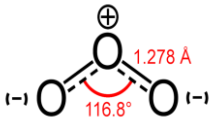


BULGARIAN MEDICAL CONSENSUS ON OZONOTHERAPY METHODOLOGY

on the initiative of the BULGARIAN ASSOCIATION FOR OXYGEN-OZONE THERAPY (BAOOT)

NOTES:

The current consensus of a methodology for working with ozone in medical practice is fully consistent with the current version of the Madrid Declaration on Ozone Therapy (2020), an official document of the International Scientific Committee on Ozone Therapy (ISCO3), summarizing the worldwide scientific interest and accumulated knowledge and experience in the field.

The current medical consensus defines a therapeutic range for the use of ozone in medical practice in the Republic of Bulgaria.

Its amendment and addition is allowed by drawing up, adopting and signing a new consensus in the future work of BAOOT.

Any newly adopted consensus with a simple majority of BAOOT members cancels the previous one.

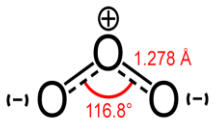
BAOOT distributes each issued medical consensus on ozone therapy to each of its members.

This medical consensus serves as a guide in the work of ozone therapists on the territory of the Republic of Bulgaria and their medical assistants.

This consensus defines the guidelines for working with ozone in medicine, the indications and contraindications for its application, the possible side effects, the types of procedures and ways of applying ozone and the corresponding doses, concentrations and courses of application.

The consensus thus adopted serves as an integral part of procedures for the approval and entry of ozone therapy into Bulgarian medical practice.

I. GENERAL PART



1. WHAT IS OZONE AND OZONOTHERAPY?

DEFINITION: **Ozone therapy** is a complementary treatment in medicine for various pathological processes through the use of established systemic, oral and local methods of applying ozone according to the appropriate treatment regimens, doses and concentrations, produced by a specially designed medical ozone generator in the form of an oxygen-ozone mixture with varying ratios of 99.95-95% oxygen to 0.05-5% ozone and based on the powerful oxidizing properties of ozone and its ability to cause controlled oxidative stress in living organism.

Ozone therapists are certified doctors, additionally qualified and certified to work with ozone in medicine, who, with the help of the relevant equipment and trained medical specialists, perform ozone therapy in the fields and specialties in which they are the only ones competent.

A medical ozone generator is a special apparatus with the technical ability to produce an oxygen-ozone mixture in appropriate ratios, concentrations, flow rate and volume, based on the passage of pressurized oxygen from a container through an electric high-voltage arc in an insulated tube.

The ozone (O_3) is a pale blue gas, an allotropic form of oxygen, composed of three oxygen atoms bonded in an irregular triangle at an angle of 116.8° from each other. At room temperature it is a gas heavier than air. Below -112°C it turns into a dark blue liquid, and below -193°C into a dark violet to black crystalline solid.

Ozone is a powerful oxidizing agent, being the third strongest after fluorine and persulfate.

Ozone is in a gaseous aggregate state in the ozone layer and in the lower parts of the atmosphere, and it is to it that we owe the blue color of the sky.

In nature, it is found in the stratosphere (the upper parts of the Earth's atmosphere bordering space) about 20-30 km from the Earth's surface, which if compressed and condensed would have a thickness of only a few millimeters. In the stratosphere O_3 can reach concentrations up to **10 ppmv** (**p**arts **per mi**llion **v**olume) or it is **1000 µg/m^3**. It is known as a natural protective ozone layer, absorbing ultraviolet rays dangerous for living organisms and humans (especially the powerful denaturing electromagnetic radiation with a wavelength of 200-300 nm with a maximum in the range of 250-280 nm).

In the atmosphere at ground level, ozone levels in the air temporarily rise from their natural formation from oxygen under the influence of electromagnetic storms, breaking waves on rocks and volcanic activity, and then its specific pungent smell can be felt, compared to the smell of coriander, grass or also known as the smell of a rain storm. Ozone is also formed as a result of urban industrial activity and is then part of air pollution in large cities. At the level of the earth's atmosphere near its surface, ozone is in an unstable form, transforming rapidly into its stable form **oxygen (O_2)**. This conversion reaction is a powerful oxidizing process and is what is used in medicine. Its ability to interact with biomolecules in living organisms and the human body is achieved not by inhaling ozone, but by introducing it in strictly defined ways and methods. Controlled and short-lived cascading biochemical reactions result from this interaction. Secondary active compounds of the oxidation are formed, which through different mechanisms of action, when properly dosed, unfold their beneficial medical effects.

2. HISTORICAL DATA

Ozone was discovered **in 1785** by the Dutch physician, physicist and chemist **Martin van Marum (1750 - 1837)** during experiments with electricity, when he noticed that an electric spark in the air led to the formation of a "peculiar electric matter" with a specific pungent odor and powerful oxidizing properties.

1840 German – Swiss professor chemist **Christian Friedrich Schönbein (1799 - 1868)** in Basel gave the name "ozone" (from the Greek word όζειν - smelly), reporting the possibility of oxygen under certain conditions to form a specific gas from three of its atoms. Schönbein established for the first time the ability of

ozone to bind to organic molecules, and in particular in regions with double chemical bonds (C = C), from which further modern studies on the effects and mechanisms of ozone are based.

In **1857**, Schönbein, together with the famous German engineer, industrialist and inventor **Verner von Siemens**, based on the principle of the "magnetic induction tube" invented the first ozone generator, mainly used to purify water for drinking purposes. Thus, industrial installations for disinfection of waste and drinking water were gradually discovered in **Europe, the first of which was in 1893** in the Netherlands, and then in cities in Germany, in Zurich, Florence, Wiesbaden, Nice, Marseille, St. Petersburg and many other cities and countries of the old continent.

The first ozone generator for medical purposes was designed and patented by the world-famous discoverer and physicist scientist with Croatian-Serbian roots **Nikola Tesla 1896** in the USA and put into mass production in **1900** by the purpose-built factory "Tesla Ozone Co". In **1957, the first** OZONOSAN medical ozone generator with the ability to precisely dose in specific concentrations and ranges was developed by the German physicist **Joachim Hänsler** (1908-1981), whose design is responsible for the operation and production of current generators for medical purposes.

Irish military physician **Dr. George Stoker** (1854 – 1920) observed **1898 – 1900** in South Africa the healing methods of local shamans of the Zulu tribe and remained intrigued by the effectiveness of healing wounds by transporting wounded soldiers to high mountain parts near the sea coast where they could stay for some time. At first, then, it was not clear what the remarkable therapeutic effects were due to. Later, returning to London, he discovered the cause and continued to apply and study the healing effects of oxygen-ozone mixtures. He published **1902-1918** several scientific articles in the prestigious journal The Lancet on ozone treatment for surgical wounds and sudden deafness, making his high contribution to the history of ozone therapy. A curious fact connects George Stoker with Bulgaria. During the Russian-Turkish war of liberation 1876-1878, he was the chief physician of the European Red Cross, responsible for the medical assistance of the Bulgarian population during the hostilities. An even more interesting fact is that Major Stoker took an active defensive pro-Bulgarian position after serving in a high medical position in the Ottoman army and witnessing the atrocities of the Ottoman troops on the peaceful Bulgarian population. He informed Europe about the situation of the Bulgarians. Thus, he became one of the figures who changed the attitude of the Great Powers towards enslaved Bulgaria at that time.

The entry of ozone more widely into clinical practice is considered to have come from **Germany**. During the First World War **1914-1918** , German doctors noticed that the wounds of soldiers in a field hospital that was located near a power plant healed faster. It turned out that the effect was due to the large amount of ozone in the air. Dr. **Anton Wolff** already in **1915 began mass application of ozone as an** antiseptic. German doctors used ozone to treat hard-to-heal and decubitus wounds, gangrene, severe burns and to stop bleeding in wounds. During this time, the Australian surgeon **Erwin Payr** (1871-1946) and the Swiss dental surgeon **E.A. Fisch** (1899-1966) actively created, worked and published valuable manuals on ozone therapy, the latter of whom wrote a dissertation in 1950 on ozone in medicine and created the CYTOZON generator, which is still present in the equipment for ozone therapy in dentistry. Scientific studies in dentistry after E.A. Fisch appeared only in the 80s thanks to the developments of **H. Kirschner** and **A. Filippi**.

1936 the French doctor **P. Aubourg** introduced the method of rectal ozone insufflation in the treatment of fistulas and chronic colitis with good success and later for systemic application and thus opened the way for the use of ozone therapy in pediatrics as well. Later, after the second half of the twentieth century, the handbooks of the surgeon **Hans-Georg Knoch** on proctology and rectal insufflation were published, which elaborated this method in detail.

During the Second World War **1939-1945**, the study of the healing effect of ozone was actively continued in Germany, where it was successfully used for local treatment of wounds, burns, after surgical interventions and amputations, other severe traumas received at the front.

After the war, however, practically for twenty-thirty years, the research was stopped, due to the appearance of antibiotics, the absence of reliable compact ozone generators and ozone-resistant materials. It was only in **the 1970s** that specialists again remembered the healing properties of ozone and included it in international scientific research programs.

Hans Wolff (1927-1980), **Joachim Hänsler** (1908-1981) **and** Siegfried **Rilling** founded in **1972** German Medical Society of Ozone Therapists. All of Hans Wolff's medical and research work has been devoted to ozone, and through his numerous publications and practice he has had an enormous share in the spread of ozone therapy throughout the world. In November **1973, the International Institute for the Study of Ozone** was founded as a public-scientific and educational organization.

Hans **Wolff 1968** is also credited with putting the major autochemotherapy (MAH) method into practice in collaboration with **Joachim Hänsler** and his company then for the production of medical ozone generators with accurate dosing in the **60-70s of the XX** century.

The development and dissemination of the bag gasification method is due to **H. Werkmeister** and **O. Rokitansky** in the second half of the past century.

In **Italy,** the orthopedic doctor **Cesare Verga** developed in **the 1980s** the discolysis method for the intradiscal treatment of hernias with ozone and the local paravertebral application of ozone using the discosan method for spinal degenerative and inflammatory diseases. His student - Italian physiologist from Siena Prof. **Velio Bocci** (1928 - 2019) later took over the development of ozone therapy in Italy under the auspices of the national association of Italian ozone therapists, developing key manuals and textbooks on the practice and theory of ozone therapy, rooted in modern understandings of the application and mechanisms of action of ozone.

A serious impetus for the development of ozone therapy, as we know it today, was given by the ozone therapy school of **Russia**. It is also due to the accumulated large database of scientific studies in the field, incl. and the introduction and demonstration of the benefits of the use of ozonated saline intravenously in practice. The State Medical Institute of **Nizhny Novgorod** in **1977** developed under the leadership of an academician cardiac surgeon from the Russian Academy of Medical Sciences (RAMN) - **Boris Al. Korolev** (1909-2010): ozonated saline as a systemic method of administration. The subsequent extensive studies and the specially published works of **Prof. Stanislav Dm. Razumovsky** (**1974** - "Ozone and its reactions with organic compounds") are the impetus for the accumulation of modern scientific data on all known mechanisms of ozone therapy. In April **1979,** for the first time in the world, an ozonated cardioplegic solution was administered in the coronary bloodstream of a patient during surgery for a congenital heart defect in Russia. **In 1986**, the same institute conducted the first extracorporeal ozonation of blood during cardiac surgery for prosthetic mitral valve. Many more avant-garde methods of ozone therapy have been developed and applied in practice by the Russian school, which are studied and used all over the world, for which Russian researchers such as Klavdia N. Kontorshchikova, Gennady A. Boyarinov and Sergey P. Peretyagin played a significant role. The latter is also the current chairman of the Russian Ozone Association and a member of the International Scientific Division ISCO3 of IMEOF.

The credit for the accumulation of a scientific database and practical manuals of the Cuban medical school is not small, especially after the establishment in **Cuba** in **1994** of the National Center for Scientific Research in the Field of Ozone Therapy. Biochemical studies of the mechanisms of ozone therapy are partly the merit of the long-term work of the Cuban institute in question, and its scientists such as Silvia Menendez Cepero and Olga Sonia Leon Fernandez stand out among them.

In **1979**, the International Medical Association of Ozone Therapists was founded.

Formed in April **2005** in New Delhi, India and actively operating on the initiative of the Italian ozone therapy school with headquarters in Brescia, Italy is also the World Federation of Oxygen-Ozone Therapy (**WFOOT**). The chairmanship in WFOOT was handed over by the Romanian orthopedist Stefan Tiron, who has been holding it until recently, to Brazil in the person of Prof. Antonio Teixeira (president of ABOZ - the Brazilian Association of Ozone Therapy) at the organization's 7th World Congress held in Bucharest, Romania, May 5-7. 2022.

2009, the International Medical Ozone Federation (**IMEOF**) was founded in Pontevedra, Spain, with headquarters in Madrid, on the initiative and under the auspices of the Spanish Ozone Medical School (**AEPROMO**). Today, IMEOF works actively under the guidance of Adriana Schwartz and Frank Shallenberger and collects all scientific articles and research results in the field of ozone therapy. Doctors from various specialties from the countries of the European Union, USA, Canada, Russia, Brazil, Cuba, Japan, China, Israel, South Africa and others participate in its international congresses.

The main international medical document and the first **international consensus** in working with ozone as methods and ways of application, indications, treatment schemes, doses and concentrations is **the Madrid Declaration on Ozone Therapy**. It was issued June **2010** by the IMEOF Scientific Council, known as **ISCO** $_3$ - International Scientific Committee of Ozonetherapy and with the assistance of the Royal Spanish Academy of Medicine. Currently, the Madrid Declaration on Ozone Therapy has two revised and supplemented editions (2nd edition June **2015** and 3rd edition May **2020**) and is the main recommended and consensus document at a globally recognized level. Behind this successful project are the efforts of the Spanish association of ozone therapists (**AEPROMO**) under the leadership and with the special assistance of Spanish obstetrician-gynecologist **Adriana Schwartz** and a large international team, among which the merit of names such as **Velio Bocci**, the current president of ISCO $_3$ - **Gregorio Martinez Sanchez**, **Nabil Mawsouf** and many others.

To date, in **over 50 countries** around the world there are active ozone practices in the fields of medicine, cosmetics, dentistry and veterinary medicine. **An international ozone therapy library** with a vast number of published scientific materials, manuals, research results and articles already in excess of 3300 documents as of May 2020 is now available to ozone therapists.

3. KNOWN OZONE ACTION MECHANISMS

Known mechanisms of action of ozone to date:

1. Direct antimicrobial action
2. Impact on exchange and cellular activation of metabolism and regeneration
3. Rheologically active effect
4. Oxygenating and antihypoxic effect
5. Antioxidant effect
6. Anti-inflammatory effect
7. Analgesic effect
8. Detoxifying properties
9. Immunomodulatory effect
10. Impact on hemostasis

3.1. Direct antimicrobial action

The powerful oxidizing effect of ozone in contact with living microorganisms denatures and damages membranes and capsules, and subsequently the cytoplasm and inner nucleus.

Ozone exhibits an immediate virucidal, bactericidal and fungicidal effect through a direct oxidation mechanism, at the same time very powerful and surpassing in comparison with other classic methods of disinfection, as well as surpassing the power of action of many antiseptic local remedies for the treatment of infected ulcers and wounds. In comparison, the antiseptic power of ozone is greater than that of chlorine and slightly weaker than that of iodine.

Unlike macro-organisms and humans, which have their own natural antioxidant defense system that kicks in and protects the cell and the organism; viruses, bacteria, fungi and other single-celled microorganisms do not have such a system, and in an environment with ozone, the latter die or lose their ability to divide and replicate.

3.2. Exchange impact. Cellular activation of metabolism and stimulation of tissue regeneration

Ozone interacts with the lipid components of the cell membrane, and more specifically with the unsaturated fatty acids of the phospholipid layer, due to preferential binding to regions with double bonds (C = C). Saturated lipids, carbohydrates and proteins also react with ozone. Cascade ozonolysis processes occur with biomolecules not only of cell membranes, but also with various biological fluids and blood. This is how various complex and powerful biologically active primary and secondary compounds are formed - ozonides, hydrogen peroxide (H_2O_2), organic peroxides, aldehydes (MDA - malondialdehyde), alkenals (HNE - 4-hodroxy-2,3-transnonenal or 4-hydroxynonenal for short). For H_2O_2 and HNE have accumulated scientific evidence to be considered major mediators of the ozone effect through their ability to activate transcriptional nuclear factors in the cell. All these highly active radicals from ozonation cause a kind of microoxidative stress, activating the body's redox defense mechanisms. These primary and secondary compounds of ozonolysis are also responsible for much of the observed effects and benefits described in ozone therapy.

Phospholipid oxidation and in general the products of lipid peroxidation signal molecular mediators in the cytoplasm (main among them is tyrosine kinase), which activate the formation of adenosine triphosphate (ATP) in the mitochondria and increase the energy reserves of the cell. ATP synthesis is additionally activated by a direct exchange of electrons and protons between the products of lipid peroxidation and the respiratory chain complexes of the mitochondria.

During ozonation, oxidation of nicotinamide (NAD) occurs, which is necessary for the beta-oxidation of fatty acids and the formation of acetyl-coenzyme A. Pyruvate oxidation is also observed, thereby activating the Krebs cycle (citric acid cycle).

The accumulated nicotinamide phosphate NADP is also involved in the pentose phosphate cycle of glycolysis, which, like the Krebs cycle, accumulates energy for the cell. NADP is also directly involved in the work of the respiratory chains of the mitochondria, determining the bioenergetic potential of the cell. It provides many necessary substances for the exchange, synthetic and dividing functions of the cell, it also helps to reduce glucose and increases its absorption. As a result, a number of exchange effects occur.

The glucose- and lipid-lowering abilities of ozone therapy are considered proven.

The ability to reduce accumulated lactic acid (lactate) in the muscles has also been established.

These effects are especially favorable and significant in the work of important exchange organs such as the liver and kidneys, as well as in the endurance of the heart and skeletal muscles, not least in the work of the brain.

Accelerating wound healing, regenerative-granulation-forming and epithelizing effects of the ozonation processes can also be attributed here. It has been established that this happens mainly through the activation of VEGF (vascular-endothelial growth factor) during ozonation. VEGF is an important factor in epithelial formation and the growth of new blood vessels. The activation of the cellular exchange of the skin, mucous membranes and connective tissue in wounds, as well as potentiation of the healing of ulcer processes on the mucous membranes of the esophagus, stomach and intestinal tract, is directly related to this metabolic mechanism.

It is now known that all this is mainly due to intracellular penetration of H_2O_2 and 4- HNE formed by ozonation as first messengers (first messengers), which activate the cytoplasmic tyrosine kinase, which as a second messenger (second messenger) includes in action an important nuclear transcription factor in the cytoplasm - NfkB. This factor is precisely responsible for activating numerous synthetic, protective and immune functions of the cell and explains the aforementioned metabolic effects and actions of ozone therapy.

It has been demonstrated how, in many ways, ozone therapy achieves an impact on cell exchange and the entire organism in a favorable direction as a result of controlled and transient micro-oxidative stress.

3.3. Rheologically active effect

The effect of ozonation on the polyunsaturated fatty acids of the biphospholipid layer of the erythrocyte membrane improves the elasticity and deformability of erythrocytes.

Ozonation restores the activity of ATP-dependent ion pumps, which favorably affects the osmotic pressure and the properties of the erythrocyte membrane.

The formed ozonides in the composition of the erythrocyte membrane make it fluid, softer and more flexible and thus improve the rheological properties of the blood. It has been proven that they reduce the rate of sedimentation of erythrocytes, reduce blood viscosity and increase erythrocyte survival.

3.4. Oxygenating and antihypoxic effect

It is known that increased levels in the cell, and in particular in the erythrocyte, of 2,3-diphosphoglycerate (2,3- DPG) from the already mentioned energetic pentose phosphate cycle, lead to a facilitated and accelerated delivery of oxygen to the tissues by the oxyhemoglobin molecule. Ozonation by the peroxide products of ozonolysis (LOPs – Lipid per-Oxidized Particles) activates the exchange of the cell in the direction of increased formation of NADP and activation of the pentose phosphate cycle by increasing the levels of 2,3-diphosphoglycerate.

Furthermore, ozonation by a systemic route leads to the activation of nitric oxide synthetase (NO-S) in vascular endothelial cells and the accumulation of nitric oxide (NO). This effect of potentiation of the NO-synthesizing enzyme is related to the effect on NfkB in ozonation in the previously described pathway through the secondary mediator tyrosine kinase. In turn, NO is a potent vasodilator of blood vessels, reducing ischemia.

Added to this is the rheologically active effect of ozonation, which further improves dewing in hypoxic tissues.

One should not forget the high penetration ability of the highly biologically active ozonides in hard-to-reach, ischemic and poorly blood-supplied areas of the body.

Thus, ozone therapy has a proven oxygen-saturating and anti-hypoxic effect. This ozonation effect is achieved even in hard-to-reach areas where the penetration of other medicinal preparations would be compromised due to lack of blood vessels or their blockage.

3.5. Antioxidant effect

It has already been emphasized the role of ozone therapy in appropriate doses and concentrations to induce in a macroorganism the so-called controlled and short-term microoxidative stress with subsequent compensatory activation of powerful antioxidant and other protective mechanisms. Among the powerful antioxidant enzyme complexes, the role of glutathione peroxidase (GHPx) , glutathione- S transferase (GST), NADP-quinone oxidoreductase (NQO-1), superoxide dismutase (SOD), hemoxygenase (HO-1) and catalase (CAT) is highlighted. In recent years, it has been shown that this powerful unlocking of the cell's defense mechanisms against oxidation is due to the ozonation-induced activation of an important transcription factor located in the cytoplasm and entering the cell nucleus, called erythroid nuclear factor 2 (Nrf2). In particular, lipid peroxidation products of ozonation (LOPs) appear to be the most potent activators of the Nrf-2 /Keap-1 associated complex. This Nrf-2 factor determines the further drive of the cell's synthetic chains in the direction of increasing antioxidant protection and some other processes of an immune nature. This, more precisely, happens by separating Nrf-2 from the Keap-1 complex in the cytoplasm under the influence of these products of lipid peroxidation and its subsequent entry into the nucleus and binding to a gene called ARE (antioxidant response element). AREs are genes with a short nucleotide sequence in the nucleus, responsible for direct transcription of the already listed antioxidant defense enzymes. This results in a powerful activation of the antioxidant defense systems under the influence of the minimal oxidative stress caused by ozone. Antioxidant enzymes controlled by ARE genes are: glutathione peroxidase (GHPx), glutathione- S transferase (GST), NADP-quinone oxidoreductase (NQO-1), superoxide dismutase (SOD), hemoxygenase (HO-1), catalase (CAT) and others. They are basically enzymes scavenging dangerous free radicals (ROS) accumulated by various life processes. Part of this mechanism also involves the other important transcription factor NfkB, inducing the production of other synthetic and protective cell proteins, including a direct increase in the levels of glutathione (GSH), known as a powerful and essential antioxidant for the human body.

Ozone therapy is basically a mechanism closely related to the oxidative-reduction balance of the body. Paradoxically, it is the ozonation-induced oxidative stress that unlocks and regularizes the achievement of this important oxidative-reduction equilibrium. In modern medicine, numerous disease processes of an autoimmune, infectious, cardiovascular and neurodegenerative nature are attributed to the loss of this balance in favor of accumulating oxidative stress. These are also a group of chronic diseases in which beneficial effects of ozone therapy as a complementary therapy are not coincidentally observed.

This balance should not be lost with incorrect and unreasonably high or hasty high dosing of ozone therapy, since an excess of reactive oxygen radicals (ROS) would disturb the balance in an undesirable direction. Therefore, future efforts are directed here to more precise dosing and improvement of ozone application methods, as well as to achieving accurate measurement of oxidative stress and correspondingly complying with this oxidative-reduction balance.

3.6. Anti-inflammatory effect

The basis of the anti-inflammatory effects is the ozonation of the double bonds (C = C) of the unsaturated fatty acids and, in particular, of arachidonic acid and the biologically active mediators of inflammation and pain produced by it, including prostaglandins, prostacyclin, thromboxane, directly related to the inflammatory cascade processes, controlled by important enzymes such as cyclooxygenase and lipoxygenase.

The specific effect of ozone on the oxidation of leukotrienes is also added here. Leukotrienes are also formed from arachidonic acid and they participate directly in the so-called slow allergic reactions. In the pathomechanisms of the inflammatory processes in bronchial asthma and other diseases, such slow allergic

type reactions are involved. The ability of ozone therapy to influence such pathomechanisms through the effect on leukotrienes is used to influence the course of bronchial asthma and other disease processes based on inflammatory reactions of slow allergic type.

Additionally, the already described antihypoxic and oxygenating, antioxidant, rheological and metabolically active mechanisms of action of ozone also contribute to reducing inflammation in the body.

3.7. Analgesic effect

The analgesic mechanism of action of ozone is related to direct oxidation and neutralization of pain receptors and pain mediators. An oxidizing effect on purinergic receptors and caspase pathways of pain transmission, but also on many other factors of inflammation and pain accumulating in the pathological focus, has been proven. This, together with the described anti-inflammatory and antihypoxic effect, ultimately contributes to the elimination of pain.

Last but not least, the described antioxidant effect by activating the redox defense systems and subsequent reduction and deactivation in the focus of inflammation and pain of the accumulated dangerous radicals from lipid peroxidation leads to an additional analgesic effect. Lipid peroxidation products accumulated from the arachidonic acid cascade inflammatory and pain processes continue to signal pain to the brain from irritated algogenic receptors. So the body continues to send out cells responding to and maintaining the inflammation. Ozonation, by including the antioxidant mechanism that neutralizes these signaling radicals, practically shortens the inflammatory processes, achieving an analgesic effect.

3.8. Detoxifying properties

The formed ozonides in the serum and in the cell membranes and specially formed during ozonation hydrogen peroxide (H_2O_2) and lipid peroxidation products (LOPs) also enter the cytoplasm of the cell, causing temporary controlled oxidative stress. This leads to a compensatory activation of the cell's antioxidant systems and, above all, to the rapid rise of the main intracellular antioxidant - glutathione (GSH). The activity of antioxidant enzymes such as glutathione peroxidase (GHPx), glutathione- S transferase (GST), NADP-quinone oxidoreductase (NQO-1), superoxide dismutase (SOD), hemoxygenase (HO-1) and catalase (CAT) also increases. A reduction of dangerous free radicals (ROS) is achieved and the oxidative-reduction balance necessary for detoxification is achieved.

The detoxifying functions of ozonation are also related to the already described metabolic activating effects in the cell, especially in the liver and kidney cells. During the ozone treatment, the accumulation of cytochrome P450 and catalase (CAT) in the hepatocyte and activation of its microsomal systems have been proven, thus enhancing the liver's detoxification and filtration capabilities. The liver's ability to intensively exchange toxins is increased as the hepatocyte accumulates more energy in the form of ATP, in addition to its increased antioxidant and microsomal functions.

In the kidneys, ozone intensifies the metabolic chain reactions of absorption of glucose, glucose-6-phosphate, pyruvate and lactate and activates gluconeogenesis. Likewise, the level of ATP in the kidney cell and hence the filtration function of the kidneys increases here.

The detoxifying mechanism of action of ozone is also the result of direct oxidation and neutralization of some of the molecules of accumulated toxins in the blood and tissues.

3.9. Immunomodulatory effect

Ozone is not an antigen and does not irritate the immune system, and by stabilizing the exchange of cells it achieves immunomodulating potential without side effects. Its metabolic regulatory properties contribute to reducing the excessive number of accumulated immunoreactive cells and antibodies and at the same time, suppressing immune cell pools to be activated and potentiated. A balancing effect on the production and ratio of anti-inflammatory to pro-inflammatory cytokines is observed. There is also an additional opportunity to achieve an oxidative-reduction balance through the dosed oxidative stress and the included

powerful antioxidant defense mechanisms. The metabolic accumulation of energy in the form of ATP in the mitochondria of immunocompetent cells is also achieved. In this way, the effects of ozonation are neither directly suppressing the work of the immune system, nor are they factors of unwanted immunostimulation, and therefore we talk about the immunomodulating and balancing properties of ozone therapy, which is widely used with success in a number of autoimmune diseases.

The penetrated intracellular hydrogen peroxide (H_2O_2) from ozonation activates the cytoplasmic tyrosine kinase and subsequently the latter as a secondary mediator stimulates the DNA transcription factor of the cell nucleus - NfkB. This factor, especially in immune cells, leads to the regulation of gene expression of relevant cytokines, incl. antibody potentiating factors, inflammatory acute phase proteins, other chemokines, adhesion molecules and very specific proteins, according to the different immunocompetence and roles of the immune system. An increase in the blood of important immune factors such as interferon beta, interferon gamma, interleukins 1, 2, 4, 6, 8 and 10 has been proven during systemic ozone therapy; granulocyte-macrophage colony stimulating factor (GM-CSF) and transforming growth factor-1 beta (TGF-1β). This is also a mechanism of influencing the immune system during ozone therapy, which keeps the immunity tense and signaled in readiness for a timely and proportionately adequate reaction.

It has been found that low and moderate therapeutic doses of ozone up to 30 μg/ml suppress the activation of T2 helper lymphocytes, which, for example, has more importance in the mechanisms of asthma, and therefore low-dose courses of treatment in bronchial asthma are preferred. While high therapeutic ozone concentrations above 30-40 μg/ml have a suppressive effect on T1 helper cells, which plays an essential role in the pathomechanisms of autoimmune diseases, and therefore there is a preference for suppressing this immune pathway with higher doses of ozone therapy.

3.10. Effect on hemostasis

It is known from studies in therapy with ozonated saline solution intravenously that ozone administered in low concentrations up to 2.5 μg/ml exhibits a hypocoagulant effect, and conversely, in high concentrations above 2.5 μg/ml, it has a thrombi-forming and blood-coagulant effect. Low doses of ozone have been shown to affect proteases in blood serum, trypsins, hemotrypsins, factors of the kallikrein-kinin system, elastase, lysine aminopeptidase and other factors of blood coagulation. This results in a clear hypocoagulant effect with prolonged clotting times, increased fibrinolytic activity and decreased platelet aggregation. Conversely, high ozone concentrations have a hemostatic effect, disrupt the integrity of the cell wall of platelets and induce their degranulation, adhesion and thrombus formation. This effect can be used for local hemostasis in bleeding wounds and ulcers with the application of highly concentrated ozone solutions topically. Systemically administered high concentrations of ozonated saline may cause unwanted intravascular hemolysis.

II. GENERAL APPLICATION RULES AND OZONE CONSIDERATIONS

“Primum non nocere!”

It should be noted that the preclinical, genotoxic, toxicological and clinical studies carried out so far in different parts of the world confirm the safe and effective application of ozone treatment methods and confirm an appropriate therapeutic range of doses and concentrations for its use in clinical practice. These methods of application and the corresponding dose regimes and ranges of therapeutic concentrations are considered in this Bulgarian consensus, are based on the accumulated worldwide scientific experience and should be taken into account by ozone therapists and their medical assistants in order to comply with the unconditional medical principle "First do no harm".

"Start low, go slow!"

" Start low doses, increase them slowly!"

Currently, there is no easy and accessible method for measuring oxidative stress. Therefore, for now, only the clinical assessment and according to the patient's condition is relied upon. Ozone treatment is based on the principle of inducing a controlled minimal oxidative stress of very short duration, which will unleash powerful antioxidant and redox-protective mechanisms plus all other effects of action without damaging the tissues.

The body reacts individually to the induced microoxidative stress during ozone therapy. Therefore, ozone treatment is usually applied with the lowest possible initial doses and gradually increased **over 2-7 days**, carefully monitoring the clinical effect. As one of the doyens of modern ozone therapy, Dr. Velio Bocci, has stated: " Start small doses, build up slowly."

It is also necessary to take into account the initial **suspected** oxidative stress in which the patient is before the start of ozone therapy. Expected high oxidative stress is reasonable to suspect in patients in severe general condition, sepsis, shock, prolonged fever, cachectic, young children and adults with prolonged illness, patients with chronic diseases in the decompensated phase of organ failure and immunocompromised. **In such patients, it is recommended to work with extremely low doses and application regimes decellerated over time with continuous medical supervision or, if possible, temporary postponement of the start of ozone therapy and possible preliminary preparation with intake of antioxidant agents.** As a rule, **the antioxidant preparation** is applied at discretion for **1-4 weeks** before the very start of ozone therapy and usually **never during** the ozone treatment itself. It can also be given **after an ozone course**. The following are suggested to be used as suitable means for the purpose: glutathione, coenzyme Q10, Vitamin C, Vitamin E, Vitamin A, beta carotene, zinc, selenium, alpha-lipoic acid, resveratrol and other flavonoids in the appropriate therapeutic doses and methods of administration - alone or in combination. In addition, advice can be given on the use of **foods rich in antioxidants** such as: avocados, blackberries, blueberries, strawberries, raspberries, carrots, broccoli, cabbage, lemons, ginger, garlic, red onions, spinach, tomatoes and others, according to indications and contraindications for accompanying diseases.

Ozone therapy is an additive (auxiliary, adjuvant) and supplementary (complementary) therapy of traditional medicine

Ozonotherapy is a **supplementary- complementary** and **additive- adjuvant or auxiliary** therapy, not alternative medicine, and is conducted together and combined with the methods and means of **traditional medicine** we know (also called **allopathic** or **conventional** medicine).

All described methods of ozone application are usually a combinatory and complementary part of the patient's overall therapy for a specific disease. Therefore, the simultaneous application of ozone methods together with the corresponding medical, surgical, physiotherapeutic and other treatment methods is a natural part of building a combined scheme of treatment in medicine.

Ozone therapy is practiced as a complementary, adjuvant and sometimes as a palliative (relieving) treatment for a number of diseases that will be mentioned in this consensus. It is part of the methods of innovative technologies that facilitate and in combination help and complement conventional treatment methods, taking into account the rule of doing no harm.

Ozone therapists extend only within their competences

Ozone therapy is a medical act and is directed and performed by **certified doctors** with additional qualifications for treating and working with ozone and by trained and instructed medical assistants under the supervision of an ozone therapist.

Ozone therapists practice ozone therapy, according to the competences of their **qualification** and **medical education**, within the scope of **a medical field** and specialty that they master and according to the received additional **qualification in ozone therapy**, issued after a training course and an exam by formed commissions of BAOOT.

Ozone therapists perform ozone therapy in appropriately **registered healthcare facilities** - medical offices, medical centers, medical departments at clinics and hospitals. Healthcare facilities are well equipped with the required medical ozone generators, medical oxygen containers and ozone-resistant medical supplies. The health care facilities where ozone therapists work have an **emergency cabinet** with an Ambu set, loaded according to medical standards. In activities involving invasive surgical methods of ozone treatment with the need for anesthesia, it is recommended to have appropriate equipment, **conditions and experience for resuscitation** and intensive care according to medical standards. In case of invasive surgical operations requiring image assistance (for example, operative intradiscal discolysis), available appropriate radiographic, fluorographic, sonographic or CAT equipment with the relevant conditions for registration, operation and qualification of such equipment in a specially designed surgical room according to medical standards are required.

Ozone therapists in activities involving invasive or systemic methods of applying ozone are guided by **the rules of good medical practice, take written informed consent from the patient** based on an approved template, which they apply as an integral part of the medical file, and strictly adhere to the rules **of asepsis and antiseptics in their work**. The materials used for ozone therapy, which have been in contact with biological fluids and tissues of the body, are disposed of according to established registration, procedure and conditions for working with biologically hazardous waste.

In the case of work methods with open exposure of ozone to the atmospheric air (gasification in a bag, vaginal insufflation, rectal insufflation, insufflation in the ear canal, intracanal insufflations, etc.), it is recommended to use a carbon mask as a **personal protective device** and ensure **good ventilation** and airing.

The WHO sanitary norm for the limit concentration of ozone in air is **0.06 ppmv** (**p**arts **p**er **m**illion **v**olume) or it is 120 **µg/m^3**. For a short exposure of up to 5 minutes, a concentration twice as high as 240 µg/m^3 (0.12 ppmv) is allowed. An advantage is that the human olfactory organ is able to register the specific smell of ozone in the air even at much lower concentrations than 0.01 ppmv (20 µg/m^3), which is far before dangerous inhalation levels are reached.

Ozone generators, oxygen containers and consumables are medical devices

Ozone therapists and medical assistants under their control work with approved medical ozone generators (**MOG**) using special containers for medical oxygen.

MOGs are standardized devices that generate ozone by means of a high-voltage electric charge in a vacuum cylinder through which passes oxygen drawn by the device's vacuum pump from a specially designed medical oxygen container.

The ozone generators used in clinical practice must be certified as medical equipment, which on the territory of the European Union, including for Bulgaria, is **CE** of class **II b** according to Directive **93/42/EEC** of the European Commission.

Medical oxygen containers have a technical requirement for their production from atmospheric air, the latter to be purified to 99% concentrated oxygen and completely dried and decontaminated.

MOGs are devices with the ability to provide accurate concentrations and ratio of the produced ozone-oxygen mixture (in µg/ml or in µg/L for some generators) and corresponding volume and flow rate (flow rate of the generator pump in ml/min or in L/h for most devices).

It is good for MOGs to have an antimicrobial sterile filter with an internal diameter below 0.2 µm, installed in the path of the formed oxygen-ozone mixture.

MOGs typically allow measuring and reporting accurate ozone concentrations ranging from **1 to 80 µg/ml** and generate a homogeneous in quality oxygen-ozone mixture with a permissible error of deviations in the concentration of the formed ozone in the range **+ /- 10%**. The new generations of medical ozone devices offer a concentration range from **1 to 100 µg/ml** with intervals of 1 to 5 µg/ml. The accuracy of the achieved ozone concentration in the mixture at the outlet of the generators should be guaranteed by at least one annual calibration of the MOG by a competent technical team of the manufacturer or commercial distributor. Devices with the possibility of automatic calibration before operating mode are now available. Modern ozone

generators for medical purposes now have a built-in photometric sensor for constant measurement and monitoring of the resulting concentration at the output of the device.

The flow rate of the generator pump provides a flow rate of oxygen-ozone gas mixture from **3 L/h (50 mL/min) to 50 L/h (833 mL/min)**, but this parameter depends on the set concentration and on the diameter of the connected drain pipe system.

The MOG vacuum pump, which draws gas from the oxygen bottle to the device, has a different pressure **from 0.5 to 2 bar**.

MOGs are stationary machines in most cases, but portable devices for home visits with a small oxygen cylinder attached to the set are now available on the market.

In the formed oxygen-ozone mixture of MOG, no other substances are present, except O_2 and O_3.

The specified concentration measured in μg/ml is sometimes noted as μg/**N**ml (N from normilized concentration). This normalized concentration is measured to ensure accuracy and uniformity under the following standard conditions: pressure **1 Atm** (equal to 760 mmHg or 1.10325 bar) and a temperature of **0°C** Celsius (equal to 273.12 K Kelvin or + 32° F Fahrenheit).

All used **consumables** (banks, bags, tubes, connections, syringes, needles) for working with ozone must be sterile for single use or with the possibility of sterilization and made **of ozone-resistant materials**: glass, silicone, stainless steel, fluoropolymer plastic, polytetrafluoroethylene (Teflon), fluorocarbon, polyvinyl difluoride, titanium, polycarbonate.

Rubber, latex, polyvinyl chloride materials are not suitable and are not recommended for use in ozone therapy, as **they are not resistant to ozone**.

Ozone dosing. Ozone is a hormetic agent

The effects of ozone therapy are dose dependent. **The hormetic effect** has been confirmed clinically and experimentally. **Hormesis** represents the phenomenon of dependence of the effect on the dose and more specifically observing a poisonous and dangerous effect in high doses and vice versa, a beneficial effect in low doses in a certain therapeutic range (or figuratively: "What kills you and is a poison can be a useful medicine for you decellerated"). In fact, in very low doses ozone has no effect and they are designated as **ineffective concentrations and doses**. Doses and concentrations at which a desired and wanted therapeutic response is achieved are called the **therapeutic range** and it is further subdivided into **low, moderate and high therapeutic concentrations and doses,** and above this therapeutic range adverse reactions and dangerous effects are observed, which are referred to as **toxic concentrations and doses**.

Ozone is applied through established systemic and local methods according to certain schemes, regimes, doses and concentrations. It is internationally accepted that **ozone** therapy concentrations and doses are given in micrograms per milliliter **(μg/ml)**. It has been proven with a large margin of certainty that **concentrations** in the therapeutic range **from 1 μg/ml to 40 μg/ml** are accepted (with some methods of administration and with certain pathology where **up to 60 - 80 μg/ml** are accepted). **1 to 6 mg** are taken as **doses in the therapeutic range for systemic ozone administration** (through established systemic methods: major autohemotherapy, minor autochemotherapy, rectal insufflation and vaginal insufflation) (rarely **up to 8-10 mg**) per procedure usually in different long courses: **1 to 20 and more** procedures in **courses** with different **frequency** from every day, every other day, 1-3 times a week, up to once every six months or up to once a month.

This is not the case when dosing ozonized physiological solution intravenously (SSO_3), which is also a systemic method of administration. With it, the treatment concentrations are several times lower than those of other systemic methods, because the previously ozonated bag of physiological solution is a significant volume expander, capable of oxidizing 3-5 liters of blood. Therefore, with **SSO_3** method the therapeutic concentrations for setting the MOG device are from **1.6 – 8 μg/ml.** This is explained in detail in the SSO_3 method description.

The total dose ozone per procedure is calculated by the formula **volume** of oxygen-ozone mixture in **ml**, multiplied by **the concentration** of ozone in the gas mixture in **μg/ml** and comes out in **μg**:

$D\ O_3$, DOSE (μg) = V, VOLUME (ml) x C, CONCENTRATION (μg/ml)

($D\ O_3$ = V x C).

The dose is sometimes determined per kilogram of body weight (**$D\ O_3$ = T,kg x C**), but more often it is determined on the basis of dose concentrations in a therapeutic range divided into low, moderate and high and according to a fixed volume of gas mixture.

The dosage, duration and frequency of treatment courses depend on the goals of the therapy, the severity of the disease process, the acuteness or chronicity of the condition, the experience of the ozone

therapist, the prognosis and according to the accompanying diseases and therapies, age, weight and degree of oxidative stress of the body in each individual case. As already noted, low doses are usually started and gradually increased over **2-7 days.** In cases of rare exceptions as for some local applications in severe infections, where a quick aseptic effect is sought, high therapeutic concentrations of 40-60 -80 μg/ml can be given at the beginning of the treatment course.

Low therapeutic doses (1 – 10 μg/ml) are used in pre-calculated and expected high oxidative stress in the respectively listed conditions and cases at the beginning or continuously for the entire course. Also for a sustaining healing effect at the end of a treatment course.

Moderate therapeutic doses (2.5 – 40 μg/ml) are the most frequently used in clinical practice through systemic and local methods of administration. They have an immunomodulating and stimulating antioxidant defense system effect, especially with a gradual progressive increase with monitoring of the clinical response according to the principle "Start low, go slow." They also exhibit excellent metabolic and detoxifying, rheologically active, analgesic and anti-inflammatory, oxygenating and antihypoxic effects.

High therapeutic doses (40 – 60 – 80 μg/ml) have a known direct and powerful oxidizing effect with bactericidal, virucidal, fungicidal action in wounds and infections, where it starts exceptionally directly with the high concentrations. They are used to prepare solutions, oils and ozonated water for oral and topical use. They are also used in the Lahodny method. Also, due to the demonstrated immunosuppressive and antibody-forming effect, high therapeutic doses can also be used in a number of autoimmune diseases (rheumatoid arthritis, scleroderma and progressive systemic sclerosis, disseminated lupus erythematosus), but with a slightly more gradual approach according to the standard ozone treatment main principle "Start low, go slow".

III. INDICATIONS FOR THE APPLICATION OF OZONE IN MEDICINE

Clinical diseases, pathological conditions and processes will be listed, arranged by body systems, for which there is accumulated experience and evidence of beneficial effects and advantages of ozone treatment.

For each nosological unit, one or two or more methods of applying ozone can be used. Usually, when combining two ozone methods, a systemic one is combined with a local or oral method of administration. Dosing in such cases is separate for each ozone method and usually takes into account the total ozone dose received for the day.

Exemplary doses, concentrations, duration and frequency of treatment courses will be indicated in the ozone application methods themselves.

1. Ozone and oncological diseases

NB! Ozone treatment at this stage has no proven direct antitumor effect in oncological diseases.

Ozone therapy in oncology can be used **additively for general supporting treatment and to increase the adaptation capabilities of cancer patients**, thanks to its known immunomodulating, oxygenating and antioxidant mechanisms of action. This also explains the delay in metastatic processes and tumor growth and the reported favorable course.

Another broad area of use of ozone in oncology is **palliative treatment to prevent and reduce side effects and consequences of radiotherapy and chemotherapy**.

Advantages of improving oncological agents side effects are indicated specifically on:

* vomiting during oncotherapy
* anemic syndrome during oncotherapy
* exhaustion and fatigue during oncotherapy
* secondary chemotherapeutic osteonecrosis
* radiation proctitis
* other radio- and chemotherapeutic epithelitis and mucositis
* skin fistulas due to radio- and chemotherapy
* tissue fibrosis due to radio- and chemotherapy

A significant beneficial effect of:

* renal,
* cardiac,
* pulmonary,
* hepatic and
* gastrointestinal complications from antitumor agents.

Moreover, the mechanisms of increasing blood filling in the target organs for oncotherapy, improving ischemia in them through increased oxygen saturation, and rheologically active effects of ozone explain **its ability to potentiate the effects of radio and chemotherapy** and thus indirectly more effectively affect the tumor.

Last but not least in this connection, the ability of ozone therapy to significantly shorten the recovery time of the surgical wound, a necessary condition for starting radiation or chemotherapy, ultimately allows an **earlier opportunity to start oncotherapy** without unnecessary delay waiting for a healing effect. A condition for starting successful chemo or radiation therapy is a previously well-healed surgical wound from the tumor resection, since a poorly healed wound will be worsened by the administered antitumor agents.

Despite **in vitro** studies of a direct antitumor effect on laboratory cell cultures from cancer-infected tissues, in vivo systemic application of ozone in the body fails to achieve this laboratory-observed effect at all. The body's powerful antioxidant redox-protective mechanisms are activated, and practically ozone fails to reach the tumor tissue and affect it in any way. There is only an unfolding of the known mechanisms of ozone, which can be used as an adjuvant therapy in cancer patients in the already mentioned situations and applications of ozone in oncology. However, **an in vivo temporary growth retardation of some tumors was observed** upon local instillation, due to the direct oxidation of the tumor cells.

2. Cardiovascular and pulmonary diseases

* Atherosclerosis
* Ischemic heart disease
* Mild degrees of heart failure up to II functional NYHA class
* Arterial hypertension
* Peripheral arterial vascular disease (PAD)
* Lympho-venous insufficiency, incl. varicose veins and thrombophlebitis
* Chronic bronchitis, incl. chronic obstructive pulmonary disease (COPD)
* Bronchial asthma
* Chronic respiratory failure

3. Endocrine and metabolic diseases

* Diabetes mellitus and its complications: polyneuropathy, diabetic foot, retinopathy
* Dyslipidemia, hypercholesterolemia, hypertriglyceridemia
* Hyperuricemia
* Hypothyroidism

4. Gastro - intestinal, liver and kidney diseases

* Chronic pyelonephritis
* Chronic gastritis and peptic ulcer with or without Helicobacter pylori
* Esophagitis and cholecystitis
* Chronic non-ulcerative colitis
* Crohn's disease and chronic ulcerative hemorrhagic colitis (UCH)
* Chronic hepatitis – viral and non-infectious

5. Neurological, rheumatological and orthopedic diseases

* Chronic cerebrovascular disease
* Central and peripheral vestibular disorders
* Migraine and other types of primary and secondary headaches
* Alcohol detoxification and alcohol withdrawal
* Parkinson's disease
* Multiple sclerosis
* Alzheimer's disease
* Nervous-vegetative dystonia
* Chronic fatigue syndrome
* Fibromyalgia
* Rheumatoid arthritis
* Disseminated lupus erythematosus
* Scleroderma and progressive systemic sclerosis
* Psoriatic arthritis
* Raynaud's syndrome
* Osteomyelitis
* Purulent arthritis and traumatic joint damage
* Septic and other infectious and non-infectious arthritis and osteoarthritis
* Degenerative joint and disc disease with or without mono or polyradiculopathy

* Compression mononeuropathies such as carpal tunnel syndrome, anterior tarsal tunnel syndrome
* Polyneuropathies, incl. diabetic and alcoholic polyneuropathy
* Pain syndromes in vertebral osteochondrosis, spondyloarthrosis and spondylolisthesis
* Myofascial syndrome, incl. plantar fasciitis
* Tendovaginitis and tendinopathies, incl. de Quervain 's tenosynovitis
* Epicondylitis and bursitis, incl. tennis elbow
* Muscle contractures
* Degenerative spinal stenoses
* Facet syndrome
* Spondylodiscitis

6. Skin and infectious diseases

* Neurodermatitis, contact eczema and atopic dermatitis
* Acne
* Skin wrinkles from age
* Cellulite
* Alopecia
* Hemorrhoids
* Psoriasis
* Skin ulcers in vasculitis and polyneuritis
* Abscesses with or without fistulas
* Infected purulent and non-purulent wounds, pyoderma, burn wounds, chronic ulcers and pressure ulcers
* Lesions from insect bites
* Cutaneous mycoses, incl. onychomycosis, tinea pedis, candidiasis
* Herpes infections- herpes simplex type 1 and 2, varicella zoster virus, cytomegalovirus
* Viral hepatitis A, B and C
* HIV and AIDS

7. Eye diseases

* Blepharitis, chalazion and hordeolum (barley)
* Meibomian cyst
* Conjunctivitis and dacryocystitis
* Keratitis, scleritis and uveitis (iridocyclitis and chorioretinitis)
* Diabetic retinopathy
* Glaucoma
* Opticoneuritis

8. ENT diseases

* External, middle and internal otitis
* Acute sensorineural hearing loss
* Acute and chronic rhinitis and sinusitis, incl. vasomotor rhinitis and sinusitis
* Tonsillitis
* Meniere's disease
* Tinnitus

9. Urological diseases

* Prostatitis
* Interstitial cystitis
* Benign prostatic hyperplasia

* Induration penis
* Erectile dysfunction

10. Gynecological diseases and obstetrics

* Bacterial and fungal vulvovaginitis
* Sclerotic lichen of the vulva (craurosis) and vulvar leukoplakia
* Atopic dermatitis of the vulva and vulvar contact eczema
* Bartholinitis
* Condyloma acuminata from papilloma virus
* Pelvic inflammatory disease and uterine adnexa
* Endometritis
* Menopausal climacteric syndrome
* Infertility from fibrosis of the Fallopian tubes
* Early toxicosis with threatened abortion
* Preeclampsia and eclampsia
* Iron deficiency anemia in pregnancy
* Fetoplacental insufficiency
* Intrauterine infection in pregnancy

IV. CONTRAINDICATIONS, INTERACTIONS, RISKS AND PROHIBITED METHODS OF OZONE APPLICATION

CONTRAINDICATIONS FOR OZONE APPLICATION

There are the following disease states and pathological processes as contraindications, in which the application of ozone, especially with systemic methods or some types of local and infiltrative methods, should be avoided or performed with great care and benefit/risk assessment:

* Severe shock, incl. with multiple organ failure and DIC syndrome
* Severe and decompensated NYHA class III and IV cardiac and/or respiratory failure
* Fresh myocardial infarctions, ischemic and hemorrhagic strokes
* Acute alcohol intoxication with severe general condition
* Acute diffuse pancreatitis in active phase
* Hyperthyroidism in decompensated phase, incl. uncompensated Graves' disease
* Acute internal bleeding
* Acute hemolytic anemias, incl. favism (glucose-6-phosphate deficiency, G6PD)
* Other hypocoagulant conditions, incl. thrombocytopenias and defects of coagulation factors
* Predisposition to hemorrhages in leukemias, taking anticoagulants, etc.
* Epilepsy beyond therapeutic control with frequent major convulsive attacks
* Hemochromatosis and patients on treatment with iron (Fe) or copper (Cu) preparations
* Severe liver and kidney failure with risk of bleeding
* Organ transplantation performed in the last 6 months
* Severe psychosis in active phase, due to lack of patient compliance

CONDITIONS AND CASES REQUIRING SPECIAL ATTENTION AND CONSIDERATION

Caution during pregnancy

Ozone therapy is used for various diseases related to pregnancy, which were already listed, but still, the application of ozone is recommended to be avoided in pregnant women with an increased risk of bleeding, with a history of previous heavy gynecological bleeding, with established placenta previa, as well as in the first trimester of pregnancy (0-13 weeks of gestation), when the embryonic development is in progress in an easily vulnerable period.

Attention in childhood

The use of ozone therapy in childhood is not prohibited. But it is limited to the application only through local surface skin-mucosal methods, and of the systemic methods, only rectal insufflation (RIO_3) is currently allowed in the corresponding doses and sample schemes given in the description of the method.

Caution when driving and working with cutting machines and electricity

None of the types of local and systemic methods of ozone treatment affect the ability to drive and work with cutting machines and electric current. On the contrary, ozone therapy, through its antioxidant, metabolotropic, oxygenating and antihypoxic effects, helps to increase concentration and attention, as well as the mental and physical endurance of the body.

Caution in professional athletes

The hematology module for blood doping could record the increased oxygen transport, hemoglobin oxygen delivery, and tissue oxygen saturation seen with ozone therapy. So, the application of ozone to

professional athletes before competitions should be timed and strictly consulted and coordinated with the team's sports doctor in order not to fail the athletes' blood doping tests.

Caution when taking anticoagulants

Applying ozone systemically and with local infiltrative methods affects blood coagulation in the direction of an increased tendency to bleed. This can be especially potentiated and risky in patients on **direct anticoagulants** (heparin, fraxiparin, enoxaparin = Clexan, fondaparinux = Arixtra) and in those taking the "old" **indirect classical anticoagulants** (warfarin = Coumadin and acenocoumarol = Sintrom), incl. with prolongation of prothrombin time (INR) and other indicators of coagulation in the direction of bleeding.

This does not apply, is not considered risky and is not taken into account in patients on antiplatelet **agents** (acetylsalicylic acid = Aspirin, ticlopidine = Ticlid, clopidogrel = Trombex, ticagrelor = Brilique, dipyridamole = Antistenocardin, nattokinase = Nataspin H and others), except in **cases** of their **combined reception** (for example, aspirin and clopidogrel together).

The new NACO indirect oral anticoagulants (rivaroxaban = Xarelto , dabigatran = Pradaxa, apixaban = Eliquis, edoxaban = Lixiana), which do not require INR control, are considered compatible with ozone therapy for now. Increased caution and attention when combining ozone with the new NACO anticoagulants in question is nevertheless required because of the lack of sufficient experience and observations of their use and few convincing data on the safety of their combination with ozone methods.

OTHER KNOWN INTERACTIONS WITH OZONE

Ozone and antioxidants

Ozone therapy relies on inducing controlled and transient minimal oxidative stress in the body, which unlocks powerful oxy-reduction and antioxidant protective and balancing mechanisms and unfolds all other effects of action through the radicals formed.

The presence in the blood of high doses of antioxidants (glutathione, coenzyme Q 10, vitamin A, C, E, flavonoids and others) during the ozone treatment itself can prevent the deployment of these mechanisms and is therefore not recommended. For this reason, only if necessary in patients with pre-therapeutically high oxidative stress, antioxidant courses can be carried out before or after an ozone course, but not during the ozone treatment itself. There are already known exceptions of simultaneous application of ozone with the antioxidant vitamin C in oncology and in infectious diseases, for which recently developed methods are still accumulating scientific data.

Mixing ozone with other agents

Avoid mixing ozone in a syringe, bank of ozonated saline, or vial of ozonated blood with other substances together, be it anesthetics, vitamin C, glutathione, homeopathic remedies, magnesium salts, etc. Ozone, as a powerful oxidizer, can neutralize them or oxidize them into secondary potentially toxic compounds.

Ozone and ACE inhibitors

Ozonation is associated with an additive and clinically significant increase in the hypotensive effects of angiotensin-converting enzyme inhibitors (ACE-inh). In patients on ACE inhibitors, it is necessary to monitor blood pressure and reduce the doses of the latter in ozone therapy.

Ozone and copper (Cu)

Patients on copper preparations cannot receive ozone therapy, because ozone interacts with copper and forms potentially dangerous and toxic copper-oxide compounds.

Ozone and iron (Fe)

For the same reason of obtaining toxic iron-oxide compounds, the use of ozone treatment methods in patients on iron preparations, especially taken intravenously, is prohibited .

Ozone and other oxidizing treatment methods

The use of other treatment methods that also cause oxidative stress, such as hydrogen peroxide treatment (**H_2O_2**), ultraviolet sessions (**UV**), procedures with **a Solux** lamp and others, should be avoided, due

to the possibility of easily getting out of control and overdosing the induced oxidative stress in over-therapeutic (toxic) doses.

Ozone and physiotherapeutic procedures

The harmless combination of ozone therapy methods with all physical procedures such as magnetic field, ultrasound, laser, infrared light, red light, electrostimulation, iontophoresis, diathermy, deep tissue oscillation, acupuncture, massages, therapeutic gymnastics and others has been established. Their combined use can bring additional benefits and speed up the desired healing results. Only a combination with the other oxidation methods already listed is avoided.

RISKY AND NOT RECOMMENDED METHODS OF OZONE APPLICATION

At this stage of development of the science of ozone therapy, methods of ozone application that are considered risky and inadvisable or are thought to have questionable results of effectiveness and insufficiently favorable benefit/risk ratio will be only mentioned.

Direct intravenous administration of ozone

It is considered a gas embolism **risk** method of administration and its use **is not currently recommended.**

The local infiltrative methods with a needle and syringe are even focused on attention and diligence to not cause an accidental unwanted fall of ozone gas mixture directly into a venous vessel, due to rapid insertion or other wrong implementation technique (non-correct angle of insertion, lack of prior aspiration and other techniques of ensuring that ozone does not fall directly into the circulatory system as a gas).

However, there are ozone therapists sharing the method of Dr. Howard Robins and his publications on Direct Intravenous Ozone (DIV) application. It is not yet widely accepted by ozone therapy practitioners and it is considered that further evidence and refinement of the methodology is needed and at this stage it is more advisable to avoid it, especially in the absence of experience.

Arterial direct application of ozone

Again, for the same reasons as for direct venous gas administration, an intra-arterial ozone administration is **not advisable** and **risky** method.

Inhalation of ozone

Ozone should never be inhaled. Inhalation of ozone is toxic and dangerous as it affects the mucus of the respiratory tract and lungs and causes serious reactions on the part of the epithelium and walls of the airways. The latter have been shown not to have a powerful enough capacity to unlock antioxidant defense systems. Inhalation of ozone has been assessed as a **strictly prohibited and high-risk** method. The effects on the lung are similar to a chemical burn and have been shown to increase the risk of opportunistic infections.

Application methods that have not received full consensus

Methods of applying ozone that have not received full consensus at this stage of development will only be listed here without description. Their application is controversial, in many cases associated with risks and with an unfavorable benefit/risk ratio, or simply not enough studies and evidence have been accumulated for their imposition. Future clinical trials will show their eventual validation and acceptance or their ultimate rejection:

* Injection of ozonated water
* Injection of ozonized glucose solution
* Hyperbaric method with repeated administration and added heparin
* Intraperitoneal introduction of ozone

* **NB!** Extracorporeal blood oxygenation (EBOO) is an accepted and consensually approved worldwide systemic method of applying ozone, but it is excluded for now in this consensus, due to the lack of the necessary special equipment currently in Bulgaria, the complexity of implementation, the high labor intensity, the long

duration, the high requirements for equipment and qualified hemodialysis personnel, and the benefit/price ratio is several times higher than the other described systemic methods of application.

V. ADVERSE REACTIONS

Adverse effects and complications due to ozone therapy are listed and divided into **mild**, **moderate** and **severe** side effects.

Ozone treatment with proper use and strict adherence to the rules of application methods and dosages extremely rarely lead to serious and severe side effects.

Most recorded adverse side events were the result of improper technique and unfollowed safety rules, choice of prohibited and risky methods, inconsistent concentrations and doses, lack of experience and unprofessional attitude when working with ozone in medical practice.

Even so, the side effects of ozone therapy are occasional exceptions and most of the cases they are completely overcoming and transient. This makes ozone therapy a sufficiently safe adjuvant and complementary treatment method in clinical practice.

MILD SIDE REACTIONS:

* Local pain, redness and heat at the injection site of a transient and bearable nature
* Local subcutaneous hematoma and hemorrhage at the injection site
* Itching and irritation on the lips and tongue quickly pass after ozone manipulation
* Short-lasting mild euphoria after an ozone procedure
* Numbness and reduced sensitivity of the extremities for several hours
* Mild flatulence with abdominal distension and constipation after rectal insufflation
* Transient mild diarrhea after oral intake of ozonated water
* Lumbar pain of short-time with irradiation to the lower limbs, with local infiltration method
* Reversible mild dyspnea soon after administration
* Mild and short-term corneal irritation after application
* Severe headache with epidural technique

MODERATE SIDE REACTIONS:

* Transient vertebro-basilar ischemia
* Vitreoretinal hemorrhage
* Other internal and external hemorrhages, incl. epistaxis, metrorrhagia
* Meningeal irritation
* Local infectious complications and viral hepatitis if aseptic rules are not followed
* Spondylodiscitis and local abscess in improper technique, dose and unfollowed aseptic rules
* Vasoconstriction during rapid infusion in major autochemotherapy
* Fibrous adhesions with intraforaminal approach

SEVERE AND FATAL SIDE REACTIONS:

* Gas embolism with risk of myocardial infarction, ischemic stroke, spinal infarction
* Pulmonary gas embolism with cardiopulmonary arrest or sudden sinus cardiac arrest
* Fulminant septicemia
* Hemolysis

VI. METHODS OF OZONE APPLICATION

Ozone therapy can be applied through **three** main **ways of administration** : **systemic** (parenteral), **oral** and **local**.

According to this classification the following **types of ozone therapy application methods** are known.

Systemic methods of application :

1. Major autohemotherapy (MAH)
2. Minor autohemotherapy (MiAH)
3. Ozonated saline solution intravenously (O_3 SS)
4. Rectal insufflation (RIO_3)
5. Vaginal insufflation (VIO_3)

Oral methods of administration :

1. Ozonated water
2. Ozonated solutions in the form of oils, emulsions and capsules

Local methods of application :

surface skin-mucosal application and local infiltrations

Surface skin-mucosal application:

1. Topical solutions (oils, emulsions, gels, creams, lotions, shampoos) and ozonized water for skin-mucosal surfaces
2. Ozone suction cup
3. Insufflation in ear canal
4. Insufflation in eye sac
5. Insufflation (gasification) in a bag
6. Vesico-urethral instillation
7. Insufflation in fistulas

Local infiltrations:

1. Paravertebral (Discosan method)
2. Intradiscal (Discolysis method)
3. Intraforaminal
4. Epidural infiltration through the sacral hiatus
5. Intra- and periarticular
6. Perivenous
7 . Glove and sock subcutaneous technique
8 . Subcutaneous technique for cellulite
9. Infiltration in trigger and biologically active points
10. Intratonsillar
11. Intraprostatic

Systemic methods of application:

1.Major autohemotherapy (MAH)

Major autohemotherapy is a systemic method of applying ozone, allowing a certain small volume of one's own blood to be ozonated and treated with an anti-clotting agent and then immediately returned to the bloodstream by intravenous infusion using special equipment.

The volume of blood required for each individual procedure is calculated based on body weight multiplied by **1.2** or **1.3**. This results in the use of no more than **1-2%** of the patient's circulating blood, and in order to avoid hemodynamic overload, it is not recommended to work with a blood volume of more than 150 ml according to the formula:

V (ml) = T (kg) x 1.2 (1.3) - up to a maximum of 150 ml.

Citrate, usually in the form of a dextrose citrate solution or sodium citrate, is preferred as the anticoagulant. The amount of anticoagulant required is **7-10 ml** citrate per **100 ml** blood. The use of heparin is avoided because of the risk of unpredictable and severe activation of platelet factor 4 in the platelet membrane and inducing dangerous thrombotic thrombocytopenia. When heparin is used as a diluent the amount required is **7,500 – 10,000 IU** for every **100 ml** of blood.

Ozone concentrations in the formed oxygen-ozone mixture of MOG for conducting major autohemotherapy (MAH) range **from 10 to 40 µg/ml**. The most commonly used low therapeutic concentrations in MAH are **10 – 20 µg/ml**, moderate concentrations **20 – 30 µg/ml** and high **35 – 40 (– 60) µg/ml**. Concentrations of 60 – 80 µg/ml are not recommended as they have been found to cause hemolysis. The most common daily dose used in practice for most pathological conditions ranges from **1 mg (=1000 µg) to 4 mg (= 4000 µg) and a maximum of 6 mg (= 6,000 µg)**. Daily doses of **5-6 (-8) mg** of MAH have a pronounced immunosuppressive effect and are therefore target doses for gradual reaching in autoimmune diseases, incl. multiple sclerosis, but one should be careful for manifestations of hemolysis.

Courses of major autohemotherapy vary widely **from 7 to 20 procedures** and frequency **from 3 times a week to once a month**, according to the different diseases and goals of the therapy. Doses are progressively increased **over 2-7 days**. The repetition of the treatment course with MAH in the case of chronic nosological units is most often **in 6 months**, but it can be as early as **3 months up to once a year**.

Major autochemotherapy is currently the most common and widely used method of applying ozone in the world and can be used in practice for all the listed indications for ozone treatment. Depending on the concentrations and treatment regimens, the different goals and effects of the therapy are also achieved.

In recent years (since 2015) Austrian gynecologist of Czech origin and long-time university lecturer in Austria and the USA **Johann Lahodny** developed a new method of major autohemotherapy with much higher concentrations and several repeated infusions of ozonated autohomologous blood in one procedure. German speakers refer to his method as OzonHochdosisTherapie **(OHT)** or in English-language literature- High Dose Ozone Therapy (**HDOT**). Lahodny uses really very high ozone concentrations outside the previous paradigm for ozone treatment of **40 - 60 - 70 - 80 - 90 µg/ml** once **a week** for **10 procedures** with withdrawal of 50-100 ml of blood at each separate reinfusion. Each procedure consists of **10 consecutive reinfusions** one after the other within about 1-2 hours, and therefore the method is also known as the **10-pass method**. The author himself calls it **L1** method (L of Lahodny). In some cases, Johann Lahodny recommends twice as many reinfusions, i.e. 20 and designates this as **L1D** (D from double) or **20- pass method**. In practice, some ozone therapists modify his technique by applying within one MAH procedure on the day - on 2 or 3, rarely up to 10 consecutive repeated reinfusions (**multi-pass method**). They run again once a week for 5-10 weeks, with the indicated or lower concentrations common for MAH and also report good results. The author is developing

more options for high-dose ozone treatment through rectal insufflation (**L2**) and through local infiltrations (**L3**).

In case of severe organ failure, Lahodny recommends daily HDOT -MAH for 1 month. He claims that in this way he managed to achieve a unique biological effect of 7-12 times **potentiation of the activity of stem cells** in the bone marrow (not achieved so far by any preparation in the world, except in part by stem cell therapeutic methods). It claims to also achieve an extreme **90% increase in the energy ATP reserve** in the cells' mitochondria (in comparison, other therapies in medicine can only reach 5-30%).

Of course, the methodology needs more studies, additional research and the collection of additional medical evidence, and at this stage it is not among the established ones. These spectacular results are yet to be scientifically substantiated by the author and the Universities of Magdeburg and Leipzig, Germany, who undertook these studies. They report success in over 2000 observed patients who have been given HDOT to date. Effects of powerful detoxification, stimulation of VEGF (Vaso-Endothelial Growth Factor) with the formation of new blood vessels, increase in the number of T-lymphocytes, eosinophilic granulocytes and T-killer cells were observed. At the same time, a decrease in the number of B-lymphocytes and a tenfold increase in prostacyclin levels as an important anti-metastatic and anti-inflammatory factor have been reported.

Another direction of development of the major autochemotherapy method is the work and efforts of the eminent German scientist, Prof. **Renate Viebahn Hänsler**. She is considered one of the modern researchers of the biochemical mechanisms of ozone application, together with the Cuban scientist Prof. Mrs. **Silvia Menendez Cepero**. Dr. Hänsler is also the chief scientific advisor to companies producing ozone generators and companies synthesizing local ozone preparations. German, Austrian and Swiss ozone therapists under her guidance combine in oncology the major autochemotherapy with high doses of Vitamin C intravenously. They are applied not simultaneously mixed, but one after the other in one day, separated by an intermediate infusion of Ringer solution 50 ml between the MAH procedure (with concentrations 10 - 15 - 20 - 25 μg/ml) and before the intravenous administration of Vitamin C (high doses of 2-10-15 g). They do not recommend concentrations of major autochemotherapy in oncology more than 30 μg/ml, because only up to this range do they get biochemical results of a desired increase in gamma interferon, so important in the oncomechanisms of cancers. They start with low concentrations of 10, sometimes even 5 μg/ml, due to the initially high oxidative stress in cancer patients. This methodology is also in the process of gathering additional evidence and studies. There are authors who defend the thesis of benefits when they are combined, but on two separate consecutive days.

2. Minor autohemotherapy (MiAH)

Small autochemotherapy is a systemic method of applying ozone with intramuscular buttock injection with a syringe of equal amounts of an oxygen-ozone mixture and one's own blood.

The volume of blood that is usually used is **5 ml** and very rarely **10 ml**. Blood from the patient is drawn from a cubital or other convenient vein into a sterile 10 or 20 ml syringe without preservatives, into which an equal amount of ozone-oxygen mixture with ozone concentrations from **5-10 to 20 μg/ml** has been previously withdrawn immediately before the manipulation. Shake vigorously for about **30 sec.** and is placed immediately deep intramuscularly at a slow rate without removing the gas from the syringe.

Thus, it can be easily calculated by the volume of oxygen-ozone mixture in ml, multiplied by the ozone concentration in μg/ml, how many micrograms of daily dose the patient will receive. With the volume and concentration variations thus set, it is seen that the patient will receive from **25 to 200 μg**.

The courses of minor autohemotherapy are usually **5 to 10 procedures**, spread over time from **every 2-3 days to once a week** or once a month and represent a kind of autovaccination, activating the immune system, which can be used as a supplement or reinforcement of other ozone application methods, especially in allergic, autoimmune, skin and oncological diseases.

3. Ozonated saline solution intravenously (SSO_3)

Ozonated saline solution intravenously is a systemic method of ozone administration, consisting of intravenous infusion of pre-ozonated isotonic (0.9% NaCl) solution with low concentrations of ozone.

The ozonation of a vial, bag or bank with saline solution is mainly achieved in two ways - "on the three needles" and "on the two needles". With the **"three needles"** method of preparation, constant bubbling of the oxygen-ozone mixture in the solution is possible during the infusion itself. In the **"two-needle" method**, there is an initial stage of about 10 minutes of saturating bubbling, the apparatus is stopped, and in the second stage, a rapid drip is infused in the shortest possible time. **A mixed approach** is most preferred, in which the constantly saturated solution is infused and towards the end, for greater safety, the bubbling is stopped and the infusion is completed without haste and with minimal risk. There are even already specially made kits for ozone generators to achieve this effect in SSO_3 by the mixed method (e.g. Dual Kit - Bexozone® by Bexen Medical, Spain) .

! For greater safety and to avoid gas embolism, it is recommended **to stop the device** and the bubbling when **the last 30-50 ml are left** from the introduced ozonated isotonic solution.

Typically, the amount of saline to be ozonated and introduced is from **200 - 250 ml** to no more than **400 ml**.

This method uses lower O_3 concentrations set on the **MOG apparatus** from **1.6 to 8 µg/ml.** Here it should be taken into account that the saline solution in the bank is saturated only in **25%** of the concentration set on the generator, and therefore it should be immediately **to recalculate** the resulting concentration in the solution, which is actually **4 times less** than the one set on the apparatus. Thus recalculated, **the actual obtained concentration** in the bank to the patient's venous system comes out from **0.4 to 2 µg/ml.** Hence, the recalculated real concentration in the solution can be used to obtain the daily dose according to the known formula **D O_3 (µg) = V (ml) x C (µg/ml)**. Thus, it is clearly seen that the daily dose received by the body when administering ozonized saline solution intravenously would normally vary from **80 to 400 µg**.

Recently, data has been published in science that the maximum saturation of the solution at SSO_3 is not 25, but **10%** of the concentration set on the MOG apparatus, i.e. not 4, but **10 times less**. If this is accepted by consensus in the future, it will mean an actual ozone concentration **of 0.16 to 0.8 µg/ml** or that for 200 ml of solution are **32 to 160 µg actually** obtained daily dose of ozone at SSO_3 method. Adoption of this correction is expected to logically recalculate or outright cancel the dosage according to kilogram of body mass. For now, the math examples below are based on the old calculation for 25% ozone saturation of the isotonic solution bank from the generator. Either way, this does not change the instrument setting, which remains the same for the accepted therapeutic range at SSO_3**: 1.6 to 8 µg/ml**.

For **low therapeutic concentration** at SSO_3 **0.4 µg/ml** is accepted, which is approximately obtained at **a dose 1 µg of body weight**, if the average weight of an adult individual is taken to be 80 kg. Therefore, in order to receive an 80 kg individual a low therapeutic daily dose of 1µg/bw. and if 80 µg in a 200 ml bank, the saline solution should be saturated with a concentration of 0.4 µg/ml (because 80 µg : 200 ml = 0.4 µg/ml). Since the saturation of the isotonic solution is 4 times less, it must be further multiplied by 4 to obtain the necessary concentration for setting the apparatus, and in this case the concentration of the apparatus turns out to be set **at 1.6 µg/ml** (0.4 x 4).

For **moderate therapeutic concentration** at SSO_3 **0.8** is assumed **µg/ml**, which is similarly obtained at **a dose of 2 µg/bw.**, where in the same way it can be calculated that for an 80 kg person and a 200 ml bag this would require setting the MOG **apparatus** to a concentration **of 3.2 µg/ml** to give the patient a daily dose of 2 µg/bw. or 160 µg (dose 2 µg/body weight x 80 kg = 160 µg; 160:200 ml = 0.8 µg/ml in solution; 0.8x4 = 3.2 µg/ml in the apparatus).

For **a high therapeutic concentration, 2 µg/ml** is accepted, which is obtained at a daily **dose 5 µg/bw.**, where again by the same mathematical path it can be calculated that for an 80 kg patient and an available 200 ml bank saline solution the concentration setting of **the generator** becomes **8 µg/ml**. (dose 5 µg/bw x 80 kg = 400 µg; 400:200 ml = 2 µg/ml in solution; 2 x 4 = 8 µg/ml in the apparatus).

This is how, with this method, there is also an additional possibility of dosing in relation to a kilogram of the patient's body weight (bw): low dose - **1 µg/body weight**, moderate dose - **2 µg/bw** and high dose - **5 µg/bw.**

! Exceeding an apparatus concentration of 8 µg/ml (which in the solution is a saturation of 2 µg/ml or even 0.8 µg/ml if we assume saturation tenfold less) at SSO_3 method is not desirable, due to the risk of damaging the venous wall and causing phlebitis and hemolysis.

Low therapeutic concentrations are used to activate the immune system mainly in cardiovascular and pulmonary diseases, in oncology, in obstetrics.

Medium doses are convenient for detoxification purposes in endocrine and metabolic diseases, incl. high sugar, cholesterol, uric acid and their complications, in chronic inflammatory and degenerative diseases.

High doses , due to the immunosuppressive effect, are convenient for autoimmune diseases, but also for the treatment of infectious diseases and burns.

The treatment course in SSO_3 is **6-12 procedures**, usually **every day or every other day** and can be repeated after **a minimum of 3 months**.

SSO_3 just as the great autohemotherapy can be used for absolutely all known indications of ozone treatment, again in concentrations, modes and doses, according to the desired effects and goals. It is more widely favored in Russia, China, Korea, Cuba and other Latin American countries, and more recently in Spain.

Ozonated saline solution intrvenously is the most widely studied method, on the basis of which all other systemic and local methods of ozone therapy, have been proven and scientifically verified. It was approved in Russia by the Ministry of Health as early as 1980, after its discovery and introduction into practice by Academician Boris Al. Korolev 1977.

The Russian scientist chemist Prof. Stanislav D. Razumovsky proves beyond doubt that the decomposition and reactions of ozone in an isotonic saline solution of NaCl does not lead to the formation of dangerous products with sodium and hydrogen and that the level of chlorine compounds (hypochlorites and chlorates) in the practically used amounts of solution and concentrations of O_3 remains negligibly low or zero and safe. In addition to detailed chemical and physico-mathematical analyses, this result has been repeatedly confirmed and confirmed in clinical practice by the application of ozonized saline solution in various diseases. After the demonstration by Razumovsky of the safe application of ozone and the determination of therapeutic doses and ranges, the wider introduction of this method in medical practice is expected.

The doses and concentrations used here are tenfold to a hundredfold lower than those of the large autohemotherapy, due to the fact that 200-400 ml of ozonated isotonic solution introduced into the bloodstream serves as a volume substitute and expander, capable of ozonating 3-5 liters of blood.

This method, compared to the major autochemotherapy, is financially more profitable, saves time, is less labor-intensive and is performed technically easier and with fewer risks. With ozonated saline intravenously, there is no risk of blood clotting or overdose and adverse reactions from the anticoagulant agent. With SSO_3 method, there is no direct contact with blood and its removal from the body, which is perceived with more fear and reservations by some patients.

4. Rectal insufflation (RIO_3)

Rectal insufflation is a mixed (systemic and local) method of applying ozone, in which a certain volume and concentration of an oxygen-ozone mixture is insufflated through a catheter into the rectal ampulla to achieve local enteral, but also through good epithelial-venous resorption and systemic effect.

It appears to be a very good system method of ozonation, well tolerated and sufficiently effective. Rectal ozone insufflation is a sufficiently equivalent alternative to major autochemotherapy, and in roughly comparable concentrations and volumes, and just like MAH, it is indicated for all diseases and pathological processes indicated for ozone treatment.

! In childhood, it is the only permitted systemic method of ozone treatment.

It is used as an alternative to the major autohemotherapy when the latter is refused, in the absence of good veins, in elderly and exhausted patients, in intestinal pathological processes, with an express preference. Still **1936 Aubourg** introduced it as a healing ozone method for fistulas and chronic colitis with good success and later for systemic application. Traditionally, many drugs are introduced systemically by the rectal route, and it is no surprise that ozonation can also be successfully implemented by this method.

Technique itself does not require a previous enema. Anesthesia is not required, only a water-based lubricant is sufficient, incl. petroleum jelly (vaseline). The irrigator tip of the rectal catheter enters 1-2 cm from the anus in children and up to 7-10 cm in adults. The position is lateral with the legs slightly bent and a sanitary pad is placed. The patient is covered, gloves are used. Clear advance instructions are given, signed written informed consent is required. The introduced amount of gaseous oxygen-ozone mixture is sufficient to be retained for 10-15 minutes. Therefore, the patient remains lying down for the indicated time and during the manipulation and after it remains relaxed with slow even breathing without straining and forcing. The maximum amount of introduced gas should not exceed **300 ml** in adults and **150 ml** in children. The rate of introduction of the gas mixture flow is slow for half to 1 minute or even slower, and 50 ml or 100 ml syringes for infusomata machine are often used. It is also possible by direct administration from the ozone device itself to the rectal catheter with a selected flow rate of the pump of **100 - 200 ml/min**.

In RIO_3 method are used concentrations in adults from **10 to 50 µg/ml** in a volume of **100-200 ml**.

Low doses are **10 – 15 µg/ml**, moderate doses are **20 – 30 µg/ml**, high doses are **35 – 50 µg/ml**. Thus, it can be seen that the maximum daily dose accepted is **10 mg (10,000 µg)**. Again, the choice of dose concentrations is in the hands of the ozone therapist and according to the disease, the clinical condition, the degree of oxidative stress, the goals and the desired effects of ozone therapy.

RIO_3 is indicated for all the listed indications for ozone therapy, with the advantage over them that it is the only systemic method in childhood. In pediatrics, the following dosages are offered:

week :	**ozone concentration (µg/ml):**		
course	low:	moderate:	high:
1st	10	15	20
2nd	15	20	25
3rd	20	25	30
4th	25	30	35

age:	**volume of $O_3 – O_2$ gas (ml):**
1 m. - 1 year	15 - 20
1 year - 3 years	20 - 35
3 years -10 years	40 - 75
10-15 years	75-120

It can be seen that slightly higher doses are used in rectal insufflation than in MAH. This need for a slightly higher concentration (about **10-20% more than** the usual **MAH** concentrations) was already established by German ozone therapists in the 80s of the last century, due to a proven slightly lower saturation of the blood circulation with products of ozonolysis. For the same reason, **daily application of the rectal ozone insufflation method** is recommended, especially in the first 5-10 days, in longer treatment courses **of 10 to 20 days or more**, with a faster progressive increase in concentrations **in 2-5 days**, with repetition if necessary after a minimum of **2-3 months**. Initial high concentrations of **40-50 µg/ml** are indicated for ulcerative hemorrhagic colitis and Crohn's disease, after the onset of improvement, their reduction follows.

5. Vaginal insufflation (VIO_3)

Vaginal insufflation is a mixed (systemic and local) method of applying ozone, in which a given volume of oxygen-ozone mixture with a certain concentration and flow rate is insufflated for a certain time by means of a catheter or a specially designed device.

2015 Adriana Schwartz published in a scientific article a comparative analysis of ozone oxidation in capillary flow in vaginal insufflation compared with MAH and RIO_3. **In the comparative analysis, it proves the same and even higher results of ozone saturation with VIO_3.** This can be expected, taking into account the highly folded crypts of the vaginal mucosa, multiplying the contact surface with the ozone gas mixture, the more pronounced vascularization, the high humidity and temperature of the vaginal mucosa, determining a significantly more intense and faster resorption of ozone. In addition, the slower insufflation (for **10 minutes**) of a larger volume of gas (**1 liter**) at a slow flow rate of **100 ml/min** also allows for a greater pressure in the vaginal cavity and this further increases the absorption of ozone in this method. The placement of a special concentric spiral vaginal tip with an attachment to prevent leakage of gas mixture at the entrance of the vagina guarantees a good distribution of ozone in the mucous crypts and the high quality and safety of the procedure. The specially designed device for VIO_3 also has two outlets – one for the introduced oxygen-ozone mixture and the second for taking excess gas to the destructor of the ozone generator, which makes it even easier to work with it.

Performing the vaginal insufflation procedure requires a pre-rinse with ozonated distilled water and the use of a water-based vaginal lubricant after the manipulation, as ozone dries the vaginal mucosa. The position is the classic Trendelenburg of gynecological examination, prior patient instruction, signed written informed consent and gloved work is mandatory.

The required therapeutic concentration ranges for VIO_3 are expectedly lower and range **from 5 to 30 µg/ml**. With a standard volume of the oxygen-ozone mixture of 1000 ml delivered for 10 min at a flow rate of 100 ml/min, it is clear that the received dose varies from **5 – 30 mg (5 000 – 30 000 µg)**.

Vaginal ozone insufflation is used in practice mainly by gynecologists for local problems and systemic inflammatory and infectious processes in the small pelvis, but it can be seen from modern studies that it can adequately replace other systemic ways of applying ozone in women.

Oral methods of administration:

1. **Ozonated water**
   and
2. **Ozonated solutions in the form of oils, emulsions and capsules**

NB! Because of for didactic reasons and due to general principles and rules of work, **oral and local methods of skin-mucosal application of ozonated water and ozonated solutions** - oils, emulsions, creams, gels, lotions and ozone capsules are given together.

Solubility of ozone in **water** is ten times greater than that of oxygen. Ozonation of water should be done in special closed glass containers by passing the oxygen-ozone mixture through it. The use of plastic containers, due to the risk of additional production of phthalates, should be avoided (meaning from polyvinyl chloride plastic products). The bubbling continues for **5 – 10 minutes** at a gas flow rate of **3 L/min** to reach the desired concentration. After this time, with the set concentration of the apparatus unchanged, no further concentration of the solution is achieved. The saturation of water with ozone here is also **4 times less** than its concentration in the gas mixture of the apparatus, as in the saline solution. So, for example, when setting a concentration of 80 µg/ml of the ozone generator, after about 5 minutes **a saturation of 25% less** or a concentration of 20 µg/ml of the water will be obtained. This should be taken into account when dosing, according to the desired effects.

The durability of ozone saturation in water is short and left open the container decreases by 25% of the initially achieved concentration in just 5-6 minutes. However, if doubly distilled water, devoid of other impurities and electrolytes, is used for ozonation and stored in a glass container with a tight silicone stopper at a temperature of 4-8°C, it will lose 50% of its initial concentration after 110 hours, and at room temperature around 20°C in 9 hours. If ozonated ordinary distilled water or filtered tap water, tightly closed at room temperature will reduce the ozone saturation concentration by 50% in 1 hour or in 20 – 30 min if not well sealed. All this must be taken into account when working with ozonated water for drinking, for lavage of wounds and various washings and drainages. **That is why it is recommended to work with freshly ozonized water within the first 15-20 minutes of its preparation.**

Low concentrations are considered **10 – 20** µg/ml, medium **40 – 60** µg/ml and high **60 - 80** µg/ml for **local administration** of ozonated water and respectively: low **2.5 – 5** µg/ml, medium **5 – 15** µg/ml and high **15 – 20** µg/ml for **oral intake** of ozonated water. For local administration, an amount of 500 ml or more is prepared, according to the need, and for oral administration, it is usually prepared in an amount of 250 ml as a single dose to drink.

Vegetable oils (sunflower, olive, coconut, sesame, palm, soybean, corn, almond, castor, avocado, jojoba and others) are used as a suitable basis for ozone therapy, obtaining stable peroxide complexes and other ozonides, aldehydes, ketones, alkenals, etc. during their ozonation. Their ozonation is more effective, the more unsaturated fatty acids the corresponding oil contains. Among vegetable oils, the most popular and most studied are sunflower and olive oil, which can be used both topically and orally. Of these, sunflower oil has more double bonds (C = C) than olive oil.

The storage of the obtained stabilized oxidized products in the oils can be preserved for up to 2-3 years in a dark and cool place (2-8 °C) in the different production processes and products. There are also manufactured ozone products stabilized for storage at room temperature.

Vegetable oils are additionally monitored for the absence of toxic compounds from ozonation such as nitrogen impurities and others. Medical ozone generators are not used for their production, which are intended only for patients and require the absence of additional contamination from toxic impurities of the diffusing oil vapor along the generator pipes, creating an additional risk of fire or explosion. For this purpose, specially designed **industrial ozonating reactor machines** are used. Their production in the form of various ozonated products such as **creams, gels, emulsions, oils, capsules, suppositories, lotions, shampoos and other solutions** is guaranteed at this stage by the pharmacological industry of ozone products for local and oral use, which is increasingly entering the market throughout the world.

The ozonation of 100 g of vegetable oil takes several hours in industrial ozonators, and the maximum ozone absorption concentration reached in the oil solution is approximately 16%, or that is 160 mg of ozone saturation per 1 g of vegetable oil.

Locally used ozonated solutions, oils and water have a pronounced antimicrobial effect, due to powerful direct oxidizing properties to which resistance cannot develop. Locally and orally, ozone solutions, oils and ozonated water also exhibit their metabolotropic, antioxidant, antihypoxic, immunostimulating, anti-inflammatory and analgesic effects. All of them lead to rapid calming of inflammatory processes and pain, accelerate the regenerative processes of granulation and epithelization, prevent tissue necrosis. A favorable environment for recovery is created, thanks to the powerful virucidal, bactericidal and fungicidal effects that destroy pathogenic microorganisms that do not have their own protective redox balancing systems.

The oral intake of ozonated water and other solutions and oils, incl. in the form of capsules, it is suitable for the treatment of esophagitis, gastritis, peptic ulcers, cholecystitis. The solutions and water are also suitable as medicinal enemas for colitis and anal fistulas. Freshly ozonated water and solutions are preferred to ensure the desired concentration.

Locally-used ozonized water and oils are suitable for a number of skin-mucosal viral, bacterial and fungal infections, wounds, ulcers, decubitus, fistulas, gangrene, abscesses, psoriatic plaques, skin lesions in dermatitis, contact eczema, alopecia, hemorrhoids, wrinkles, cellulite and others. They are applied locally by surface application, lavage and drainage.

Studies have proven that a satisfactory antimicrobial effect on viruses, bacteria and fungi from local ozone solutions and ozonated water is realized at minimum concentrations of **30 µg/ml** at the output of the ozone generator and with a duration of impact of **at least 30 minutes. Although, for the purpose of antimicrobial effects, high concentrations of at least or above 40 µg/ml** are preferred for more certainty. In case of necrotic and severe purulent processes, an initial start with maximum concentrations of **70 (80) - 60 - 40 µg/ml is required**, and after achieving a granulating and epithelizing effect, they are gradually reduced to regenerating concentrations **of 5 - 20 µg/ml**. Regarding effects in gastrointestinal diseases, oral and local concentrations of **2.5 - 4 - 10 -20 µg/ml are sufficient**.

Concentrations of vegetable oil solutions for topical and oral use are popularly reflected in a **peroxidation index (PI)**, according to measured lipoperoxide ozonolysis products. The peroxide index reflects the concentration of peroxides formed in ozonated oils in (**mmol/kg**) **or more precisely, the equivalent amount of oxygen atoms in the composition of peroxides per 1kg of oil product**(**mEqO_2/kg**), also referred to as **peroxide number (PV** – peroxide value), is calculated. Currently, other measurable indicators such as the level and concentration of aldehydes, acid value, iodine index and iodine numbers, saponification index and others, which have not yet entered the production practice for quality analysis of ozonated oils, are being standardized.

For now, the peroxide index (PI) values can be used to determine the dosage and indications for use of these products. For **low** levels **values of peroxide number** (**PV**) **200-400** mEqO_2 /kg are determined; for **medium** levels **400 – 600** mEqO_2 /kg and for **high** levels **800 – 1200** mEqO_2 /kg.

The products with **low levels** (200 – 400) are suitable for oral use in gastrointestinal diseases, locally for stimulating granulations and epithelization of healing wounds and for a whitening and skin cleansing effect for cosmetic purposes.

Preparations of **moderate levels** concentration (400 – 600) are the main ones in the treatment of skin-mucosal wounds with or without infection, insect bites, alopecia, hemorrhoids, acne, cellulite, wrinkles, burns, ulcers and wounds in the healing phase.

High levels of peroxide index (800-1200) are applied to heavily infected wounds with purulent and necrotic complications, severe burns, deep trophic ulcers and decubitus, massive and irritated psoriatic lesions and lesions from herpes simplex and herpes zoster infections, as well as mycotic infections.

Local methods of application: skin and various local infiltrations

Surface (topical) skin-mucosal application

1. Locally-used solutions (oils, emulsions, gels, creams, lotions, shampoos) and ozonized water for skin-mucosal surfaces

NB! Because of for didactic reasons and due to general principles and rules of work, **oral and local methods of skin-mucosal application of ozonated water and ozonated solutions** - oils, emulsions, creams, gels, lotions and ozone capsules are given together.

LOOK ABOVE!

2. Ozone suction cup

Ozone cupping is a local method of ozone application that uses a cup or bell-shaped tip with the ability to provide a vacuum on the surface of the skin. The cup is filled with an oxygen-ozone mixture. It has a superficial massage effect on the skin, subcutaneous tissue, and the underlying muscles. For better contact, easier movement on the skin surface and for a more pronounced effect, ozonized or other oil can be used.

It increases blood flow and **recently another metabolic effect of ozone has been shown to significantly reduce lactate levels in the muscles**. This is actively used in ozone cupping method. Accumulation of lactic acid in the muscles leads to fatigue, muscle spasm ("muscle fever") and pain. The combined massage and ozone effect of the suction cup contribute to the reduction of gathered lactate and accelerates recovery processes in musculoskeletal and peripheral neurological diseases associated with accompanying muscle spasm and pain and in cellulite. It can also be used to potentiate the effects of techniques for local infiltration of ozone in tissues, to increase the resorption of local skin preparations and to potentiate the effects of other combined physiotherapeutic procedures.

The concentrations used range **from 15 to 60 µg/ml** for ozone suction cup.

The duration of the procedure is **5-20 minutes**.

The courses are **7-10-12**, maybe **every day** or **every other day**.

3. Insufflation in ear canal

This is a local method of insufflation through a catheter of an oxygen-ozone mixture into the ear canals, mainly used for local problems of the ear. It can be combined with any of the systemic application methods, but it can also be used alone.

Requires prior confirmation by otoscopy of the integrity of the tympanic membrane. To achieve a better effect, pre-moistening of the ear canal is practiced immediately before the procedure.

High concentrations are preferred for herpesvirus infections of the ear canal and purulent inflammatory processes of the middle and outer ear. Moderate doses are preferred in the recovery phase, and low doses for a maintenance effect or at the beginning in people with high oxidative stress.

A 50 ml syringe and adapted silicone tubing with a Y branch from a stethoscope, from another catheter or Luer Lock Connector – Female. For better ozone resorption, insufflation is done very slowly and is usually done manually.

5 - 10 - 25 µg/ml and an application time of **5 minutes** are recommended.

Low concentrations **5 µg/ml**, average **10 – 15 µg/ml**, high **20 – 25 µg/ml**.

The volume of introduced gas is **50 ml**.

The courses are **7-10-12** or more if needed, maybe **every day** or **every other day**.

The requirements for proper room ventilation and the use of a carbon protective mask need to be considered for this open local procedure with the possibility of ozone entering the room air.

4. Insufflation in eye sac

In a similar way to otic insufflation, insufflation is performed in the eye sac.
Due to the irritating effect of ozone, preliminary anesthesia with eye drops is recommended.

It is used to treat a number of listed eye diseases, and can be combined with a systemic method of ozone administration or with local infiltration of an oxygen-ozone mixture into the eye surrounding tissue by an experienced ophthalmologist. There are already manufactured eye drops containing ozone solutions.

10 – 30 µg/ml and an application time of **5 minutes** are recommended.

The courses are **7-10-12** or more if needed, with a frequency of **2-3** times **a week**.

The requirements for strict asepsis, for good ventilation of the room and the use of a carbon protective mask should be considered in this procedure as well.

5. Insufflation (gasification) in a bag

Bag gasification is the insufflation of an oxygen-ozone mixture tightly sealed to the skin of an extremity of the body. It is usually used for purulent wounds, ulcers, fistulas, gangrene and decubitus, including the various forms of diabetic foot. The aim is to create saturated contact of the skin and the surface of the wound itself with ozone to achieve antimicrobial and regenerating - healing local effects. To achieve an even higher result, it is recommended to pre-moisten the treated skin surface and the lesion with a sterile saline solution, and perhaps best with fresh ozonized water or another ozone solution. It is often combined with a topical dressing, also including an ozonated topical agent, and combined with a systemic method of ozone application as needed.

An ozone resistant plastic bag is used. The air in the bag is previously removed and after the manipulation it is again necessary to withdraw the residual oxygen-ozone mixture.

Low concentrations are accepted **10 – 20 µg/ml**, moderate **30 – 50 µg/ml**, high **60 – 80 µg/ml**.

In case of purulent-necrotic processes and infected wounds, start with maximum concentrations.
It is started with **80 – 60 µg/ml** in the first days and for a shorter time **5 – 15 min**.
When regenerative processes of granulation and epithelization begin to be observed, continue with a decreasing dosage of **50 – 40 – 30 – 20 – 10 µg/ml** already for a longer duration of the procedure **20-30 min**. The volume of oxygen-ozone mixture is according to the filling of the entire capacity of the plastic bag.

The courses are **until a satisfactory effect** is achieved. The frequency is at the beginning **every day**, in the healing period they are decellerated **every other day** to **2 times a week**.

The requirements for strict asepsis, for proper ventilation of the room and the use of a carbon protective mask are in full force during this procedure.

6. Vesico-urethral instillation

Vesico-urethral instillation is a local method of introducing freshly ozonated double- distilled water through a urethral catheter into the bladder in specific concentrations and volumes.

Insufflation of an oxygen-ozone gas mixture in the bladder is no longer consensually used, due to the strong sensitivity and reactivity of its mucosa from the direct impact and from the pronounced drying effect of ozone.

Only sterile double- distilled water is used to ensure the absence of any impurities and microorganisms, which is prepared immediately before manipulation.

The ozonated amount of bidistilled water is usually **500 ml** in concentrations **5 – 20 µg/ml**.

The flow rate is **100 – 200 ml/min** for **5-10 minutes**.

It is good to leave 50 ml of ozonated water intravesically at the end of the procedure.

Vesico-urethral instillation is preferred for local infectious-inflammatory processes of the bladder.

7. Insufflation in fistulas

Insufflation of an oxygen-ozone gas mixture in various skin fistulas, perianal fistulas and other surgically formed fistulas is practiced as a local method with a suitable catheter. It can also be done manually with larger syringes or with a catheter connected to the ozone generator.

At the beginning, the cavities, channels and possible cystic deformities of the treated fistula are washed. It is best for this purpose to use freshly ozonated double- distilled water before insufflation.

Insufflation is carried out not for volume, but for a time of **5 to 20 minutes** at a slow speed, if it is automatic, the flow rate of the gas flow is set to **100-200 ml/min**.

Special care is taken to ensure that some of the cavities, cystic deformities and labyrinthine channels of the complicated fistulas do not have direct contact with the airways to avoid direct entry of the gas mixture into the lungs.

In purulent and highly infected fistulas, it is started with maximally high concentrations from **80 - 60 µg/ml** in the first days and for a shorter time of **5 - 10 min**. When regenerative processes of recovery and reduction of exudate, a drop in temperature begin to be observed, then continue with a descending dosage **50 – 40 – 30 – 20 – 10 µg/ml** already for a longer duration of the procedure **15 – 20 min**.

And here, as low concentrations are read **10 – 30 µg/ml**, moderate therapeutic ranges: **30-50 µg/ml** and high therapeutic concentration ranges: **60-80 µg/ml**.

The combined use of this local ozone method with a systemic one is also possible.

And with this procedure, the requirements for strict asepsis, for proper ventilation of the room and the use of a carbon protective mask are mandatory.

Local infiltrations

Local infiltrations are the injection of a certain volume and concentration of ozone into the tissues of the body using appropriate microinvasive or even surgical techniques.

In some more severe cases, the treatment could be combined with a systemic method of ozone application. But due to the large enough amount of introduced ozone gas mixture often required with these infiltration methods, it is good to consider the total dose that the individual will receive, especially if combined with more than one ozone method. **In general, the combination of local infiltration techniques with a systemic ozone method is allowed only exceptionally after a careful assessment** of the degree of oxidative stress and the benefit/risk ratio.

Good medical practice for local ozone infiltrations is to obtain from the patient written consent form after careful and clear explanation of the method, expectations, benefits and possible harms of the procedure. Strict adherence to the rules of asepsis is one of the duties here.

The following is a subdivision of local infiltration methods, according to the areas of introduction and implementation technique:

1 . Paravertebral (Discosan method) infiltration

Paravertebral ozone infiltration is a popular local injection method of introducing, with a needle and syringe, ozone in a certain volume and concentration near the vertebral processes. It is also known as the **discosan method** or discosan, from disk and ozone.

It was introduced by one of the doyens of modern ozone therapy - the Italian orthopedist Cesare Verga in 1979 specifically for the treatment of spinal disc herniation; for distinguishing it didactically from its surgical alternatives - the direct intradiscal insertion technique called the discolysis method of Cesare Verga and the epidural intrahiatal injection for hernias with/without radiculopathy.

Later it became clear that the method was discussed has positive effects not only for disc herniations, but also for a number of other degenerative and inflammatory diseases of the spine.

Paravertebral introduction of ozone can be practiced with comparable results subcutaneously or superficially intramuscularly at **2-4-5 cm** in the paravertebral musculature. It combines with excellent results with other local physiotherapy methods such as laser exposure, magnetic field, ultrasound, diathermy, deep tissue oscillation and other electrostimulating and electroanalgesic procedures.

However, the simultaneous (mixed in one syringe) as well as on the same day and place local infiltration of ozone into the paravertebral musculature together with other preparations (such as paraspinal blockades with medicinal cocktails of anesthetics, analgesics and steroids, collagen, homeopathic agents, hyaluronic acid, glucosamines, etc.) should be avoided. The possibility that the strong oxidizing effect of ozone will neutralize or change the molecules of other agents found together in one treatment area is the reason why it is not recommended to work with them simultaneously in the same area of the body.

Therapeutic concentrations for paravertebral injection range from **5 to 20 µg/ml**.

The volume of injected oxygen-ozone gas mixture is **5-10 ml** per puncture with a total of 4 points of infiltration of 1 disc herniation - about 2 cm above and 2 cm below its level, always bilaterally. This for 1 disc herniation is 4 standard injection points and according to the classic disc method makes a daily dose **of 100 to 800 µg** ozone. The tip of the spinal process of the corresponding vertebra, palpably fixed, serves as a reference point. Injection points are **1 cm apart** (or approximately one finger) from the tip of the processus spinosus for **the cervical zone** and **2 cm** (or two fingers) from this line for **the lumbosacral and thoracic area**. In the cervical region and the first 6 vertebrae of the thoracic spine, less often **5 ml is used** per puncture, and for lumbosacral and the last 6 vertebrae of the thoracic department **10 ml** of each injection.

This is the classic implementation of the discosan method. Its enrichment and supplementation is allowed for a better effect in subcutaneous or muscle trigger points paravertebrally or along the course of an affected peripheral nerve according to the principles of neural therapy. Its additional combination with trigger and neural local methods is necessary for persistent pain and spasm and long-lasting lack of effect from the application of the discosan method alone. As trigger points for additional local infiltration, places of strong pain on pressure or places of marked muscle spasm can be chosen by palpation, also points of facet joint, sacro-iliac joints or in reflexogenic points of Vallée.

Typically, the distance between the infiltration points is at least 4 cm apart to avoid excessive tissue infiltration of the gas mixture. This distance is also observed with other local infiltration methods, where 2 to 10 cm distances are allowed.

The needle enters the skin at a 90° angle, the syringe is held in sight of its scale to accurately assess the dosage. No anesthesia is required. The introduction of the oxygen-ozone mixture is manual and **always at a very slow speed** after ensuring that the tip of the needle has not entered a blood vessel. Preliminary antiseptic treatment of the skin surface in the area of interest is mandatory. Three-component syringes of 10 or 20 ml are preferred in order not to waste the amount of gas mixture and to ensure ease and security of smooth slow introduction. The needles are standard needles for subcutaneous or intramuscular injections.

The position of the patient is lying on his stomach or on his side. Admission to a sitting position is exceptional in case of inability to lie down due to pronounced antalgic pose syndrome.

The discomfort experienced from the infiltration of ozone paravertebrally is a slight soreness resembling burning, warming, pinching, pricking, a slight sharp pain. This discomfort is tolerable, easily overcome by patients and transient.

The procedure is outpatient and does not require any prerequisites for preparation on the part of the patient, nor the need to observe special rules after the manipulation, apart from the restrictions imposed for the relevant pathological process.

The patient was previously informed, instructed and signed an informed consent form.

Usually, the discosan method is started independently at the beginning of the therapeutic course. In other degenerative and inflammatory diseases in the spine and complicated or persistent radicular complaints, the treatment course can be enriched with local infiltration in trigger zones or along the course of an affected peripheral nerve. Doses and volumes can be gradually and stepwise increased, monitoring the effect achieved and tolerability of the organism.

The frequency of the manipulations is different according to the severity of the patient's condition and the goals of the therapy. It can vary from **every 5 days of a calendar week** in the first 2-3 weeks to courses requiring ozone placement **every other day, 1-2 times a week** to **once every two weeks** or **once a month** as a maintenance therapeutic tail.

The duration of the course is also different: **from 7 to 20 procedures**, according to the obtained results.

2 . Intradiscal (Discolysis method) infiltration

Intradiscal ozone therapy or discolysis method is a local surgical method of ozone application in the intervertebral disc under image control with the aim of direct local impact on the cartilage structure of the disc itself in case of disc herniation.

Introduced 1979 by Cesare Verga.

Discolysis is, in practice, a microinvasive surgical procedure performed by neurosurgeons, anesthesiologists, general surgeons, or orthopedists under X-ray, fluoroscopic, ultrasound, or CAT-assisted control. It requires anesthesia and appropriate operating room conditions, additional equipment and experience with the necessary imaging equipment, instrumentation and available trained specialists. This makes it more expensive, requiring a more complex organization of team, apparatus and room and increases the risks, adding those of the anesthesia itself, of the imaging assistance and the passage of the needle through important spinal-cerebral structures, roots and vessels. On the other hand, discolysis is performed once or twice and is also a very efficient local ozone method.

Antibiotic prophylaxis is recommended on the day of the procedure.

The volume of oxygen-ozone mixture for intradiscal introduction is **5 ml** for higher spine divisions or in more gracile patients and **10 ml** for lower divisions and more massive individuals.

The concentrations used range from **25 – 35 μg/ml**.

The length of the spinal needles required for the manipulation is **9-20 cm**, according to the constitution of the patient and the judgment of the ozone therapist.

Although the desired effect is a lysing effect on the collagen structure of the cartilage tissue of the vertebral disc and reduction of the hernia volume, it is not recommended to work with high concentrations above 40 μg/ml. It was established in animal models that the intradiscal insertion of ozone in concentrations of 50 μg/ml and more leads to disruption of the annular integrity of the disc, subsequent gross fibrous growth in it and the surrounding tissue, which poses a risk of relapses, complications and the opposite of therapeutic results.

As already mentioned, the frequency of conducting is **once** or, if necessary, **twice** in a **1-3 month** interval from the first one.

3 . Intraforaminal infiltration

Intraforaminal ozone infiltration into the intervertebral foramen in radiculitis and radiculopathies due to partial foraminal stenosis from disc-osteophyte complexes is also well performed under imaging X-ray or ultrasound control by an orthopedist, neurosurgeon, general surgeon, and sometimes by an experienced anesthetist.

Sufficient volume to infiltrate the intervertebral foramen is **5 ml** in concentrations of the oxygen-ozone mixture set to MOG **5 – 20 μg/ml**. The spinal needle has a length of **4 to 9 cm**, according to the area and the constitutional features of the patient.

The frequency of intraforaminal infiltration is **1-3 times a week** in a course lasting **7-20** procedures.

4 . Epidural infiltration through the sacral hiatus

Epidural infiltration through the sacral hiatus is a surgical microinvasive technique of introducing ozone into the epidural space through approach via the hiatus sacralis. It is performed under local anesthesia and requires imaging assistance with a B- mode ultrasound probe to establish successful access to the peridural space of the hiatus sacral canal. It is performed by an experienced neurosurgeon, orthopedist, general surgeon or anesthesiologist.

It may prove beneficial in multiple herniated discs at more than 2 levels or in polyradicular damage of a degenerative-inflammatory nature and in search of a less invasive and even lower risk surgical ozone method.

The required volume of gas mixture is **10 – 20 ml** with concentrations **5 – 20 µg/ml**.

Usually, **3-4** infiltrations **once every 2 weeks** are enough. It can also be used **twice a week** for more pronounced symptoms. Spinal needles with a length of **9 - 20 cm** are more suitable.

5 . Intra- and periarticular infiltration

With the already listed joint diseases of a degenerative, infectious, traumatic and autoimmune inflammatory nature, suitable for treatment with ozone therapy, intra- or peri-articular application of ozone can be used. The manipulation of local infiltration of joint spaces or surrounding spaces and bursae is performed by experienced specialists, usually orthopedic traumatologists, general surgeons, rheumatologists, anesthesiologists.

Depending on the size of the treated joint, the required **volume of** oxygen-ozone gas mixture during **intra-articular** administrations varies from **1-2-5-10-20 ml** (from the smallest joints of the phalanges to the hip and knee joints) in concentrations **5 – 20 µg/ml**. Antibiotic prophylaxis is appropriate on the day of the manipulation.

According to the therapeutic goals, low doses are **5 µg/ml**, medium **10 µg/ml**, high **15 - 20 µg/ml**.

In **peri-articular** infiltrations, **the volume** of gas per injection is **1-5 ml** in **a minimum of 4 points** of injection in two upper and two lower quadrants immediately around the joint with **the same concentrations** as intra-articular **5 – 20 µg/ml**. Peri-articular infiltrations are practically easy-to-perform subcutaneous or muscular injections with a syringe and needle with a 45° inclination and a direction away from the joint, with a very slow speed of introduction and after preliminary antiseptic treatment and aspiration. Peri-articular procedures do not carry the risks of intra-articular injection of substances, do not require antibiotic prophylaxis and can be performed by all ozone therapists, regardless of specialty, and are often a good alternative or first choice method to intra-articular.

The frequency of their implementation is usually **from 1 to 3 times a week** with a gradual decelleration over time with a positive effect and the duration of the courses from **7 to 20** procedures.

6 . Perivenous infiltration

Perivenous injection is used as a local ozone method for subcutaneous or muscular tissue infiltration around superficial visible venous vessels affected by varicose deformity with thrombophlebitis and trophic venous insufficiency problems.

No anesthesia is required.

It is performed manually with a subcutaneous or thin muscle needle and a three-component syringe with a capacity of 10 or 20 ml to achieve a very slow and smooth way of introduction.

Infiltration takes place very slowly at an acute angle of the needle of 20-30° parallel to the course of the treated vein about **0.5-1 cm** from the venous wall and after careful aspiration to check that it does not enter the vein or another blood vessel.

The amount of gas required varies in volume from **2 to 5 ml** of each puncture with concentrations of **5 – 20 µg/ml** in at least **2 points bilaterally**, i.e. on both sides along the course of the affected vein.

It can be performed **every other day to once a week** in a course **of 5 to 15** procedures, depending on the severity of the condition, the goals of the therapy and the results achieved. It is permissible in severe cases of acute thrombophlebitis with impaired general condition and febrility, combining it with a systemic method of ozone administration of choice after taking into account the total daily dose resulting from the combination and assessed pre-therapeutic oxidative stress in the patient. It is also successfully combined with local skin application of ozone oils and solutions to enhance efficacy.

7 . Glove and sock subcutaneous technique

The technique was developed by Adriana Schwartz 2012-2015.

It is successfully used in polyarthritic and polyarthrotic changes in the small joints of the hands and feet, neuropathic pain in distal polyneuropathies and in Raynaud 's syndrome.

It represents a slow under the skin infiltration of a relatively larger volume of oxygen-ozone mixture with the effect of inducing subcutaneous emphysema in the acral parts of the limbs.

It is performed with a subcutaneous needle and **50 ml** or **20 ml-** syringe after grasping with two fingers and lifting a skin fold on the dorsum of the hand or foot and subsequent manual introduction at a slow speed with the **direction** of the needle less than 45° **to the fingers**.

The volume required varies according to the size of the hands and feet in different individuals and is usually **10 to 40 ml** in concentrations **from 5 to 20 µg/ml**.

The frequency is assumed to be **2 times a week** with the duration of the course until the pain subsides, usually **6** procedures are required.

8 . Subcutaneous technique for cellulite

This is a subcutaneous infiltration of an oxygen-ozone mixture, in which two implementation techniques are known.

In **the first technique** of subcutaneous infiltration in multiple points with a needle and syringe through a distance of **2 - 4 - 10 cm** from each other and a volume of **2 - 3 ml** at a point with concentrations of **5 – 20 µg/ml** and reaching a total volume for the day **not exceeding 100 ml**. It is suitable for acne, limited area or milder cellulite and some other skin diseases.

The second technique is by slow hypodermic insufflation of ozone to induce a subcutaneous emphysema effect similar to the glove and stock procedure. Only it is performed with a catheter directly connected to the ozone generator to the subcutaneous needle with a slow flow rate of **20 - 50 ml/min** up to **not exceeding** a total volume of **200 ml** with concentrations of **5 - 20 µg/ml**. This technique is suitable for large areas of cellulite and for the treatment of skin wrinkles, and is therefore usually performed by experienced cosmetic surgeons and dermatologists.

9 . Infiltration into trigger and biologically active points

Infiltration in trigger and biologically active points is the cutaneous, subcutaneous or muscular introduction of an oxygen-ozone mixture with a needle and syringe into points of the human body determined by anatomical atlases, where it is believed that a reflexogenic effect would be achieved.

Here, the irradiating reflexogenic points of Vallée of nerve plexuses and nerve roots, incl. paravertebral points and the three radiating points for the facial trigeminal nerve; points along the course of peripheral nerves according to the type of neural therapy, known acupuncture points, viscero-dermatome points from the Zacharin- Head reflexogenic zones, painful points in fibromyalgia, points of maximum prominence from underlying muscle spasm, points of vertebral facet joints, vertebral intraforaminal points, etc.

Essential for their correct determination is their good knowledge by practicing ozone therapists, their careful palpatory differentiation and orientation with practical atlases and handbooks available.

Some of these points are quite superficial intradermally and require intracutaneous infiltration, while others are quite deep, sometimes supraosseous, and require deeper muscular infiltration.

The concentrations used are from **5 - 20 µg/ml** for subcutaneous and muscular infiltration in a volume of **2.5 - 5 ml**, and for cutaneous injection (intradermal technique of the "skin horn") at acupuncture and other reflexogenic points, a more limited range of therapeutic concentrations is used **from 6 to 9 µg/ml** and smaller volume of each puncture **up to a maximum 1 ml**.

The frequency of application for some requires **a single** treatment, while for others it is in standard courses of **5 to 20** procedures **every other day to once a week** or less often. Infiltration itself can be done quickly. The maximum total volume for the day does not exceed **100 ml**.

10 . Intratonsillar infiltration

Intratonsillar infiltration is used as a local ozone method in various tonsillitis and hypertrophy of the tonsils.

It is usually performed by ENT specialists or surgeons.

Infiltrate with a suitable needle and syringe an oxygen-ozone mixture in concentrations of **5 – 20 µg/ml** and a volume of **2 - 3 ml** per puncture in **2 to 3 points** of introduction in 1 tonsil.

It can be combined, if necessary, with a system method of optional ozone application.

The courses are **4-6** procedures **every day to once a week**, according to the therapeutic needs.

As a variant, **peritonsillar infiltration** is also practiced in similar doses and volumes.

The introduction of ozone with a needle and syringe into the base of **a nasal polyp** with a volume of **2 ml** in a lysingly high concentration of **50 µg/ml** several times until its removal can be counted as a local infiltration method and approved and applied by ozone therapists - ENT specialists.

11 . Intraprostatic infiltration

Intraprostatic infiltration is a local method of injecting ozone into both lateral lobules of the prostate gland in men with prostatitis or benign prostatic hyperplasia.

It requires performance by a urologist or an experienced surgeon in the field.

Usually, **5 ml** is practiced for each individual lobule with a concentration of **20 µg/ml** and a frequency of **once a week** in a course of a total of **10** procedures.

In prostatic hypertrophy, application of an ozone mixture in a larger volume of **20 ml** has been developed for each lobule and with a higher concentration than previous technique of **30 µg/ml** and frequency **once a week** or **less often** in a course lasting **10** procedures.