reduced proteoglycan content in old and degenerated discs may also facilitate the ingrowth of nerves and capillaries because aggrecan can inhibit their growth *in vitro* (28, 29). Ingrowth of nerves has been thought as a possible base for the evolution of acute pain in chronic persistent pain.

Radicular pain often is the result of nerve root inflammation and irritation. Clinical practice and research demonstrate that mechanical compression on the nerves may cause only motor deficits and altered sensation but will not cause pain. Inflammation in the epidural space and nerve roots provoked by a herniated disk is a significant factor in causing radicular pain. Chronic compression of a nerve root can induce axon ischemia, impede venous return, promote extravasation of the plasma protein, and cause local inflammation. If dorsal root ganglia are chronically compressed and irritated, this theoretically can lead to their sensitization and resultant radicular pain. Similar mechanisms of radicular pain are postulated to occur in the thoracic and cervical spine as well. In summary, clinical practice and animal research suggest that radicular pain is the result of inflammation of the nerve root in the epidural space provoked by leakage of disk material, compression of the nerve root vasculature, or irritation of dorsal root ganglia from spinal stenosis.

Electromyography

The classical instrument for studying nerve functioning alteration is EMGraphy. Altered EMGraphic tracings indicate the dysfunction of muscles, due to inadequate transmission of electrical impulses along a peripheral nerve. A compressed or metabolically altered nerve loses its capability of active progressive point by point production and transmission of electrical impulses towards muscles. The instrument will record the dysfunction in the muscles, allowing to understand if the alteration has a central or peripheral nervous system origin.

EMGraphic recording is normally associated with Electo Neurographic Recording. This consists in the recording of the conduction quality and velocity in a nerve and allows to demonstrate the anatomical site where obstacle exists. The anatomical location of nerve roots, with respect to the disc level is to be kept in mind:

L3 EMERGES FROM L2-L3 CONJUGATION FORAMEN

L4 EMERGES FROM L3-L4 CONJUGATION FORAMEN

L5 EMERGES FROM L4-L5 CONJUGATION FORAMEN

S1 EMERGES FROM L5-S1 CONJUGATION FORAMEN

Table III. *In the series of 125 patients: number of patients showing EMGgraphic alterations before and after the treatment.*

	PRE-TREATMENT	POST-TREATMENT	
EMG alterations		4 months	12 months
Discreet	82	49	32
Minor	43	12	10

Patients and methods

Among patients treated by intradiscal oxygen-ozone administration, in the period from January to June 18, because of disco radicular conflict, we randomly selected a group of 200 cases for this study. Among these, 125 patients showed EMGraphic signs of mono or pluriradicular nerve dysfunction upon enrolment for treatment (125 among 200, that is 62.5%). The details of the dysfunction are given in Table II. Repeated EMGraphic control during the treatment gives a valid parameter to quantify nerve root dysfunction: this is an objective, repeatable and precise data.

The treatment consisted in intradiscal 0203 gas mixture administration at the doses of 5 to 15 ml (this depends on the fissuration of the annulus and subsequent diffusion of the gas in the peri radicular and peridural space) and was performed by the senior author. The apparatus used for performing the Treatment is the Oxygen-Ozone generator of Medica, Srl (Bologna, Italy). EMGraphic controls were performed on enrolment, and at 4 and 12 months postoperatively, by the electophysiologist (A.Z.) as an independent observer.

RESULTS

The evolution of EMGraphic picture does not always correspond to the clinical situation. In several cases the normalization of the last will coexist with residual signs of EMGraphic dysfunction. This is notorious in radicular pathology, as well as in peripheral nervous system pathology in general, which ever kind of medical treatment was undertaken. EMGraphic data will express the nerve structure dysfunction because of relative Ischemization given by mechanic compression and by the mentioned biochemical factors which make the basis of the problem. Persistence through the time of signs of damage will express the fact that some nerve fibres are definitively altered. This may exist also in the absence of any clinical manifestation. EMGraphic improvement on the other hand will indicate the recovery of the nerve root function, thanks to the normalized nourishment.

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