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# INTERNATIONAL JOURNAL OF OZONE THERAPY

formerly RIVISTA ITALIANA DI OSSIGENO-OZONOTERAPIA

The Official Journal of  
WFOOT - World Federation of Oxygen-Ozone Therapy,

FIO - Italian Federation of Ozone Therapy,

SEOT - Spanish Association of Ozone Therapy,

Hellenic, Indian, Slovach and Chinese National Societies

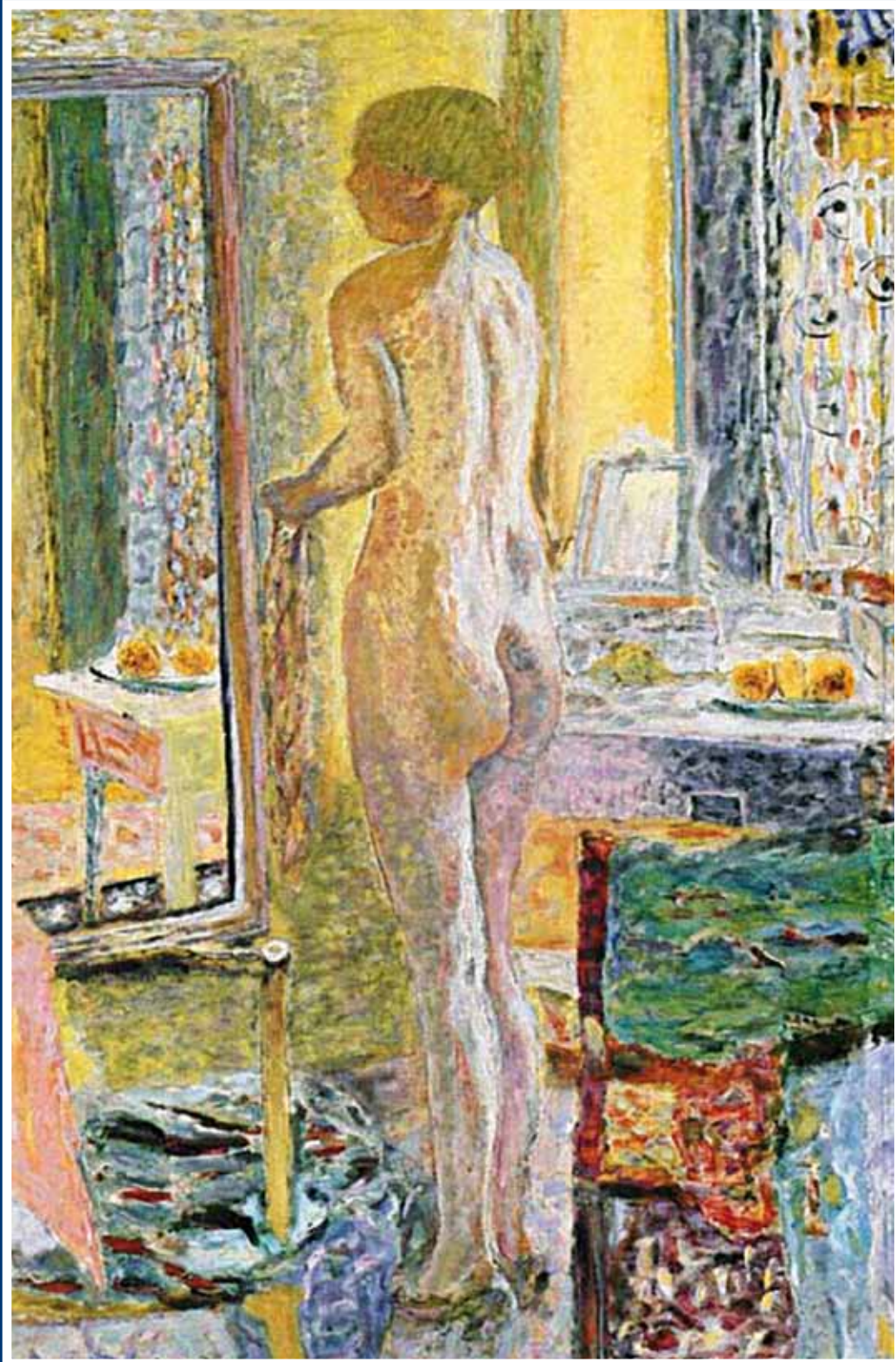
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II Congreso de la International Medical Ozone Federation (IMEOF)

Cancún, México. 10 al 12 de noviembre 2011

## "Por la Integración de la Ozonoterapia en la Medicina Convencional"

### CIRCULAR No. 2 CONVOCATORIA

Estimado/a Colega:

La Asociación Mexicana de Ozonoterapia (AMOZON) conjuntamente con la International Medical Ozone Federation (IMEOF) tienen el placer de anunciarle la celebración del III CONGRESO MEXICANO DE OZONOTERAPIA Y EL II CONGRESO DE LA IMEOF, a realizarse en Cancún, México del 10 al 12 de noviembre del año en curso.

Se ha concebido un programa científico que incluye las principales temáticas tratadas hasta el presente por Especialistas nacionales e internacionales que trabajan e investigan sobre este tema en los últimos años.

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El evento contará con la presencia de reconocidas personalidades nacionales e internacionales en este campo de las ciencias médicas, destacándose entre ellos los principales Directivos de varias de las Asociaciones que forman parte de la IMEOF, así como eminentes investigadores y profesores de diversas universidades mexicanas y de otros países de América, Europa y Asia.

Bajo el lema "Por la Integración de la Ozonoterapia en la Medicina Convencional" este Congreso propiciará el intercambio y la discusión sobre diversos temas enfocados a la aplicación de esta práctica en múltiples especialidades, a la vez que será el marco propicio para compartir las experiencias de los profesionales dedicados al tema.

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Dr. Froylán Alvarado Güémez

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# Major Ozonated Autohaemotherapy in the Treatment of Limb Ulcers not Responding to Conventional Therapy

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**Key words:** leg ulcer, chronic ulceration, ozone therapy, oxidative stress, regenerative medicine

**SUMMARY** - Chronic leg ulceration significantly impacts on the individual's health status. It is also a major financial burden on the national healthcare system. The maintenance of chronic ulcer is the result of an equilibrium imbalance between local tissue demand and systemic metabolic supply. The effect of this pathological condition leads to an increase in free radical production overwhelming the scavenging capacity of physiological antioxidant defence mechanisms. The reduction of oxygen free radicals concentration removes one of the major causes hindering the healing of chronic ulcer. Blood ozonation is recognised to induce a strong oxidative stress which causes an oxidative preconditioning that increases the efficacy of endogenous oxygen free radicals' scavenging properties. After a brief review of the pathophysiology of cicatrization and ozone properties, we document a significant number of patients who did not respond to conventional treatment, yet were successfully cured with major ozonated autohaemotherapy.

## Introduction

Chronic leg ulceration (CLU) is defined as *any wound below the knee that does not heal within a six-week period*<sup>1</sup>. It is a common pathology affecting approximately 1% of the population, increasing up to 3% or more when considering people aged 65 and over<sup>1-11</sup>. CLU has a major impact on an individual's quality of life, as well as healthcare system costs. Health financial reports estimate that 1% of annual healthcare budgets in European countries are absorbed by leg ulcer treatments. In the USA, CLU patients account for more than six million people and the financial impact approaches 2 billions Euro<sup>1,4</sup>. In Italy approximately two millions people have CLU<sup>5,6</sup>. Regardless of the etiology, CLU leads to tissue inflammation produced by inadequate blood supply, which results in tissue anoxia, oedema, induration and extravasation of serum, cytokines and blood formed elements. The final step of this pathophysiological cascade is a structural change in the surrounding tissue and cellular death<sup>1,3,10</sup>.

Therapeutic management of CLU includes a multidisciplinary approach involving medical, paramedical, surgical and technological competencies<sup>8-11</sup>.

Major ozonated autohaemotherapy (O<sub>3</sub>-MAHT) has been reported as an effective treatment

of CLU. This therapy was first introduced into the clinical field by Hans Wolff in 1974. Since then, much progress and many new discoveries have been made. Although there have been many contributors involved in the research, we gratefully acknowledge Professor Velio Bocci of Siena University whose relentless enthusiasm and research has created a major awareness and has brought to the forefront the significant medical application of O<sub>3</sub>-MAHT<sup>12,15,35-46</sup>.

This review presents the results obtained in a series of patients with chronic leg ulceration who did not respond to conventional medical and surgical therapy, but subsequently were successfully treated with O<sub>3</sub>-MAHT.

## Healing Process

Before examining the mechanism of oxygen-ozone treatment, it is beneficial to briefly refresh the pathophysiology of the healing process.

Regardless of the etiology, an ulceration implies a breach in the epithelial layer covering the skin so that the underlying tissue is exposed and damaged. The physiological responses include the activation of the healing process reacting to the interaction between platelets, exposed collagen and other

extracellular substances. Even if the healing process is a *continuum* that begins immediately after injury, conventionally it is divided into four overlapping phases: inflammatory, migratory, proliferative and remodelling.

The *inflammatory* reaction is mainly supported by platelets which attract the neutrophils and monocytes in the affected region. These activated cells produce chemotactic agents which amplify the attraction of other inflammatory cells like fibroblasts and other mesenchymal cells<sup>18,19</sup>.

During the *migratory phase*, an increase in the local concentration of inflammatory cells, cytokines, growth factors and other mediators aid in improving and creating an advantageous environment for tissue proliferation. Fibroblasts produce extracellular matrix and neoangiogenesis is stimulated by local hypoxia and specific growth factors.

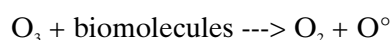
During the *proliferative phase*, the regenerative process requires not only growing enzymes but also a proper delivery of O<sub>2</sub> and vitamin C, a strong antioxidant agent, and a vigorous enzymatic activity. The contractile action of myofibroblasts will contribute to restrict the wound area and to pull together the edges of the lesion. Only if this phase is uninterrupted, will the final *remodelling stage* take place and the collagen structural links be rearranged in a normal structure<sup>16-23</sup>. If the metabolic demand for damaged tissue is not properly met, the healing cascade is interrupted at the inflammatory phase and the lesion becomes chronic.

A chronic lesion is characterized by an increased concentration of vasoactive substances such as histamine, bradykinin, serotonin, PAF, TNF, cellular growth factors, ADP, derivatives of arachidonic acid (prostacyclin, thromboxane, leukotrienes), interleukin, etc. Chronic inflammation is also responsible for persisting platelets and coagulation cascade activation, vascular permeability, oedema, capillary compression, hypoperfusion, etc.

Local ischemia is a particularly critical factor, which hinders the healing process because it perpetuates hypoxia, low pH, high lactic acid concentration and lipo-peroxidation. Oxidant metabolites such as free radicals and other reactive O<sub>2</sub> species (ROS) increase dramatically in chronic hypoxic areas and become major and essential factors of chronic lesion maintenance<sup>16-23</sup>. In physiological conditions, ROS synthesis is balanced by the breakdown activity of endogenous antioxidant defence mechanisms which include several agents like superoxide dismutase, catalase, albumin, etc. When this equilibrium is lost, the toxic effect of ROS becomes clinically recognizable in different forms, up to permanent tissue damage.

## Ozone Properties

The ozone (O<sub>3</sub>) molecule has an extremely unstable structure composed by three atoms of oxygen. In its gaseous state O<sub>3</sub> is heavier than O<sub>2</sub>; at 20°C its half-life is around 40 minutes. Structural O<sub>3</sub> decay enters the same metabolic pathway of oxygen, its triatomic molecule breaks down into the O<sub>2</sub> molecule and atomic oxygen according to the following reaction<sup>12-15</sup>:



Atomic oxygen is a very reactive atom which immediately reacts with the surrounding environment and contributes to generate the same ROS regularly produced by O<sub>2</sub> decay during normal physiological processes. It is noteworthy to underline that 1-5% of oxygen metabolized by the mitochondria, leaks the oxidative phosphorylation to undergo univalent reduction and originate free radicals which have to be detoxified by endogenous antioxidants defence mechanisms to prevent ROS injuries<sup>24-30</sup>. Contact with ozone originates a short and moderate oxidative stress that raises the ROS concentration and triggers an increase in endogenous antioxidant system response.

Repeated controlled exposure to O<sub>3</sub> gives rise to an oxidative preconditioning with the final effect of upregulating the body's antioxidant defence mechanisms<sup>6-11</sup> aimed at maintaining the cellular redox balance and increasing the scavenging efficacy of ROS and free radicals<sup>31-33</sup>. As a consequence, because of the rapid disappearance of O<sub>3</sub> due to its short half-life, the enhanced action of endogenous antioxidant agents is mainly targeted toward the ROS population distributed across the organism rather than toward the volatile and unstable O<sub>3</sub>.

Other reported direct and indirect effects of O<sub>3</sub> on O<sub>2</sub> delivery and improved microcirculation include the modification of cell wall membrane characteristics and the increase in 2-3 DPG and nitric oxide production. The direct effect of 2-3 DPG increase is a reduced affinity of haemoglobin for O<sub>2</sub> that shifts to the right the oxygen-haemoglobin dissociation curve and makes it easier for the haemoglobin to release bound oxygen. Several authors have reported a rise in fluidity and deformability of the erythrocyte membrane after ozone treatment whose ultimate effect is an improvement in blood rheology<sup>12</sup>.

Finally, the increment of nitric oxide generation is another factor improving tissue oxygenation. Nitric oxide (NO) is a potent signal transmitter involved in nearly every phase of cellular activity, including inflammation processes and host





A



B



C



D

Figure 1 Case 1. A) Right leg; B) Left leg; C-D) Final result.

defence. NO has also a potent direct vasodilating effect and therefore it may play a crucial role in CLU pathophysiology<sup>34</sup>.

### Materials and Methods

The cases of 17 patients (12 men and 5 women) are described below. Their mean age was 74.7 years ranging from 37 to 82. From the onset of the first examination, these patients had had chronic leg ulcerations for several months or years and all were poorly responding to their standard conventional medical treatments. Any indications for open surgery or percutaneous revascularisation had been excluded in all patients. Three patients were on regular insulin treatment. Due to dramatic worsening of the lesion and an inability to control extreme pain, amputation was considered in six

of the patients. After signing an informed consent form patients began the O<sub>3</sub>-MAHT therapy. The majority of ulcers were of multifactorial origin. The average ulcer area ranged from 1 to 150 cm<sup>2</sup> (mean 30 cm<sup>2</sup>). Ulcers were reported to have been opened and exposed varying from three to 60 months (median 10). Deep ulcers involving the muscles were observed in nine cases. Pictures of the lesions were taken at the beginning of treatment, every five-six sessions and again at the end of O<sub>3</sub>-MAHT therapy.

The O<sub>3</sub>-MAHT was performed as described by Bocci<sup>12,13</sup>. With the patients lying on a treatment couch, a sterile technique was used and a large bore peripheral vein cannulated with a butterfly 16G needle, 225 ml of the blood was withdrawn in a dedicated ozone-resistant glass vacuum bottle (B-Braun) containing 20 ml of sodium citrate 3,8% as anticoagulant. Again with a sterile technique,

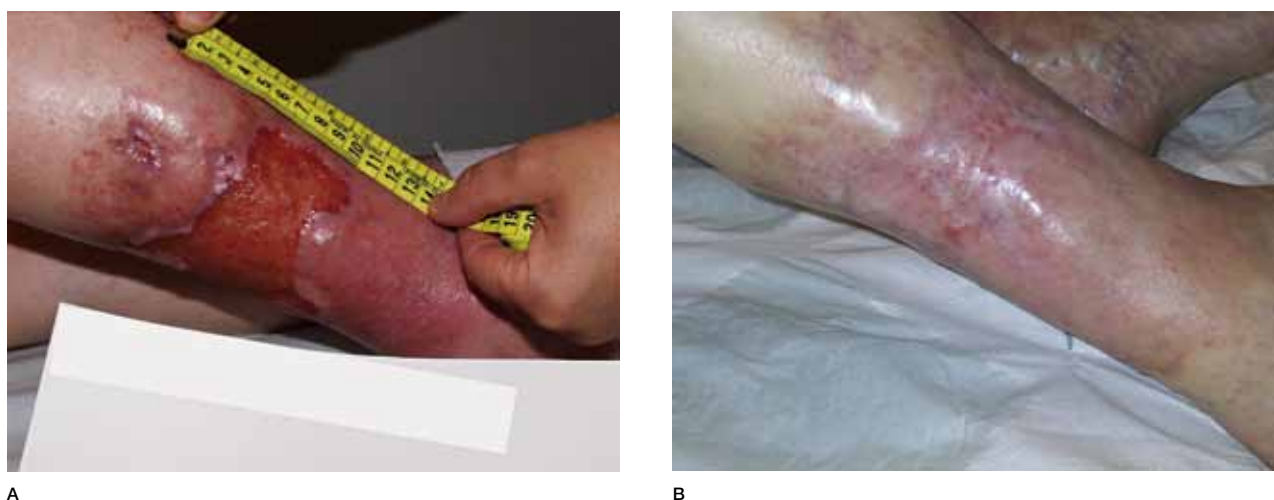


Figure 2 Case 2. A) Reports the effect of 18 treatments. B) Final result.

225 ml of a oxygen-ozone mixture was introduced through a dedicated inlet into the bottle containing the collected blood. The oxygen-ozone mixture concentration was gradually increased in the following four to five treatments to reach a maximum of 50 mcg/ml of ozone, corresponding to an  $O_2$  concentration of 95%. To produce the ozone an  $O_3$  generator (Dr. Haensler Ozonosan photonik) was used, which synthesized the desired oxygen-ozone mixture by treating medical  $O_2$  with a high voltage discharge. After the addition of ozone, the blood containing bottle was gently mixed for ten minutes to allow complete blood ozonation. Finally, using a transfusion drip the blood was slowly reinfused to the patient over a 20-30 minute period.

These patients were treated twice a week during the first four-five weeks, followed by weekly administration.

Patients' current therapy including topical dressings was uninterrupted in all cases. Therefore the only therapeutic change made in these patients was the administration of  $O_3$ -MAHT.

### Case Reports

**Case 1.** A 57-year-old man; non-smoker and non-drinker. There were no other associated pathologies reported. Two years earlier he started to suffer a progressive worsening of deep ulcerations on both legs. The patient was evaluated by several specialists, including a dermatologist, internist, allergologist, plastic and vascular surgeon. Allergic, vascular and autoimmune disease was excluded. A vasculitis of unknown origin was diagnosed.

Even a course of cortisone did not produce any

effect. The pain was unmanageable despite a combination of major and minor analgesic agents. The patient's quality of life was extremely poor due to the continuous pain and the extreme limitation of deambulation which was only possible with the help of crutches.

An epidural catheter was placed for a continuous bupivacaine (25 mg/day) and morphine (3 mg/day) administration. The pain became controlled, however, the lesion worsened and became infected. As an extreme measure, the surgeon took into consideration a bilateral leg amputation. As a last resort,  $O_3$ -MAHT was initiated. After 62 treatments the left leg wound was completely healed while a total of 120 sessions of  $O_3$ -MAHT were required for a complete recovery of the right leg as well (Figure 1).

**Case 2.** A 62-year-old type 2 diabetic woman was being administered XV units of insulin twice a day. Prior to our intervention, the patient had undergone a chemical lumbar sympatectomy, and she was wearing an epidural catheter for continuous administration of bupivacaine (18 mg/day) and morphine (2 mg/day). For three years there had been a large chronic ulcer on her right leg which was regularly medicated and debrided by a dermatologist. Due to the worsening of the clinical course a  $O_3$ -MAHT treatment was initiated. The effect of 18 treatments is reported in Figure 2A. After 40 sessions the wound was healed.

**Case 3.** A 72-year-old woman developed a non-healing chronic ulcer on the medial aspect of her left malleolar zone 12 months prior to our intervention. Due to the poor results of con-



A



B

Figure 3 Case 3.

ventional medical treatment, the patient became discouraged, and was non-compliant in applying the recommended dressings on the wound. After 22 sessions of  $O_3$ -MAHT the lesion had healed (Figure 3).

**Case 4.** A 77-year-old man had been a heavy smoker for 50 years (40 cigarettes a day) with a history of lung emphysema and the features of a pink puffer. He presented a persistent productive cough, with abundant excretion and sputum. The patient had been receiving home  $O_2$  therapy for a few months (3 litres/min). His gait was limited due to exertional dyspnoea and lower limb pain, and his walk was assisted with crutches. A chest x-ray revealed cardiomegaly, despite being on diuretic treatments for four years.

The patient suffered lower limb arterial insufficiency and pain-related insomnia. A painful chronic ulcer had extended over the right lateral malleolus for the last 18 months (Figure 4A). The ulcer showed a marked red discolouration, the edges of the ulcer were rolled and the base was covered by a frankly purulent material and debris. A smaller ulcer was also located on the lateral portion of the foot. A severe exfoliative watery discharge erythema affected the surrounding area of the foot and ankle. He was classified in stage IV of the Leriche – Fontaine scale. A contrastographic arteriogram excluded surgical revascularisation indications. He underwent percutaneous angioplasty, and intravenous prostaglandin (80 mcg twice a day) was infused for 15 days without any significant result. An epidural catheter was positioned in the lumbar spine and connected to an elastomeric pump with 0.5 ml/h of delivery rate for

pain and sympathetic block treatment. The analgesic mixture was titrated to infuse morphine 3 mg/day and bupivacaine 35 mg/day. This therapy was efficacious for pain control, but the ulcers did not seal in spite of routine surgical toilette and topical medication. After five months of epidural catheter placement, major ozonated auto-haemotherapy was initiated twice a week. After 20 treatments he was no longer receiving  $O_2$  treatment. Five weeks later, a reduction of epidural morphine and catheter removal was scheduled. After a total of 46  $O_3$ -MAHT treatments, the ulcers were completely sealed, the surrounding erythema disappeared and normal skin colour was recovered (Figure 4B).

**Case 5.** An 82-year-old man had suffered with an ulcer for nine months and was scheduled for an autologous skin graft for ulcer repair. The plastic surgeon initially proposed  $O_3$ -MAHT to explore the possibility to debride the bottom of the wound before plastic surgery. After 13  $O_3$ -MAHT treatments the lesion was completely healed without requiring any surgery (Figure 5A-B).

**Case 6.** The clinical history of this 43-year-old patient reported a kidney infection since the age of twenty. This condition led to renal failure requiring chronic haemodialysis. He underwent two kidney transplants, in both cases experiencing graft failure resulting in removal of the organ. Subsequently he first underwent percutaneous angioplasty dilation of a critical femoral artery stenosis and then a percutaneous insertion of a stent. Despite this surgical treatment, a chronic painful ulcer appeared on his left big toe and heel approximately three years prior to our intervention. A partial toe amputation



A



B

Figure 4 Case 4.



A



B

Figure 5 Case 5.

was required due to osteonecrosis (Figure 6A,C). The chronic ulceration did not heal and continuous pain persisted with intensity after dialysis treatment. The patient refused a complete big toe amputation proposed by the surgeon. After two months no evident improvement was noted. The patient came for major ozone autohaemotherapy, in which he received 25  $O_3$ -MAHT allowing a complete healing of ulcers (Figure 6B,D).

*Case 7.* A 75-year-old woman with depression and a sedentary lifestyle. The patient developed venous stasis ulcers on her left leg. Her surgeon administered topical medication and curettage. However, the clinical picture worsened with the enlargement of ulcers and an increase in pain (Figure 7A). After three months, under pressure

from her relatives, she looked for an alternative therapeutic approach. Ozonized autohaemotherapy was initiated and complete healing of ulcers and pain relief were obtained after only 10  $O_3$ -MAHT treatments (Figure 7B).

*Case 8.* A 70-year-old male carpenter with type-2 diabetes and receiving XXXV units of insulin treatment daily had had a chronic small but extremely deep painful ulcer in his left heel for the past 12 months. The ulcer appeared purplish, with rolled margins, a non-granulating base and a watery discharge (Figure 8A). Twice a week the patient underwent major ozonated autohaemotherapy. The pain disappeared after five treatments, a total of 15  $O_3$ -MAHT was required before the ulcer healed completely.



A



B



C



D

Figure 6 Case 6.



A



B

Figure 7 Case 7.



A



B

Figure 8 Case 8.



A



B

Figure 9 Case 9.



A



B

Figure 10 Case 10.



A



B



C



D

Figure 11 Case 11.

**Case 9.** A 65-year-old woman with osteoarthritis. Three years earlier, a severe deformity of the left first metatarsophalangeal joint required excision and amputation of the left big toe. Subsequently, in the insertion site of surgical nails, an osteomyelitis developed. The patient underwent several antibiotic treatments and the nails were removed. However, a chronic deep ulceration remained at the distal extremity of the first metatarsal bone. Figure 9 (arrow indicates a corn that developed as a consequence of the altered transmission of forces on the weight-bearing foot). All the medical and surgical attempts to heal the ulcer failed. After 25  $O_3$ -MAHT the wound had healed.

**Case 10.** A 42-year-old man presented with a rare case of Merkel tumor. The tumor was excised from the patient's gluteus. Insufficient healing of this area existed despite multiple surgical treat-

ments. Subsequently, the planned radiotherapy was not feasible due to an incomplete healing of the excised region. The patient came to our observation eight months after the initial operation. The results achieved were astonishing. After only 10  $O_3$ -MAHT treatments, the surgical wound was perfectly healed and the patient was able to continue his therapeutic plan (Figure 10).

**Case 11.** A 74-year-old obese man with type 2 diabetes. For several months he had presented a rapid worsening of a devastating ulcer, which deepened into the muscular plane of the right leg (Figure 11A,C). The pain suffered by the patient was scored as 100 on a Visual Analogic Scale and the patient was unable to walk or stand on a weight-bearing foot. Angiography excluded any critical vascular stenosis susceptible to surgical or percutaneous intervention. Ozone treatment was



A



B



C



D

Figure 12 Case 12.



A



B

Figure 13 Case 13.





A



B

Figure 14 Case 14.

proposed as the only alternative to amputation. An epidural catheter was positioned in the lumbar spine for continuous administration of bupivacaine 25 mg/day and morphine 3 mg/day. The results displayed in Figure 11B,D were obtained after 50  $O_3$ -MAHT treatments.

**Case 12.** A 68-year-old man suffered a motor-bike accident which led to the formation of an extended haematoma in his right calf. The surgeon followed a conservative therapeutic protocol and the lesion was left undrained. The haematoma became infected and a surgical incision was performed to drain the copious pus. Systemic antibiotic administration to prevent staphylococcus aureus infection was initiated and antiseptic dressing was applied locally. Despite this protocol, a loss of muscular tissue (Figure 12A,C) was evident due to an extended necrotic area which involved a large portion of the calf. Nine months after the accident, the surgeon recommended ozone treatment as a last resort to save the leg. After 35  $O_3$ -MAHT treatments the results depicted in Figure 12B,D were achieved. Ten subsequent treatments did not yield any further significant results. This allowed the patient to undergo a successful skin autologous graft transplantation.

**Case 13.** A 70-year-old moderately obese diabetic man underwent surgical excision of a cutaneous tumour on the foot two years prior to our intervention. The surgical wound only partially healed despite various treatments, including topical application of autologous platelet-derived growth factors. Figure 13A, taken before commencing ozone therapy, depicts the extent of healing after

$O_3$ -MAHT. Figure 13B depicts the complete healing of the wound after 20  $O_3$ -MAHT treatments. The results obtained in this case should be emphasized since the healing was achieved where scar tissue existed. Moreover, during surgery all the soft tissue close to the bone had been excised, limiting the physiological capabilities of a normal healing process. As a reaction to the healing process, a strong adhesion to the underlying anatomic plane occurred hindering an adequate amount of collagen deposition and neo-angiogenesis.

**Case 14.** A 55-year-old male suffered a traumatic fracture of his left ankle. A surgical fixation of the fracture was performed by a plate application. A few months later, the plate and screws were removed due to an intolerance. Wound healing was difficult and an alternation of opening and closure of the wound became part of the patient's life for the next two years. He underwent several courses of antibiotic treatments due to a recurrent wound infection. He developed the clinical manifestation of algodystrophy with pain, vasomotor disturbances and trophic changes, with a marked swelling of the lower left leg on standing. Prior to  $O_3$ -MAHT, his wound had been constantly open during the last nine months and he was under Ciproxin treatment because the culture of exudative effusion grew pseudomonas colonies (Figure 14A). After 29  $O_3$ -MAHT treatments we observed closure of the wound as shown in Figure 14B.

**Case 15.** A 38-year-old man. Fifteen years ago the patient underwent two surgeries for arthrodiesis. A few years later an ulceration formed on his heel, which was initially treated conservatively



A



B

Figure 15 Case 15.



A



B

Figure 16 Case 16.



A



B

Figure 17 Case 17.

with success. Subsequently, due to chronicization of the lesion, he underwent autologous skin grafting twice. Six months after the last operation the ulceration reappeared and the plastic surgeon decided to follow a conservative approach with tissue growth stimulating dressings.

A few months later, a decision was made to implement the treatment with  $O_3$ -MAHT. Figure 15A was taken after eight months of wound opening. Figure 15B depicts the result obtained after 20 sessions of  $O_3$ -MAHT, 11 months after the appearance of the lesion.

**Case 16.** An 82-year-old woman with no reported concomitant pathologies. Over the past three years, she has presented an extended chronic ulceration on the lateral aspect of her right leg. She underwent regular medicated dressings and surgical curettage, but the maximum results achieved are shown in Figure 16A. She decided to implement ozone treatment as part of her therapy. Figure 16B shows the result obtained after 22  $O_3$ -MAHT treatments.

**Case 17.** The last case we present here does not concern leg ulcerations but a failing musculo-cutaneous free flap implanted after oncologic laryngectomy in a 71-year-old man. Figure 17A depicts the clinical aspect three months after the operation.

Surgeons were pessimistic and dismantling of the implant was seriously considered. The daughter, an anaesthesia nurse, forced her father and the surgeons to attempt ozone therapy as a last resort. Major ozonated autohaemotherapy treatment was initiated and after 30  $O_3$ -MAHT treatments the results obtained are shown in Figure 17B. The tracheostomy tube was removed. The necrotic tissue and a complete even if retracting cicatrization was obtained. We considered it imperative to report this case to support the efficacy of  $O_3$ -MAHT not only for the treatment of leg ulcerations, but for any lesion where there is an imbalance between metabolic delivery and demand.

## Conclusions

The cases we have presented are the most significant and impressive in our experience with  $O_3$ -MAHT. Several other patients with lesions less advanced than those presented in this paper have benefitted from ozone treatments. According to the extremely favourable cost/efficacy ratio, we support the application of  $O_3$ -MAHT at least in those patients suffering chronic ulcers poorly responsive to traditional medical and surgical therapy.

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# Effects of Ozone Therapy on Oxidative Stress Biomarkers in Coronary Artery Disease Patients

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**Key words:** ozone therapy, coronary artery disease, oxidative stress

**SUMMARY** - Coronary artery disease is considered a major cause of death in the western world and its primary pathological manifestation is myocardial damage due to ischemia-reperfusion phenomena. Ozone has been used as a therapeutic agent and beneficial effects against the damage induced by renal or hepatic ischemia-reperfusion have been observed in vivo. The present study evaluated the behaviour of oxidative stress biomarkers in coronary artery disease patients after 20 sessions of ozone (50 µg/mL ; 200 mL) by rectal insufflation. Blood samples of 40 patients and 50 healthy subjects were tested by spectrophotometric techniques. Indicators of biomolecular damage, enzymatic antioxidant activity and total antioxidant status were determined. We demonstrated that ozone therapy reduced the oxidative stress index as reflected by the increase in superoxide dismutase and catalase activities, the reduction of malondialdehyde, total hydroperoxides and advanced oxidation protein products concentration. Furthermore, an increase in glutathione levels was noted. The results of the present study show that repeated administrations of ozone in non-toxic doses play a positive role in the control of antioxidant/pro-oxidant balance in coronary artery disease patients.

## Introduction

Coronary artery disease (CAD) remains a major cause of death in the western world<sup>1</sup>. Myocardial ischemia-reperfusion (IR) injury is the principal contributor to the morbidity and mortality associated with CAD<sup>2</sup>.

The level of IR-induced myocardial injury can range from a small insult resulting in limited myocardial damage to a large injury culminating in cardiomyocyte death<sup>3</sup>. Despite the complexity of

the mechanisms responsible for the IR-induced myocardial damage, essential factors leading to cellular injury have been delineated. Evidence indicates that several interrelated factors, including a decrease in cellular ATP levels, accumulation of hydrogen ions<sup>4</sup>, production of reactive oxygen species (ROS)<sup>5</sup>, calcium overload<sup>6</sup>, and leukocyte activation, contribute to IR injury<sup>7</sup>.

The production of ROS during both ischemia and reperfusion has been confirmed using electron paramagnetic resonance<sup>5</sup> and experimental evidence has addressed the role of ROS in myocardial IR injury<sup>7-9</sup>. Given the worldwide prevalence of CAD and the associated IR-induced cardiac injury, the evaluation of strategies to provide cardioprotection is an important research area. Recently, emphasis and attention have focused on the use of medical ozone<sup>10</sup>.

In the light of more recent pharmacological knowledge, ozone can be considered a pro-drug which at certain non-toxic doses can induce a rearrangement of the biochemical pathways with the activation of a second messenger in a cascade with a multiple system action<sup>11</sup>.

## Abbreviations:

CAD: coronary artery disease

IR: ischemia - reperfusion

ROS: reactive oxygen species

SOD: superoxide dismutase

CAT: catalase

H<sub>2</sub>O<sub>2</sub>: hydrogen peroxide

O<sub>2</sub><sup>•-</sup>: superoxide anion radical

MDA: malondialdehyde

GSH: glutathione

PP: peroxidation potential

TH: total hydroperoxides

AOPP: advanced oxidation protein products

GPx: glutathione peroxidase

Evidence that antioxidant enzymes, nitric oxide pathways and other subcellular activities could be modulated by low ozone doses is now proven and could support the effects of ozone in many pathological conditions such as diabetes mellitus, hepatic and renal IR and disc hernia<sup>10-14</sup>.

The present work considered that treatment with ozone at repeated low doses in CAD patients could reduce the oxidative stress and provide an antioxidant status, which can serve to compensate the CAD-derived cardiovascular complications and also to enhance patients' quality of life. Our results confirmed this hypothesis and demonstrated that ozone compensated the antioxidant/pro-oxidant balance in CAD patients.

## Materials and Methods

### Study design

The clinical study was reviewed and approved by both the National Institute of Angiology and Vascular Surgery and the Pharmacy and Food Sciences College (University of Havana) Committees for Research on Human Subjects, and the procedures were in accordance with principles of the Declaration of Helsinki<sup>15</sup>.

All patients gave their informed consent to be enrolled after receiving adequate information about the study (characteristics of the study, benefits and possible side-effects).

Medical personnel were instructed to report all adverse experiences whether or not described for the medication used. Adult patients of both genders and different ethnicity with a diagnosis of CAD who attended the National Institute of Angiology (Havana, Cuba) from October 2009 to October 2010 were eligible to participate in the study.

Exclusion criteria were: severe septic conditions, hypersensitivity to the medication to be used, hepatic dysfunction, renal failure (serum creatinine level  $>1.32 \mu\text{mol/L}$ ), pregnancy, cancer or other serious disease, inability to cooperate with the requirements of the study, recent history of alcohol or drug abuse, current therapy with any immunosuppressive agent or anticonvulsant, concurrent participation in another clinical study, or current treatment with an investigational drug.

The study included 50 gender- and age-matched healthy subjects.

Ozone was generated by OZOMED equipment manufactured by the Ozone Research Center (Havana, Cuba) and was administered by rectal insufflation. Ozone was obtained from medical grade oxygen, and was used immediately upon generation and represented only about 3% of the

gas mixture ( $\text{O}_2+\text{O}_3$ ). The ozone concentration was measured by using a built-in UV spectrophotometer set at 254 nm. The patients were treated with 200 mL of  $\text{O}_2-\text{O}_3$  containing 50  $\mu\text{g/mL}$  of ozone once a day for 20 days.

Blood samples for biochemical determinations were obtained after 12 h overnight fast, at the beginning of the study, and 24 h after the last ozone administration. These samples were immediately centrifuged at 3000 g, at 4°C for 10 min. The serum was collected and aliquots were stored at -70 °C until analysis.

### Biochemical determinations

All biochemical parameters were determined by spectrophotometric methods using a Pharmacia 1000 Spectrophotometer (Pharmacia LKB, Uppsala, Sweden) and a microplate reader (SUMA, Center of Immunoassay, Havana, Cuba). Superoxide dismutase (SOD) activity was evaluated by using Randox Ltd. Kit Cat. No. SD125 (Diamond Road, Crumlin, UK).

The method employs xanthine and xanthine oxidase to generate the superoxide radical ( $\text{O}_2^-$ ), which reacts with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride (INT) to form a red formazan dye. SOD activity was measured by the inhibition degree of this reaction<sup>16</sup>.

Catalase (CAT) activity was determined by following the decomposition of hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) at 240 nm at 10 s intervals for one minute<sup>17</sup>. After precipitation of thiol proteins, the reduced glutathione (GSH) levels were measured according to the method of Sedlak and Lindsay<sup>18</sup> with Ellman's reagent (5,5'-dithiobis-2-nitrobenzoic acid) 10-2 M (Sigma St Louis, MO, USA), the absorbance was measured at 412 nm.

Purified GSH (Sigma St Louis, MO, USA) was used to generate standard curves. The advanced oxidation protein products (AOPP) were measured as described previously<sup>19</sup>.

Briefly, the technique consists in treating 100  $\mu\text{L}$  of serum in PBS (1 mL) with 50  $\mu\text{L}$  of potassium iodide 1.16 M followed by 100  $\mu\text{L}$  of acetic acid. The absorbance of the reaction mixture was immediately read at 340 nm. AOPP concentrations were expressed as  $\mu\text{M}$  of chloramine-T (Sigma St Louis, MO, USA).

Concentration of malondialdehyde (MDA) was determined using the LPO-586 kit obtained from Calbiochem (La Jolla, CA, USA). In the assay, the production of a stable chromophore after 40 min of incubation at 45 °C was measured at a wavelength of 586 nm. For standards, freshly prepared solutions of malondialdehyde bis [dimethyl acetal]

(Sigma St Louis, MO, USA) were employed and assayed under identical conditions<sup>20,21</sup>.

Quantification of total hydroperoxides (TH) was measured by Bioxytech H<sub>2</sub>O<sub>2</sub>-560 kit (Oxis International Inc., Portland, OR, USA).

The assay is based on the oxidation of Fe<sup>2+</sup> to Fe<sup>3+</sup> by hydroperoxides under acidic conditions. Ferric ions bind with the indicator xylenol orange (3,3'-bis(N,N-di(carboxymethyl)-aminomethyl)-o-cresolsulfone-phtalein, sodium salt) to form a stable colored complex, which can be measured at 560 nm.

Finally, to determine susceptibility to lipid peroxidation the peroxidation potential (PP) was calculated. Samples were incubated with a solution of copper sulfate (2 mM) at 37 °C for 24 h and then MDA concentration was determined<sup>22</sup>.

#### Statistical analysis

The OUTLIERS preliminary test for detection of error values was initially applied. Data were subsequently analyzed by one-way analysis of variance (ANOVA) followed by a homogeneity variance test (Bartlett-Box). Student's t-test (two-tailed) was used to determine differences between groups. Data were expressed as the mean ± standard deviation (SD). The level of statistical significance employed was at least  $P < 0.05$  for all determinations.

## Results

### General characteristics of subjects involved in the study

In relation to the baseline characteristics (Table 1), both groups were similar at randomization ( $P > 0.05$ ). More than 60% of subjects in both groups were older than 60 years and males were the majority. The medical history of patients was characterized mainly by CAD, but also by hypertension, ischemic cardiopathy and myocardial stroke.

Patients presented a prevalence of risk factors for cardiovascular diseases (CVD) such as hypercholesterolemia and obesity.

The conventional treatments were those used to control hypertension (captopril in 40%, nitrosurbide in 45%, and atenolol in 60% of patients, respectively), hypercholesterolemia (ateromixol® in 70% of patients), and CVD (aspirin® in 90% of patients).

### Biomarkers of oxidative stress

Table 2 shows the behaviour of oxidative stress biomarkers. After 20 sessions of ozone treatment, TH levels in CAD patients were similar to the control group.

At the beginning of the study, MDA concentration was higher in patients ( $P < 0.001$ ) compared

**Table 1** Baseline characteristics of control subjects and CAD patients.

Characteristics	Control group (n=50)		CAD Patients (n=40)		
	n	%	n	%	
Age (years)	50-60	11	18.00	12	30.00
	61-70	23	38.00	20	50.00
	71-80	19	32.00	7	17.50
	> 80	7	12.00	1	2.50
Gender	Female	19	38.00	14	35.00
	Male	31	62.00	26	65.00
History	CAD	–	–	40	100.00
	Hypertension <sup>a</sup>	–	–	32	80.00
	Myocardial stroke	–	–	3	7.50
	Ischemic Cardiopathy	–	–	37	92.50
Risk factors	Hypertension	–	–	32	80.00
	Hypercholesterolemia <sup>b</sup>	–	–	14	35.00
	Obesity <sup>c</sup>	–	–	17	42.50
	Smoking	–	–	21	52.50
Complementary diagnostic criteria	TC (mM)	3.18 ± 0.50		6.8 ± 1.31	
	BMI (kg/m <sup>2</sup> )	22.10 ± 2.30		34.5 ± 9.67	

*Legend:* <sup>a</sup> Hypertension was defined as elevation of systolic (>140 mmHg) and/or diastolic (>90 mmHg) blood pressure. <sup>b</sup> Hypercholesterolemia: increase in total cholesterol >6.7 mM. BMI, body mass index: weight (kg)/height (m<sup>2</sup>). No significant statistical differences between groups ( $P > 0.05$ ) were found for age and gender. CAD: coronary artery disease. TC: total cholesterol.

**Table 2** Effects of ozone therapy on biochemical parameters of oxidative stress.

Oxidative stress biomarkers	Control group (n=50)	CAD Patients (n=40)	
		Time 0 (Conventional therapy)	20 days after Ozone therapy
MDA ( $\mu\text{M}$ )	3.87 $\pm$ 0.91a	14.78 $\pm$ 5.03b	9.21 $\pm$ 3.30c
TH ( $\mu\text{M}$ )	63.00 $\pm$ 8.09a	92.96 $\pm$ 7.70b	65.45 $\pm$ 6.52a
AOPP ( $\mu\text{M}$ of chloramines)	9.19 $\pm$ 0.64a	25.63 $\pm$ 6.93b	15.84 $\pm$ 3.34b
GSH (mmol/L)	789.45 $\pm$ 97.30a	378.73 $\pm$ 54.12b	517.17 $\pm$ 81.36c
CAT (U/L/min)	231.80 $\pm$ 11.33a	804.70 $\pm$ 108.67b	584.10 $\pm$ 94.77c
SOD (U/mL/min)	11.35 $\pm$ 1.97a	39.75 $\pm$ 8.86b	22.17 $\pm$ 12.36c
PP ( $\mu\text{M}$ of MDA)	9.17 $\pm$ 0.82a	18.93 $\pm$ 6.50b	8.48 $\pm$ 1.73a

The table shows the means  $\pm$  SD of all measured biomarkers. At time zero the patients only received their conventional medication for the CAD, during the experiment, patients received the ozone therapy concomitantly with the conventional medication. Different letters represent statistical differences ( $P < 0.05$ ) between the same set.

to healthy individuals, but after the last ozone administration this parameter was positively modified. MDA levels were reduced in CAD patients compared to time zero, with statistical differences ( $P < 0.05$ ).

Similar behaviour was observed for AOPP concentrations. In patients, ozone therapy induced a significant reduction of AOPP levels ( $P < 0.05$ ) in comparison to the high levels determined before the gas administration.

In relation to the antioxidant enzyme activity, both CAT and SOD showed a significant increase in CAD patients ( $P < 0.001$ ) compared to control group. Ozone treatment positively modulated the activity of these enzymes. At the end of the study the enzyme activity was significantly lower than values at time zero in the same patients ( $P < 0.001$ ). The concentration of low weight molecular antioxidant GSH in CAD patients was significantly lower compared to reference values in control group ( $P < 0.001$ ). It was important to note that the ozone administration promoted a significant increase ( $P < 0.05$ ) of GSH in patients after 20 sessions of treatment.

In order to measure the susceptibility to lipid peroxidation, as indicator of total antioxidant activity, the PP was determined.

The results showed a highly oxidative damage on lipids in patients with CAD respect to control group, with statistical differences ( $P < 0.001$ ). However, after ozone treatment, the antioxidant status of CAD patients was higher than in time zero, as reflected the measured PP and back to the normal interval.

## Discussion

In addition to traditional risk factors for CVD (HTA, obesity, hyperlipidemia, diabetes, cigarette smoking and age), oxidative stress (OS) and inflammation are now being considered significant risk factors for CVD and other diseases. Under OS conditions take place a disruption of redox balance, affecting the antioxidant status and favored the ROS generation<sup>23,24</sup>.

Ozone therapy is capturing attention all over the world since the basic studies clarified the main biochemical mechanisms of action and the real possibility of taming ozone toxicity. There are good reasons to believe that the therapeutic efficacy of ozone therapy consists in simultaneously improving circulation and oxygen delivery, in enhancing the release of autacoids, growth factors and cytokines and in reducing chronic OS<sup>10</sup>. This last effect has been approached in the treatment of many diseases, such as diabetes<sup>13</sup>, hepatic ischemia/reperfusion injury<sup>14</sup> and others.

An efficient clinical diagnostic of the redox balance in CAD patients is of paramount importance to control the degenerative damage associated with OS, or to monitoring the effect of a nutritional or therapeutic regimen. In order to establish the behaviour of antioxidant status after ozone therapy we determined the serum activity of SOD and CAT enzymes and also the non enzymatic antioxidants GSH and water soluble reduced substances.

SODs are metal-containing proteins that catalyze the removal of superoxide anion, generating  $\text{H}_2\text{O}_2$ <sup>25</sup>. Then, this ROS is converted in



water and oxygen in a CAT-catalyzed reaction<sup>26,27</sup>. In the present study ozone treatment produced an increase in antioxidant enzymes activity. Overexpression of SOD and CAT in cultured cells and animal models has provided protection against the deleterious effects of a wide range of OS paradigms<sup>27</sup>. These observations suggest that the treatment with therapeutic agents, which promote the expression of endogenous antioxidants, represent a plausible alternative in the treatment of those OS-mediated diseases such as CAD<sup>28</sup>.

The SOD and CAT activity trends observed in the present work could be associated with the observations that ozone treatment may promote a moderate OS which, in turn, increases antioxidant endogenous systems protecting against oxidative damage<sup>10,12</sup>. It is known that the protective mechanism of ozone may involve protein synthesis<sup>14</sup>. Elevated ROS concentrations induce gene expression in many cells, whose products exhibit antioxidant activity. A major mechanism of redox homeostasis is based on the ROS-mediated induction of redox-sensitive signal cascades that lead to increased expression of antioxidants<sup>29,30</sup>.

Another assayed component of the endogenous antioxidant system was GSH. The ozone therapy positively modulated the GSH level in patients. GSH plays an important role in mediating several redox-based signaling processes as well as gene expression within the cell<sup>31</sup>. GSH is also essential for the activity of the powerful glutathione peroxidase (GPx) antioxidant enzyme family<sup>32,33</sup>, in antioxidant gene transcription<sup>33</sup> and in transthiolation reactions<sup>34</sup>. The importance of GSH in vascular pathology was highlighted by studies showing that it protects the macrophages from oxidized low-density lipoprotein (ox-LDL)-induced cell injury within the atherosclerotic lesion<sup>35</sup>.

Biomarkers of ROS-induced damage have the potential not only to determine the extent of oxidative injury, but also to predict the potential efficiency of therapeutic strategies aimed at reducing such an OS<sup>36</sup>. In line with the increase in antioxidant enzymes there was a reduction of biomolecules damages. Although MDA levels in CAD patients were higher than in control group, was very satisfactory the result after the ozone treatment, because at the end of the study there was a significantly reduction of this end-product of lipid peroxidation. It has been reported that the plasma MDA-low density lipoprotein levels are useful not

only as an indicator of OS, but also as a marker of progression of atherothrombotic risk in CAD patients<sup>37</sup>. Also, total hydroperoxides levels were reduced after ozone treatment which suggests that lipid peroxidation was positively modulated by O<sub>2</sub>-O<sub>3</sub>.

Amino acids, peptides, and proteins are vulnerable to the attack of a variety of ROS and related oxidants. According to our results it is evident that the high AOPP levels in the CAD patients plasma could be reduced by ozone therapy. The trend of this parameter mediated by ozone administration is very positive because is know that the oxidative damage on proteins is correlated with the extent of atherosclerotic lesions and the occurrence of adverse cardiovascular events<sup>24</sup>.

As an expression of the total antioxidant-oxidant balance, the PP was positively affected at the end of the study respect to the beginning in those patients who received the ozone therapy. A significant decrease compared to the initial level was found after ozone administration. The fact that the values return to normal intervals can be considered a normalization of the redox status.

## Conclusions

Repeated rectal insufflations of ozone contributed to enhance the activity of antioxidant enzymes and non enzymatic defenses. In addition, the treatment reduces the oxidative injury of lipids and proteins. These observations suggest that ozone may be used in combination with the conventional drugs for CAD. Consecutive and spaced treatments, in a regular form, are necessary to obtain an effective antioxidant activation status in CAD patients. Normalization of the antioxidant / pro-oxidant status in these patients could be a positive influence to avoid future complications and fatal events. Future clinical trials will be necessary to establish how long the antioxidant status is maintained after therapy and how often it will be necessary to repeat the ozone treatment.

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# Oxygen-Ozone Treatment of Verrucas

T. GASTALDI

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**Key words:** oxigen-ozone, verrucas

**SUMMARY** - *This paper describes the oxygen-ozone treatment of verrucas using bags and washing with ozonized water resulting in a complete clinical resolution.*

## Introduction

Verrucas are skin warts caused by human papilloma virus (HPV) belonging to the Papovaviridae family. They are benign lesions made up of a nucleus of skin striations fed by blood vessels and covered in different layers of epithelial tissue. The virus penetrates the epidermis causing infection and triggering excessive skin replication. Verrucas are spread by surface contact (the virus remains in the skin and is not found in blood) usually in communal areas like showers, gyms and swimming pools where the warm atmosphere and moist surfaces favour survival of the virus in active form. Without these environmental conditions the virus will not survive long outside the skin.

Verrucas can develop on any part of the body but often only affect specific areas, e.g. hands, feet, elbows and knees. These parts of the skin are those most commonly subject to mechanical trauma and contact with the external environment and those most likely to have microlesions the virus can attack.

The appearance of verrucas varies depending on the body area affected and the virus strain involved. Verrucas can be divided into common (or vulgar) verrucas, and plantar, flat and filiform verrucas. Common verrucas have the same colour as the skin and are usually distinguished by their typical rough often crinkled surface. Plantar verrucas are confined to the soles of the feet and usually alter the skin striae. For this reason and the fact that verrucas may also contain tiny black petechiae, plantar warts are readily distinguished from calluses and corns. The black petechiae represent tiny haemorrhages under the skin formed by stretching the papillae. Verrucas of this type tend to be soft, flat and covered by calluses and tend to be painful on application of pressure on walking.

## Materials and Methods

Between June 2005 and April 2011 we treated 55 patients presenting verrucas on different parts of the body: 45% on the hands, 40% on the feet and 15% with lesions elsewhere.

Two methods were used:

1. Intralesional injection of an oxygen-ozone mixture at a concentration of 40 micrograms/ml using 5 ml syringes and 27 or 30 G needles. Injections were administered every three to four days until the verrucas disappeared.
2. Washing with ozonized water at a concentration of 25 micrograms /ml associated with bags containing oxygen-ozone at a concentration of 50 micrograms/ml. Treatments were repeated every three to four days until the verrucas disappeared.

## Results

Thirty-five patients underwent treatment with ozonized water and bags while 20 received oxygen-ozone injections.

All patients fully recovered with a disappearance of the verrucas in a period ranging from 14 to 35 days (average 21, average 17 with oxygen-ozone injections, 23 with water and bag treatment). Multiple verrucas were present in 41 patients.

The verrucas regressed progressively and on detachment no scarring occurred. Both techniques were well-accepted by the patients as they are completely painless.

## Conclusions

To date, human papilloma virus has not been the topic of methodical in-depth studies. For this reason a treatment guaranteeing complete



Figure 1



Figure 2



Figure 3



Figure 4



Figure 5



Figure 6



Figure 7



Figure 8



Figure 9



Figure 10



Figure 11



Figure 12



Figure 13



Figure 14



Figure 15

cure was not available until recently. Leading medical journals<sup>1</sup> claim that no treatment has a likelihood of success greater than 73%. The treatments utilized to date include:

- Surgical resection.
- Cryosurgery.
- Keratolytic chemicals.
- Intralesional injections: consisting in the injection of interferon into the verruca.
- Laser surgery.
- Vitamin E application.
- Fig tree latex.

Among the many treatments proposed, oxygen-ozone therapy yielded particularly significant outcomes without scarring after therapy or pain during treatment. No patients suspended treatment. Oxygen-ozone treatment is an innovative therapy recommended for its simplicity and success rates.

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# A New Infiltrative Paravertebral Approach for the Cure of Disc Herniation Using O<sub>2</sub>/O<sub>3</sub>

R. VIGLIOLI

Brescia, Italia

**Key words:** oxygen-ozone, paraventricular, disc herniation

**SUMMARY** - *This paper describes a new infiltrative paravertebral approach for the cure of disc herniation using O<sub>2</sub>-O<sub>3</sub>.*

## Introduction

A high number of patients apply for *infiltrative* paravertebral therapy, or 'complaine' for sciatica and/or severe *cervicobrachialgia* given the crippling effect of the pathology. However, it is still the doctor's duty to try to reduce the emotive impact caused by the pain of the event (even if its invasive nature is limited), maintaining or even improving the favourable outcomes of this method.

## Materials and Methods

At the Chirotherapeutic Center in Brescia, Italy approximately 850 patients have undergone this new operative model over the last two years. The treatment protocol consists in paravertebral monofiltration of the side of the hernia or protrusive lesion, indicated by clear radial symptomology, using a 0.4×40 mm needle and injecting 4/5 ml of O<sub>2</sub>-O<sub>3</sub> gas at an ozone concentration of -30 mcrg to a depth of O<sub>3</sub>-ml.O<sub>2</sub> another followed by 2-3 ml to the more surface muscle for a total of six to eight sessions at three week intervals. With no clear radial symptoms, the scheme is identical, being sure however to alternate the infiltrative site (left-right) at each session and possibly also increase the total number of sessions to eight or ten. The patient can be allowed to stand immediately after the bandage is put in place, so as to reduce the clino-orthostatic time, which will drastically reduce postural hypotension which occurs above all in elderly patients.

## Results

This method has been carried out for two years with very consistent results. Patients' symptomological improvement, meaning a reduction of

at least 50% of the VAS score, was achieved in approximately 80% of patients, in line with previous findings. There was also a complete absence of adverse side-effects.

## Conclusions

The shorter 'therapeutic time', low traumatic nature of the method together with the brevity of the cycle (maximum therapy three weeks) all contribute to lowering anxiety in patients and at the same time making the O<sub>2</sub>-O<sub>3</sub> therapy safer and more practical while still maintaining its well-established therapeutic effects. This approach is considerably less invasive than traditional treatment methods, partially due to the thinner needles, and the smaller volume of gas used in each session. However, it is closely linked to the use of needles at least 40 mm long and three weekly treatment sessions.

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## Letters to the Editor

Dr Matteo Bonetti  
Scientific Director  
International Journal of Ozone Therapy

*Caro Direttore*

La informo di aver ricevuto nei giorni scorsi la sentenza del TAS di Losanna, dove sono stato ascoltato come esperto- Mi sembra opportuno dare spazio a questa informazione, importantissima per chi ha creduto e crede nell'ozono terapia. Informazione nella quale si attribuisce il giusto riconoscimento pubblico all'uso medicale dell'ozono.

*Lamberto Re*  
www.lambertore.com

### TRIBUNALE ARBITRALE DELLO SPORT

Arbitro unico:  
*Prof. Ulrich Haals, Zurigo, Svizzera*

*Omissis*

9.31. Tornando sul punto concernente l'adeguatezza del ricorso all'ossigeno-ozono terapia per la cura delle patologie sofferte dagli atleti, bisogna constatare che, anche qui, non vi è concordanza di opinioni tra gli esperti di parte. Ed infatti, mentre il consulente tecnico del Ricorrente ha fermamente sostenuto l'opportunità dell'impiego della terapia per tali patologie, gli esperti dell'UPA-CONI si sono mostrati decisamente scettici al riguardo. Anche il consulente tecnico d'ufficio ha espresso la sua opinione sul punto, rilevando che più che escludersi categoricamente l'utilità della terapia per la cura delle patologie indicate, si può constatare che non vi sono dei dati

scientifici che mostrino, in maniera inequivoca, l'efficacia della stessa.

9.32. Orbene, mentre, riscontrando una divisione di opinioni quale quella appena descritta, sembra opportuno evitare di emettere verdetti scientifici sulla questione dell'efficacia della terapia per il trattamento delle patologie indicate, pare potersi affermare che, per l'indagine volta ad esaminare l'intento del Ricorrente, vi sia un altro elemento che può ritenersi essere rilevante. Al di là della reale efficacia della terapia, infatti, deve constatarsi che essa viene effettivamente impiegata dai medici che ne sostengono l'utilità, per il trattamento delle patologie allegate. Questo dato -che si evince dalle perizie prodotte dal Ricorrente e dalle dichiarazioni rilasciate dal Prof. Re in udienza- non è stato smentito dai consulenti tecnici dell'UPA-CONI che, come detto, si sono limitati a mettere in discussione l'efficacia della terapia. E' possibile, dunque, che il Ricorrente fosse realmente convinto dell'utilità della terapia nei casi in cui essa sia stata praticata. Su questa base, allora, non sembra che il fatto che il Ricorrente abbia utilizzato la terapia in oggetto per la cura delle patologie di cui soffrivano gli atleti, che a lui si erano rivolti, possa essere interpretato automaticamente come un'intenzione di sottoporre gli stessi a pratiche dopanti.

*Omissis*

Losanna, 2 Agosto 2011





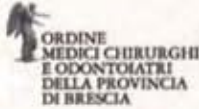
COMUNE DI BRESCIA



World Federation Oxygen  
Ozone Therapy



Federazione Italiana di  
OSSIGENO-OZONOTERAPIA



# III WORLD CONGRESS of Oxygen-Ozone Therapy

V° CONGRESSO NAZIONALE F.I.O.



Federazione Italiana di  
OSSIGENO-OZONOTERAPIA



**World Federation Oxygen  
Ozone Therapy**



**FNOVI**

FEDERAZIONE NAZIONALE  
ORDINE VETERINARI ITALIANI



*Il Ministro dell'Istruzione,  
dell'Università e della Ricerca*

Roma, 13 Aprile 2011

Egregio Professore,

desidero innanzitutto esprimere i miei sinceri apprezzamenti per la promozione del 3° Convegno Internazionale di Ozonoterapia che in questi ultimi anni ha trovato sempre più ampia ed efficace applicazione in campo medico.

La veste internazionale dell'iniziativa che ha un ruolo di alta rilevanza scientifica, la presenza di autorevoli relatori saranno sicuramente motivo di apporto di esperienze concrete e convalidate e sono pertanto spiacente di non poter essere presente a causa di inderogabili impegni di Governo.

Nel ringraziare per il cortese invito, invio un cordiale saluto e formulo i migliori auguri per il successo dell'importante evento.

Mariastella Gelmini

Prof. Matteo Bonetti  
Brescia



Dott. CARMELO SCARCELLA  
Direttore Generale ASL di Brescia



Dott. ENZO MONTEMAGNO  
Questore di Brescia



Dott. RAFFAELLO MANCINI  
Presidente Ordine dei Medici



Dott. MARCO TOMA  
in rappresentanza del Sindaco  
di Brescia Dott. Adriano Paroli



**RASSEGNA STAMPA**

**SALUTE.** Dal 14 aprile congresso internazionale al Museo Mille Miglia

# Ozonoterapia: Brescia si scopre leader mondiale

A confronto sulla terapia oltre 700 specialisti  
Sarà l'occasione per fare il punto su tutte le novità

**Elisabetta Bentivoglio**

Dopo Pechino e Madrid, Brescia per tre giorni sarà la capitale mondiale dell'ozonoterapia. Dal 14 al 16 aprile, nella sala congressi del Museo Mille Miglia, si terrà il terzo World Congress of Oxygen-Ozone Therapy (congresso mondiale di ossigeno-ozono terapia), dedicato a una disciplina medica che negli ultimi anni, e proprio a partire da Brescia, si è sempre più affermata nella cura di patologie infiammatorie acute e croniche delle piccole e grandi articolazioni, fino al suo utilizzo in campo infettivologico per il trattamento dell'ulcera di Buruli, una malattia estremamente grave e mutilante che colpisce le popolazioni dell'Africa subsahariana.

**PATROCINATO** dal Comune e dall'Ordine dei Medici di Brescia, il Congresso, al quale parteciperanno più di settecento medici provenienti da tutti i cinque continenti, sarà l'occasione per presentare tutte le novità mondiali in materia di ossigeno-ozonoterapia e di fare il punto della situazione sugli studi randomizzati presentati di recente sulle riviste internazionali di medicina. La più illustre del settore è nata proprio in Italia - l'International Journal of Ozone Therapy - dalla volontà di un team di medici italiani tra i quali spicca il bresciano Matteo Bonetti, responsabile del servizio di Neuroradiologia della Città di Brescia. Sarà proprio lui il padrone di casa e presidente del Congresso, incarico ricevuto quattro anni fa a Pechino, a testimonianza del riconoscimento che Bonetti ha guadagnato a livello mondiale.

Quando si parla di ozonoterapia, cosa si intende? «L'ozono è un gas, dal punto di vista chimico è semplicemente una molecola costituita da tre atomi di ossigeno e come tale è il più potente antimicrobico che esiste in natura - spiega il professor Marco Leonardi, presidente di Fio, la Federazione Italiana di Ossigeno-Ozonoterapia -. Se inizialmente l'ozono veniva utilizzato in campo medico per le sue grandi capacità disinfettanti, oggi grazie alla scoperta del suo potere ossidativo è in grado di rompere i grossi componenti macromolecolari che sono alla base dell'integrità vitale di cellule batteriche, funghi, protozoi e virus e di curare così ulcere e gangrene cutanee, oltre ad aver ottenuto ottimi risultati anche nel trattamento delle maculopatie retinee».

Un potere disinfettante talmente forte da essere applicato anche a livello industriale per la disinfezione delle acque e la potabilizzazione. L'ozono è largamente usato anche nel-

l'igienizzazione delle piscine - come alle Olimpiadi australiane del 2000 - consentendo di risparmiare fino all'80 per cento di cloro.

**MA CIÒ CHE HA** realmente consacrato l'ozonoterapia a livello mondiale è il trattamento del mal di schiena e dell'ernia al disco. «In Italia esistono 100 mila nuovi pazienti all'anno che soffrono di mal di schiena - annuncia il responsabile di Fio Toscana Gianantonio Pellicani -. Pazienti che, se trattati con una terapia all'ozono, hanno l'80 per cento delle possibilità di guarire senza ricorrere ad interventi chirurgici o trattamenti invasivi». Fino ad oggi Fio ha monitorato 30 mila pazienti trattati con ozonoterapia per indagare eventuali sintomi da post-trattamento: nessuno ha manifestato effetti collaterali.

Oltre all'applicazione umana, da dieci anni l'ozonoterapia viene utilizzata anche sugli animali di piccola taglia per curare sindromi da stress post-chirurgico o traumatico e tutte le patologie legate a fattori infiammatori. Al Congresso, che dedicherà un'intera sessione alle applicazioni veterinarie, interverranno in qualità di relatori i maggiori esperti mondiali del settore, tra i quali il professor Vjiay Kumar, il presidente italiano Marco Leonardi, il cinese He Xiaofang, autore dei più importanti lavori scientifici di ricerca ozonoterapica su animale, e il professor Velio Bocci, autore di oltre cinquecento pubblicazioni sull'utilizzo e gli effetti dell'ozonoterapia. Durante la giornata inaugurale, i quattro luminari sopracitati riceveranno il premio «Francesco Riccardo Monti» alla carriera. ♦

**Viene praticata  
con successo  
per guarire  
il mal di schiena  
e per chi soffre  
di ernia del disco**

**Nella tre giorni  
spazio anche  
per l'utilizzo  
come antistress  
su animali  
di piccola taglia**



Il congresso che il 14, 15 e 16 aprile al Museo Mille Miglia porta a Brescia i maggiori esperti a livello mondiale

BRESCIA  
OGGI



## Matteo Bonetti Un pioniere al servizio della ricerca mondiale

Matteo Bonetti (nella foto), nato a Cremona, ha ottenuto il diploma di maturità scientifica presso il liceo "Gaspare Aselli" nel luglio 1980. Dopo la laurea in medicina conseguita nel 1987 ha frequentato continuativamente la scuola di specializzazione in radiodiagnostica dell'Università di Brescia, specializzandosi il 6 luglio 1992. Dal 1990 al 1998 è stato assistente medico presso il Servizio di Radiologia degli Spedali Civili di Brescia diretto dal prof. Chiesa nella

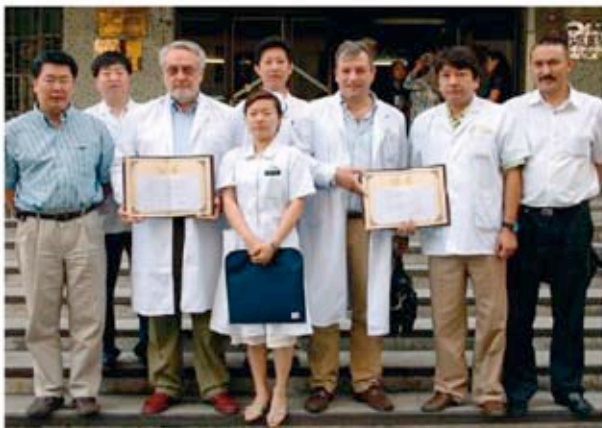
sezione di Neuroradiologia; in questi anni Matteo Bonetti si è dedicato alla neuroradiologia interventistica spinale, un settore che ha continuato a seguire anche una volta diventato responsabile del Servizio di Neuroradiologia dell'Istituto clinico Città di Brescia di cui tutt'oggi è il responsabile. A questo incarico il dott. Bonetti affianca quello di prof. (a.c.) Università degli studi dell'Insubria, responsabile del centro d'Interventistica spinale del Poliambulatorio Oberdan, reviewer

for Ajnr (American Journal of Neuroradiology), segretario Wfoot (World federation of oxygen-ozone therapy), segretario Fio (Federazione italiana di ossigeno-ozono terapia), direttore scientifico Rivista Italiana di ossigeno-ozonoterapia 2001-2005, scientific director international Journal of Ozone. Therapy dal 2006, vincitore premio Isico per l'anno 2005, premio riservato ai migliori lavori scientifici di ricerca dedicati alla colonna vertebrale pubblicati su rivista internazionale indicizzata.

**DO**  
Diagnostica  
& Riabilitazione

SPECIALE OZONOTERAPIA

A CURA DI VOCEMEDIA PUBBLICITÀ



IL DOTTOR MATTEO BONETTI IN CINA

Brescia Dal 14 al 16 aprile al museo della Mille Miglia

# Brescia, capitale dell'ozonoterapia

Prevista la partecipazione di delegazioni di oltre 70 Paesi del mondo

**D**opo Pechino e Madrid, Brescia è per tre giorni la capitale mondiale dell'ozonoterapia. Si tiene, infatti, presso la sala congressi del museo delle Mille Miglia dal 14 al 16 aprile, con il patrocinio del Comune di Brescia e del locale Ordine dei medici la tre giorni dedicata a questa disciplina medica che negli ultimi anni si è sempre più affermata in diversi campi d'applicazione a partire dalla cura dell'ernia del disco per passare alla patologia infiammatoria acuta e cronica di piccole e grandi articolazioni fino all'utero in campo infettivologico come

**Una tre giorni dedicata a una disciplina medica che negli ultimi anni ha conosciuto sempre maggiori campi di applicazione per la cura di diverse patologie**

per esempio nel trattamento di una malattia estremamente grave e mutilante quale l'ulcera di Buruli che colpisce nell'Africa sub sahariana. Il dottor Matteo Bonetti, responsabile del Servizio di neuroradiologia

dell'Istituto clinico Città di Brescia è il presidente del Congresso, incarico ricevuto quattro anni fa in Cina a Pechino. Le giornate del Congresso sono state pensate per presentare tutte le novità mondiali nella materia, con una sessione interamente dedicata alle sue applicazioni in veterinaria. Cos'è l'ozonoterapia? L'ozono è un gas, dal punto di vista chimico è semplicemente una molecola costituita da tre atomi di ossigeno. Come tale è il più potente antimicrobico che esista in natura. Inizialmente le principali applicazioni dell'ozono si basavano soprattutto sulle grandi capacità disinfettanti che questo gas

aveva. Il trattamento che ha consacrato definitivamente l'ozonoterapia è stato quello relativo al mal di schiena (ernie e protrusioni lombari). L'ozono è stato introdotto in Italia agli inizi degli anni 80. Al Congresso sono state invitate oltre 70 delegazioni nazionali da tutto il mondo ed interverranno i maggiori esperti mondiali, fra questi: il professor Vjiay Kumar neurochirurgo indiano, presidente della World Federation of Ozone Therapy, il professor Marco Leonardi presidente della Federazione italiana di ozonoterapia e ordinario di neuroradiologia all'Università di Bologna,

il professor He Xiaofang, presidente della Società cinese di ozonoterapia e ordinario di radiologia interventistica a Guangzhou (ex Canton) nonché autore dei più importanti lavori scientifici di ricerca su animale a livello mondiale e il professor Vello Bocci direttore dal 1971 dell'Istituto di farmacologia dell'Università di Siena autore di oltre 500 pubblicazioni sull'utilizzo e sugli effetti dell'ozonoterapia. Durante la giornata d'apertura saranno consegnati i premi "Francesco Riccardo Monti" riconoscimento alla carriera proprio ai quattro medici sopra citati.

# LA VOCE del POPOLO



## Ozonoterapia: Brescia capitale mondiale

■ Pechino, Madrid ed ora Brescia. La nostra città diventerà dal 14 al 16 aprile la capitale mondiale dell'ozonoterapia per il terzo convegno internazionale dedicato a una disciplina medica che ha visto l'Italia in prima fila nell'approfondimento scientifico (nella foto, la presentazione di ieri). Nella sala congressi del Museo Mille

Migliaia sono attesi circa 700 medici suddivisi in una settantina di delegazioni nazionali per valutare e discutere dei più recenti risultati nelle molte aree di applicazione dell'ozonoterapia. Anche per dare a questa disciplina basi scientifiche sempre più solide. Patrocinato da Comune di Brescia e Ordine dei Medici di Brescia, il

convegno sarà coordinato da Matteo Bonetti ed è promosso dalla Federazione Italiana di Ossigeno-Ozonoterapia, presieduta da Marco Leonardi. L'ozono è definito dagli organizzatori «il più potente antimicrobico che esiste in natura»: attualmente è molto sfruttato nelle terapie contro l'ernia al disco.

GIORNALE di  
BRESCIA





SPECIALE OZONOTERAPIA

## Terzo congresso di ozonoterapia a Brescia Un trattamento introdotto in Italia negli anni '80

Uno sforzo non indifferente, ma anche una grandissima soddisfazione: l'organizzazione del 3° congresso mondiale di ossigeno-ozono terapia a cura del dottor Matteo Bonetti (nella foto) è stato un successo sia nei numeri (oltre 700 i medici presenti) sia nei contenuti. Normale che la soddisfazione sia stata tanta al termine della convention durata tre giorni. "L'incarico di organizzare questo evento - commenta Bonetti - ricevuto in Cina quattro anni fa, mi ha riempito di orgoglio, caricandomi

di un' elevata responsabilità: devo ringraziare il mio staff che è riuscito ad organizzare un evento completo in tutte le sue sfaccettature." Per capirne di più, quando parla di ozonoterapia, cosa si intende? "L'ozono è un gas, una molecola costituita da tre atomi di ossigeno. Come tale è il più potente antimicrobico che esiste in natura. L'ozono è in grado di rompere i grossi componenti macromolecolari che sono alla base dell'integrità vitale di cellule batteriche, funghi, protozoi e virus. Questa sua potente azione

disinfettante - spiega ancora Bonetti - viene utilizzata anche a livello industriale sia nella disinfezione delle acque reflue." E nella medicina? "Il trattamento che ha consacrato questa terapia è quello del mal di schiena (ernie e protrusioni lombari) l'ozono è stato introdotto in Italia agli inizi degli anni '80 anche se il precursore per scopi medici fu Werner Von Siemens che nel 1857 costruì il primo tubo a induzione per la distruzione di microorganismi."

A CURA DI VOCEMEDIA PUBBLICITÀ

Brescia Un convegno ospitato dal museo della Mille Miglia

# Ozonoterapia cura per il mal di schiena

A confronto oltre 700 medici provenienti da 68 Paesi del mondo

DI LORENZO ROMANO



DA SINISTRA TOMA, MONTEMAGNOLI, BONETTI, LEONARDI E SCARCELLA

**S**i è svolto a Brescia nelle giornate dal 14 al 16 aprile il terzo congresso mondiale di ossigeno-ozonoterapia. Nella splendida cornice del museo delle Mille Miglia, oltre 700 medici provenienti da tutto il mondo si sono confrontati sulla materia. Presidente del congresso Matteo Bonetti, responsabile del Servizio di neuroradiologia dell'Istituto clinico Città di Brescia, considerato a livello mondiale uno dei pionieri nei trattamenti con ossigeno-ozono per il mal di schiena. Il congresso ha visto la partecipazione di medici giunti da 68 nazioni diverse con tut-

**Per tre giorni la città è stata capitale mondiale di una disciplina medica che ha conosciuto maggiori applicazioni per diverse patologie**

ti i continenti rappresentati, aperto nella giornata di giovedì con il patrocinio del ministero dell'Istruzione, dell'università e della ricerca con la lettura dei saluti inviati dal ministro Maria Stella Gelmini. La cerimonia

di apertura ha visto la presenza del Marco Toma in rappresentanza del sindaco di Brescia Adriano Paroli, di Carmelo Scarcella direttore generale dell'Asl e di Raffaello Mancini presidente dell'Ordine dei Medici di Brescia.

Alla presentazione è intervenuto anche il questore di Brescia Enzo Montemagnoli. Durante la prima giornata di lavori sono state presentate le letture magistrali di Vijay Kumar, presidente della World Federation of Oxygen-Ozone Therapy e di Vello Bocci dell'Università di Siena sullo stato dell'arte. A seguire sono stati consegnati i premi "France-

sco Riccardo Monti" alla carriera a Marco Leonardi, ordinario di Neuroradiologia presso l'Università di Bologna, fondatore insieme al prof. Bonetti dell' "International Journal of Ozone Therapy", rivista scientifica indicizzata completamente dedicata all'ozonoterapia, al prof. He Xiaofung di Guangzhou, autore dei più autorevoli lavori di ricerca sperimentale su animale, al professor Vijay Kumar ed al prof. Vello Bocci. Il congresso è proseguito nella giornata di venerdì e sabato con la presentazione dei più importanti lavori scientifici dedicati all'ossigeno-ozonoterapia a partire dai trattamen-

ti ormai consolidati per l'ernia del disco e la patologia infiammatoria acuta e cronica di piccole e grandi articolazioni e con una sessione completamente dedicata alla veterinaria. Dal 1857, anno 0 dell'ozonoterapia, sono passati circa 150 anni e la ricerca italiana nella materia è oggi considerata al primo posto a livello mondiale: gli studi scientifici e la tecnologia sono unanimemente riconosciuti come il meglio in assoluto, come è stato ampiamente ribadito durante questo terzo congresso mondiale di ossigeno-ozonoterapia ospitato dal museo della Mille Miglia.

LA VOCE del  
POPOLO

QUI  
BRESCIA

# P IONIERI CONTRO IL MAL DI SCHIENA

A BRESCIA IL TERZO CONGRESSO MONDIALE DI OSSIGENO OZONO TERAPIA.

Si è svolto a Brescia nelle giornate dal 14 al 16 aprile il Terzo Congresso Mondiale di Ossigeno-Ozono Terapia. Nella splendida cornice del Museo delle Mille Miglia, oltre settecento medici provenienti da tutto il mondo, si sono confrontati sulla materia. Presidente del Congresso il Dottor Matteo Bonetti, responsabile del Servizio di Neuroradiologia dell'Istituto Clinico Città di Brescia a cui venne conferito l'incarico a Pechino (Cina) quattro anni orsono, considerato a livello mondiale uno dei pionieri nei trattamenti con ossigeno-ozono per il mal di schiena. Il congresso ha visto la partecipazione di medici giunti da sessantotto Nazioni diverse con tutti i continenti rappresentati dall'Australia al Canada per passare alla Cina, all'India fino alla Siria e all'Egitto. Molto nutrita la rappresentanza Sudamericana (Brasile, Argentina, Uruguay, Bolivia, Cile, Colombia, Venezuela, Costa Rica, Cuba, Guatemala). Anche l'Europa è stata ampiamente rappresentata oltre che dall'Italia padrona di casa, infatti, erano presenti Spagna, Svizzera, Grecia, Austria, Germania, Croazia, Ucraina, Russia ed Albania. Il Congresso si è aperto nella giornata di giovedì con il Patrocinio del Ministero dell'Istruzione, dell'Università e della Ricerca con la lettura dei saluti portati dal Ministro Maria Stella Gelmini. La cerimonia di apertura ha visto la presenza del Dottor Marco Toma, in rappresentanza del Sindaco di Brescia Adriano Paroli, del Dr. Carmelo Scarcella, direttore generale dell'Asl di Brescia ed del Dottor Raffaello Mancini, Presidente dell'Ordine dei Medici di Brescia. Alla presentazione è intervenuto anche il Dottor Enzo Montemagno, Questore di Brescia.

Durante la prima giornata di lavori sono state presentate le letture magistrali del Professor Vijay Kumar, neurochirurgo indiano, nonché Presidente della World Federation of Oxygen-Ozone Therapy, relative alle prospettive future dell'Ossigeno Ozono Terapia e quelle del Professor Vello Bocci dell'Università di Siena sullo stato dell'arte. A seguire sono stati consegnati i premi alla carriera "Francesco Riccardo Monti" al Professor Marco Leonardi, Ordinario di Neuroradiologia presso l'Università di Bologna, Fondatore insieme al Professor Bonetti di International Journal of Ozone Therapy, rivista scientifica indicizzata completamente dedicata all'Ozono Terapia distribuita in tutto il Mondo, il Professor He Xiaofung di Guangzhou (ex Canton), direttore del Servizio di Radiologia Interventistica dell'Università di Guangzhou ed autore dei più autorevoli lavori internazionali di ricerca sperimentale su animale, il Professor Vijay Kumar, presidente della World Federation ed infine il Professor Vello Bocci, autore di oltre cinquecento lavori scientifici pubblicati su riviste Internazionali dedicati all'Ossigeno Ozono Terapia.

Il Congresso è proseguito nella giornata di venerdì con la presentazione dei più importanti lavori scientifici dedicati all'Ossigeno Ozono Terapia, a partire dai trattamenti (consolidati da oltre vent'anni di attività) per l'ernia del disco e la patologia infiammatoria acuta e cronica di piccole e grandi articolazioni per poi passare ad analizzare i più importanti studi randomizzati e controllati pubblicati in Letteratura Internazionale.



## PIONIERI CONTRO IL MAL DI SCHIENA

Una sessione del Congresso è stata interamente dedicata al confronto internazionale delle varie esperienze in tutti e cinque i continenti.

I lavori sono proseguiti anche nella giornata di sabato con una sessione completamente dedicata alla veterinaria, molto interessanti sono state le presentazioni dedicate allo studio del trattamento dell'ernia discale nel cane, in particolare nel bassotto, così come gli studi dedicati al trattamento della mastite della vacca.

Ritornando al campo medico, molto interesse hanno destato le presentazioni dedicate al trattamento delle malattie infettive, in particolare grazie alla Federazione Italiana di Ossigeno Ozono Terapia: oggi, infatti, abbiamo centri di ossigeno ozono terapia dislocati nell'Africa Subsahariana, ad Haiti e nel Pakistan per la cura di malattie estremamente invalidanti quali l'ulcera di Buruli (dovuta al micobatterio *Ulcerans*), ma anche per il trattamento di ulcere e piaghe da decubito.

Il Congresso, che ha suscitato grande interesse per la sua rilevanza scientifica, si è chiuso nella serata di sabato con un arrivederci a Buenos Aires in Argentina nel 2013 dove a far gli onori di casa sarà il Professore Osvaldo Pepe.

Per capirne di più, quando parliamo di ozonoterapia, cosa intendiamo? Lo abbiamo chiesto al Presidente del Congresso, il Dottor Matteo Bonetti.

"L'ozono è un gas, dal punto di vista chimico è semplicemente una molecola costituita da tre atomi di ossigeno invece che dei due atomi di cui normalmente è costituita la molecola di ossigeno. Come tale, è il più potente antimicrobico che esiste in natura.

Inizialmente le principali applicazioni dell'ozono si basavano soprattutto sulle grandi capacità disinfettanti che questo gas aveva. L'ozono, grazie al suo grande potere ossidativo,



è in grado di rompere i grossi componenti macromolecolari che sono alla base dell'integrità vitale di cellule batteriche, funghi, protozoi e virus, quindi l'utilizzo principale era nelle ulcere e gangrene cutanee. Questa sua potente azione disinfettante ad ampio spettro viene, oggi, utilizzata non solo in medicina, ma anche a livello industriale sia nella disinfezione delle acque per la potabilizzazione, sia nel trattamento delle acque reflue. Molti studi hanno inoltre dimostrato che l'ozono è più efficace del cloro nell'eliminazione di alcuni virus che trovano grande vitalità nelle acque potabili (come ad esempio il virus EBOLA). Per questo motivo non ci si deve meravigliare se l'ozono è largamente usato anche nell'igienizzazione delle piscine (Olimpiadi Australiane del 2000) dove induce un risparmio dell'80% di cloro, una riduzione del reintegro dell'acqua e non necessita di alcun intervento di personale in quanto l'impianto è automatizzato. Per quanto riguarda, invece, il trattamento che ha consacrato questa terapia, ovvero quello del mal di schiena (ernie e protrusioni lombari), l'ozono è stato introdotto in Italia agli inizi degli anni 80, anche se il suo utilizzo in medicina risale negli anni 30 in Germania. Il precursore dell'uso dell'ozono per scopi medici fu Werner Von Siemens che nel 1857 costruì il primo tubo a induzione per la distruzione di microorganismi. Nella seconda decade del XX secolo un altro tedesco, il chimico Justus Barone Von Liebig, fu il primo a studiare le applicazioni dell'Ozono per uso umano, approfittando della tecnologia sviluppata da Siemens.

Dal 1857 sono passati circa 150 anni e la ricerca italiana nella materia è oggi considerata al primo posto a livello mondiale: gli studi scientifici e la tecnologia sono, infatti, unanimemente riconosciuti come il top in assoluto, come è stato ampiamente ribadito durante questo III° Congresso Mondiale di Ossigeno-Ozono Terapia".

# III World Congress of Oxygen - Ozone Therapy V° Congresso Nazionale

Museo della Mille Miglia  
from 14th to 16 th April 2011  
Brescia Italy

Nello splendido scenario del Museo delle Mille Miglia di Brescia si è svolto il III^ Congresso Mondiale di Ossigeno Ozono Terapia dal 14 al 16 Aprile.

Presidente del Congresso il Prof. Matteo Bonetti, responsabile del Servizio di Neuroradiologia dell'Istituto Clinico Città di Brescia, nonché segretario della World Federation of Oxygen-Ozone Therapy, incarico ricevuto quattro anni fa in Cina a Pechino durante il 1^ Congresso Mondiale di Ossigeno Ozono Terapia.

Il Congresso che ha avuto il patrocinio del Ministero della Pubblica Istruzione dell'Università e della Ricerca, nonché del Comune di Brescia e dell'Ordine dei Medici di Brescia, ha fatto sì che Brescia diventasse per tre giorni la capitale mondiale dell'ozono terapia.

Pratica medica che si è sempre più affermata in diversi campi di applicazione negli ultimi anni a partire dalla cura dell'ernia del disco per passare alla patologia infiammatoria acuta e cronica di piccole e grandi articolazioni fino all'utilizzo in campo infettivo logico come per esempio nel trattamento di una malattia estremamente grave e mutilante, quale l'ulcera di Buruli che colpisce nell'Africa Subsahariana. Chiediamo al Prof. Bonetti: "quando è venuto



BONETTI PROF. MATTEO

a contatto per la prima volta con l'ossigeno ozono terapia?"

Ho iniziato a lavorare con ossigeno ozono nel 1993 ,ero un giovane neuroradiologo che lavorava presso il Servizio di Neuroradiologia degli Spedali Civili di Brescia e mi chiesero di occuparmi di trattamenti mini invasivi del rachide. Venni perciò a contatto con il mondo dell'ozono terapia quasi casualmente,da allora sono passati quasi vent'anni e il percorso che l'ozono terapia ha fatto è stato importante sia in Italia che nel Mondo.

Sono state colmate le lacune più importanti tanto che l'ozono terapia oggi è riconosciuta in tutto il Mondo grazie agli articoli pubblicati su riviste internazionali come American Journal Neuroradiology ,grazie al Professor Leonardi Direttore della Cattedra di neuroradiologia dell'Università di Bologna che al Professor Andreola di Bari.

Una cosa,a cui tengo particolarmente e che voglio sottolineare come passo importante della crescita dell'ozono terapia,è stata la fondazione di International Journal of Ozone Therapy rivista che io e il Professor Leonardi abbiamo creato nel 2003 e che oggi rappresenta lo strumento ideale per l'aggiornamento scientifico di tutti gli ozono terapeuti.



Ma per capirne di più le chiediamo : "quando parliamo di Ozonoterapia ,cosa intendiamo?"

L'Ozono è un gas ,dal punto di vista chimico pè semplicemente una molecola costituita da 3 atomi di ossigeno invece che dei due atomi di cui normalmente è costituita la molecola di ossigeno.

Come tale è il più potente antimicrobico che esista in natura .

Inizialmente le principali applicazioni dell'ozono si basavano soprattutto sulle grandi capacità disinfettanti che questo gas aveva.L'Ozono ,grazie al suo grande potere ossidativo,è in grado di rompere i grossi componenti macromolecolari che sono alla base dell'integrità vitale di cellule batteriche,funghi,protozoi e virus,quindi l'utilizzo principale era nelle ulcere e cancrene cutanee.

Questa sua potente azione disinfettante ad ampio spettro viene,oggi,utilizzata non solo in medicina,ma anche a livello industriale ,sia nella disinfezione delle acque,per la



potabilizzazione, che nel trattamento delle acque reflue. Molti studi hanno inoltre dimostrato che l'Ozono è più efficace del cloro nell'eliminazione di alcuni virus che trovano grande vitalità nelle acque potabili (come ad esempio il virus EBOLA).

Per questo motivo non ci si deve meravigliare se l'Ozono è largamente usato anche nell'igienizzazione delle piscine (Olimpiadi Australiane del 2000) dove induce un risparmio dell'80% di cloro, una riduzione del reintegro dell'acqua e non necessita di alcun intervento di personale in quanto l'impianto è automatizzato.

Per quanto invece riguarda il trattamento che ha consacrato questa terapia ovvero quello del mal di schiena (ernie e protusioni lombari) l'Ozono è stato introdotto in Italia agli inizi degli anni 80; tuttavia il suo utilizzo in medicina inizia negli anni 30 in Germania.

Il precursore dell'uso dell'Ozono per scopi medici fu Werner Von Siemens, che nel 1857 costruì il primo tubo a induzione per la distruzione di microorganismi.

Nella seconda decade del XX secolo un altro tedesco, il chimico Justus Barone Von Liebig, fu il primo a studiare le applicazioni dell'Ozono per uso umano, approfittando della tecnologia

sviluppata da Siemens.

Dal 1857 sono passati circa 150 anni e la ricerca italiana nella materia è oggi considerata al primo posto a livello mondiale, gli studi scientifici e la tecnologia sono, infatti, unanimemente riconosciuti come il meglio in assoluto.

Sappiamo che il Congresso ha avuto un grande successo oltre forse le aspettative, "cosa ci può dire a tale proposito?" In effetti è stato un successo veramente notevole, abbiamo avuto oltre 68 delegazioni Nazionali provenienti da tutti i continenti cominciando dall'Australia, dove la pratica è stata introdotta alcuni anni orsono per passare ovviamente all'Asia dove l'Ozono è molto diffuso soprattutto in Cina ed in India, ma molto nutrita è stata anche la rappresentanza Sud Americana con pressoché tutti i Paesi Sud americani presenti dal Brasile all'



Argentina, all'Uruguay, alla Bolivia, al Cile, alla Colombia, al Venezuela, alla Costa Rica, al Guatemala ,etc

Ovviamente l'Europa con a capo l'Italia a fare la padrona di casa, era ampiamente rappresentata; oggi paesi come la Spagna, la Svizzera, l'Austria, la Germania, la Grecia e la Russia sono all'avanguardia nell'utilizzo dell'ossigeno ozono terapia in ambito medicale.

Vorrei concludere ringraziando le Autorità Comunali con a capo il nostro sindaco Adriano Paroli, e ovviamente Asl e Ordine dei Medici di Brescia.



BRESCIA  
DIP

FRANCESCO BIGGARDO MONTI prize

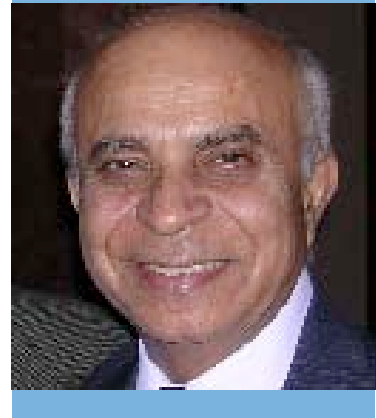


2011

**Francesco**  
**Riccardo**  
**MONTI**  
prize



The **Francesco Riccardo Monti** prize for life time achievements is a recognition for scientific work done to spread Oxygen-Ozone Therapy practice in Italy and around the world.



**Professor Vjiay Kumar**

Professor **Vjiay Kumar** (India), a Neurosurgeon and President of the World Federation of Oxygen-Ozone Therapy has the important merit of having spread the practice of oxygen-ozone therapy with scientific rigour throughout Asia starting from his native city New Delhi. Vjiay Kumar is currently a world reference for the treatment of disc herniation.



**Professor Marco Leonardi**

Professor **Marco Leonardi** (Italy) is full professor of Neuroradiology at Bologna University, President of the Italian Federation of Oxygen-Ozone Therapy and founder of the Italian Journal of Oxygen-Ozone Therapy, currently the International Journal of Ozone Therapy, a journal indexed in Elsevier's Embase database and internationally acknowledged as the only scientific journal devoted to ozone therapy.



**Professor He Xiaofung**

Professor **He Xiaofung** (China) is head of the Interventional Radiology Service at Guangzhou University and President of the Chinese Society of Ozone Therapy. His prize is awarded for his research activity, mainly on animals.



**Professor Velio Bocci**

Professor **Velio Bocci** (Italy) has been head of the Pharmacology Institute at Siena University since 1971. He is the single person who has contributed most to international scientific research into oxygen-ozone therapy worldwide. His career has been crowned by more than five hundred articles published in the international literature.

# 2013

## FIRENZE



Federazione Italiana di  
OSSIGENO-OZONOTERAPIA

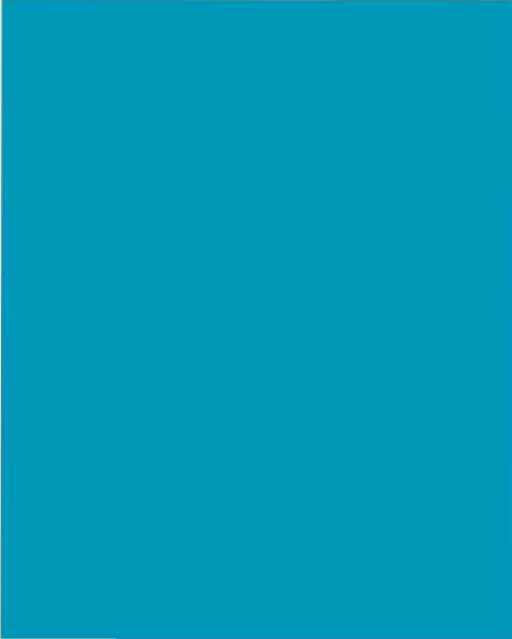
## II° FRANCESCO RICCARDO MONTI PRIZE

Presidente **Gianantonio Pellicanò** Firenze (Italy)





# Francesco Riccardo MONTI prize



SOME COMMENTS





**PROF. MARCO LEONARDI**

University of Bologna, Italy  
Chair of Neuroradiology - Bellaria Hospital - [www.neuroradiologia.unibo.it](http://www.neuroradiologia.unibo.it)

Complimenti per il grande successo ottenuto, un Congresso ad altissime livello scientifico.

Complimenti ancora Matteo”

Marco



**VIKRAM AND VIJAY KUMAR**

Dear Prof. Bonetti

Thank you again for the wonderful conference in Brescia. It was a tremendous show! Congratulations to all of you. Thank you.

Warm regards  
Vikram and Vijay Kumar



**ANGELES ERARIO AND ANIBAL GRANGEAT IAOT**

[www.iaot.com.ar](http://www.iaot.com.ar)

Dear Matteo,  
thank you very much for inviting me. Congress and the entire organization were perfect and I look forward to participating with my boss in the WFOOT. You can count with us.

Once again, it was our pleasure to be part of the conferences. A big hug from Buenos Aires, Argentina.



**HE XIAOFENG M.D.,PH.D.**

**Nanfang Hospital, Southern Medical University**

Guangzhou, China

I, on behalf of the Chinese Society of Ozone Therapy, warmly congratulate the holding of the Third World Congress of Oxygen-Ozone Therapy, and wish the conference a complete success, and wish the delegates good health and happiness!



**PROF. MASSIMO GALLUCCI**

Professor and Head  
Dept of Neuroradiology, University-Hospital - 67100 L'Aquila - ITALY  
[massimo.gallucci@cc.univaq.it](mailto:massimo.gallucci@cc.univaq.it)

Complimenti per il grande lavoro fatto per questo III° Congresso Mondiale di Ozonoterapia e per il successo ottenuto, un salto di qualità per il mondo dell'ozonoterapia.



## ALFONSA MARTÍN

Dear colleague,

It has been a pleasure meeting you in Brescia.

Our Association, the ACEOOT, is very grateful to you for your kind invitation to participate at the Congress, which was most interesting. We wish you luck for your recent joint cooperation agreement with ISCO.

We hope to be able to maintain, in future, the kind and respectful relationship we have been having so far.

Sincerely yours



## GIANNI PELLICANÒ

Grazie di cuore Matteo, organizzazione perfetta, un grandissimo congresso ad alto livello scientifico, grazie davvero e sentiti orgoglioso del grande lavoro fatto.



## YVES BERGERON

Dr. Bonetti,

first of all, I want to thank you and congratulate you for the excellent organization of the congress.

It would be interesting to gather together the presentations about the musculoskeletal system on one side, and the presentations pertaining to internal medicine on the other side.

Also, presentations varied a lot on a scientific point of view. Some had high scientific standards whereas others were more personal and less critical about this therapeutic resource.

I myself believe it is important to keep an objective, critical mind, about ozone therapy instead of presenting it like a panacea appreciated by some, and little-known by others.

I'll be glad to further discuss this matter with you. Thanks once again.

Regards



## DR. MARIAN SIMKO

Fribourg, Svizzera

Dear Matteo,

Thank you very much for the invitation.

The meeting, was wonderful.

La ringrazio ancora di tutto e il piacere di incontrarci in futuro.



### **STAFA ALTIN**

Caro Matteo,  
volevo di nuovo ringraziarti e farti i complimenti per il tuo lavoro nell'organizzazione del Congresso. Un bel Congresso, con sostanza e dettagli curati.  
Mi è piaciuto molto.

A presto!  
Altin



Medicina e Chirurgia Ancona  
Classe 2008

#### **D.I.S.M.A.R.**

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Mobile: + 39 339 5372953

### **LAMBERTO RE, MD**

Carissimo Matteo,  
a distanza di alcuni giorni dal Congresso da te coordinato e splendidamente organizzato, invio queste mie note in merito all'evento.  
Desidero innanzitutto complimentarmi per l'alto spessore scientifico degli argomenti trattati che hanno riguardato tutti i campi di applicazione dell'Ozono Terapia con approfondimenti tematici che saranno di grande supporto per il futuro di questa nuova branca della Medicina.  
Forse per la prima volta, e dinanzi ad una platea di elevato impatto internazionale, sono stati discussi argomenti e dati scientifici che confermano la crescita di questa terapia e l'impatto che potrà avere nella pratica clinica. Veramente complimenti e auguri per un futuro che, sono certo, riserverà all'Ozono Terapia uno spazio sempre più ampio soprattutto se supportato da Convegni e Aggiornamenti all'altezza di quest'ultimo evento bresciano.

Saluti vivissimi a te e al nostro caro Presidente Marco.  
Lamberto Re



### **ALESSIO ZAMBELLO**

Caro Matteo,  
complimenti vivissimi per questo III Congresso Mondiale, so quanto lavoro ti è costato! Un congresso che segna una svolta, per la prima volta si è cambiato passo. Relatori di grande spessore scientifico, professionale ed umano. Relazioni serie, ben preparate, casistiche numerose e proposte terapeutiche razionali.  
L'ozono sta acquisendo una meritata autorevolezza, la strada è questa.

Un saluto  
Alessio



### **RAMIRO ALVARADO**

Mi Caro Matteo,  
Just coming back to my country, I want to Thank You for the invitation to participate in the wonderful congress on Ozone Therapy, really was very nice CONGRATULATION for your big and hard effort I hope to see you again very soon. Let me know about all the plans and programs you have

With My best Regards



## JOSIP BURIC

Caro Matteo

innanzi tutto ti volevo ringraziare ancor una volta per avermi dato la possibilità di presentare le mie relazioni

Il convegno è stato di alto livello e non lo dico solo per cortesia. Ho incontrato persone di diverse nazionalità il che conferma la internazionalità del convegno e la diffusione del ozono stesso. Nella stragrande maggioranza dei casi delle presentazioni cui ho avuto modo di assistere devo confessare che non mi aspettavo un livello così elevato di preparazione scientifica ma anche di metodologia della ricerca clinica che ha portato alla presentazione stessa. Inoltre, la condotta del intero convegno nella lingua inglese ha dato quel tocco in più al congresso che certamente se lo è meritato. Congratulazioni per l'organizzazione meticolosa e attenta dell'evento. Al prossimo anno.

Sinceri saluti



## GIULIANO FABRIS

Caro Matteo,

ancora complimenti per l'organizzazione e la riuscita del Congresso Mondiale. La presenza di personalità di indubbia autorevolezza nel mondo dell'ozonoterapia e la qualità delle relazioni sono state di elevata valenza scientifica. Il 3° Congresso Mondiale di Brescia, è un effettivo e forte punto di riferimento, un confronto delle varie esperienze mondiali, un ulteriore traguardo per l'ozonoterapia, che come è stato confermato è di grande efficacia terapeutica, basso costo ed esiguo rischio nell'ernia del disco e nel dolore muscolo-scheletrico. L'ampia e qualificata partecipazione di vari specialisti in branche mediche e chirurgiche diverse, ma complementari, ha apportato certezze per i medici che si dedicano all'ozonoterapia.

Un grazie per tutto il lavoro che con passione hai svolto.



## ANNUNZIATA IZZO E ANTONELLA BERTOLOTTI INTERMED ONLUS

La riuscita del III World Congress of Oxygen-Ozone dell'aprile 2011 a Brescia, è stata eccezionale, soprattutto per la possibilità di confrontarci con i colleghi e di trovare riscontro in alcune scelte che, soprattutto nei Paesi in via di sviluppo, sono a volte difficili. Il nostro punto di vista è stato supportato anche dalla dott.ssa labichella, che ci ha suggerito di applicare la TOA anche sull'Ulcera di Buruli. Anche la collega Schwartz Tapia che ha parlato di una patologia che noi trattiamo spesso nei nostri dispensari in Africa, ci ha dato suggerimenti importanti ed utili. Inoltre il trattamento con olio ozonizzato è risultato utilizzabile sulle lesioni che stiamo curando.

## **MARIA LETIZIA IABICHELLA**

Il recente consenso mondiale di ossigeno-ozonoterapia ha offerto la possibilità di interscambio di esperienze pluridisciplinari ed al tempo stesso ha stimolato in noi ricercatori la collaborazione nella programmazione di protocolli necessari a documentare ulteriormente i risultati clinici con i dati ottenuti in laboratorio. All'impegno di Matteo Bonetti un encomio di merito.



## **DANIEL ROUBINI**

Grazie per lo splendido congresso.  
Ottima organizzazione con relazioni originali e stimolanti. Ho appreso fatti nuovi e consolidato le mie conoscenze.



**ISTITUTO CLINICO S. ANNA**

## **F. ALBERTINI**

Servizio di neuroradiologia  
istituto Clinico S. Anna - Brescia

L'utilizzo dell'ozonoterapia nelle varie discipline mediche suscita sempre maggiore interesse sia nel mondo scientifico che da parte dei pazienti. Grande occasione di incontro è stato quest'anno il Congresso Mondiale di Ozonoterapia organizzato a Brescia, con numerose sessioni scientifiche dedicate a tutti i settori di impiego e alle novità. L'impatto e il livello scientifico del congresso sono stati notevoli permettendo un utile confronto tra gli specialisti e evidenziando come tale terapia sia spesso in grado di influenzare molti aspetti e la qualità di vita dei pazienti.



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## **ROBERTO DALL'AGLIO**

il congresso di Brescia è stato contrassegnato da due grandi elementi: da una parte si è assistito alla sempre più raffinata definizione delle tecniche interventistiche neuroradiologiche e neurochirurgiche dell'ozono sulle patologie del rachide; dall'altra si è osservata una nuova gamma di applicazioni dell'ozono legate all'attività antalgico-antiinfiammatoria che è stata un primo progresso clinico delle dimostrazioni di interferenza positiva col sistema immunitario dimostrate in vitro: dalle premesse biologiche e speculative di Bocci e Re, la lectio magistralis di Fahmy sulle applicazioni reumatologiche ha segnato una impostazione innovativa in questo campo, foriera di promettenti esperienze da espletare anche in studi comparativi coi farmaci antireumatici.

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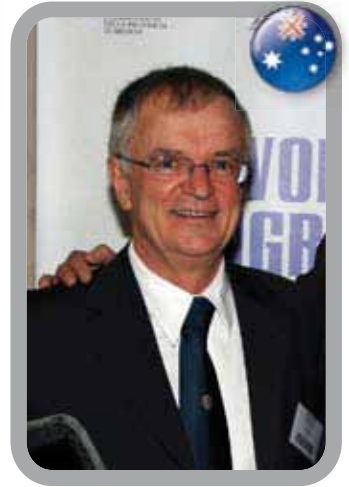
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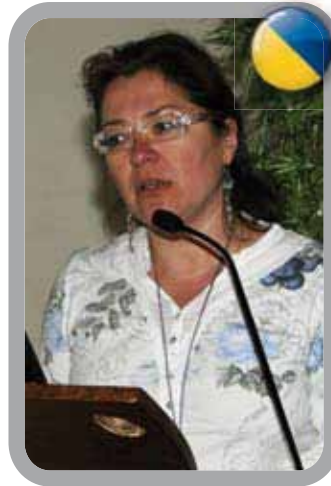
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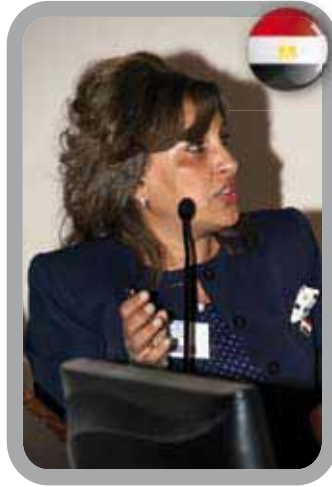




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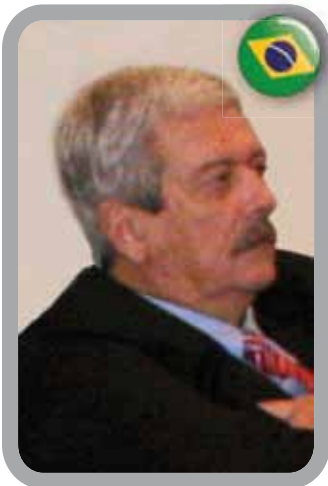
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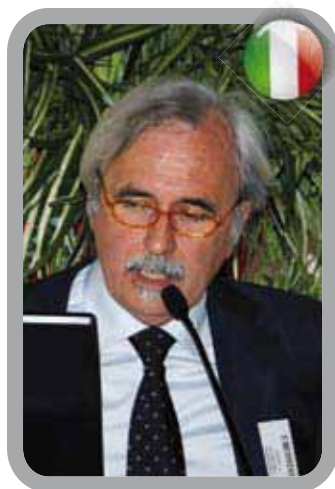
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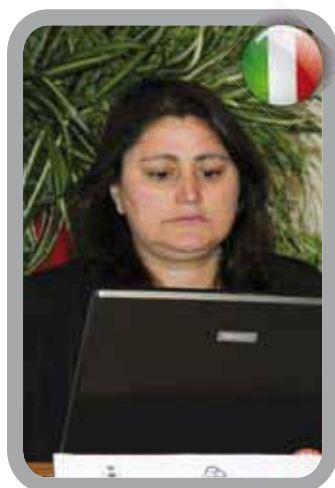
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## I SABATI di OSSIGENO-OZONOTERAPIA 10 DICEMBRE 2011

L'evento si terrà presso la sala congressi del  
"RIZZI AQUACHARME HOTEL & SPA"

Via G. Carducci 11 – 25047 Boario Terme (BS)

Tel. 0364531617

La mattinata si propone di fornire ai partecipanti adeguate conoscenze sulla pratica clinica ed ambulatoriale dell'ossigeno ozono terapia secondo le linee guida prefisse dalla Federazione Italiana di Ossigeno Ozono Terapia.

Evento riservato ai medici di base e accreditato dal Ministero della Salute programma E.C.M.

L'iscrizione è gratuita ed è aperta fino ad una settimana prima del Corso.

### Relatori

Dr. Matteo Bonetti

Dr. Marco Moretti

Dr. Alessio Zambello



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### PROGRAMMA

ore 9.00: *APERTURA CORSO*

Registrazione partecipanti

ore 9.15: Dr. Matteo Bonetti

*L'Ozono Terapia: stato dell'arte.*

ore 9.45: Dr. Marco Moretti

*Efficacia del trattamento con O2-O3 e acido Yaluronico nelle lacerazioni parziali del tendine del sovra spinato.*

ore 10.15: Dr. Alessio Zambello

*Autoemoterapia ozonizzata nel trattamento delle ulcere vascolari non responsive al trattamento tradizionale.*

ore 10.45 – 11.00: Coffee Break

ore 11.00: Dr. Marco Moretti

*Efficacia delle infiltrazioni con ossigeno ozono e acido Yaluronico nella tendinopatia da sovraccarico del tendine d'achille nello sportivo.*

ore 11.30: Dr. Alessio Zambello

*L'ossigeno ozono nella pannicolopatia edematosa degli arti inferiori: trattamento estetico o terapeutico?*

ore 12.00: Dr. Matteo Bonetti

*L'ossigeno ozono terapia nel trattamento del mal di schiena.*

ore 12.30: Tavola rotonda/dibattito

ore 13.30: TEST DI VALUTAZIONE

ore 14.00: CHIUSURA CORSO



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World Federation Oxygen - Ozone Therapy

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Segreteria FIO

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