Expert consensus of Chinese Association for the Study of Pain on the application of ozone therapy in pain medicine

Zhi-Gang Zhuang, Li-Juan Lu, Bao-Gan Peng, Ke Ma, Zhen-Yu Cai, Zhi-Jian Fu, Guang-Zhao Liu, Jin-Feng Liu, Wen-Tao Liu, Xiao-Hong Li, Tao Song, Da-Sheng Wu, Jing Yao, Peng Yao, Jian-She Yu, Yan-Qing Liu

ORCID number: Zhi-Gang Zhuang 0000-0002-6038-7628; Li-Juan Lu 0000-0002-6571-8529; Bao-Gan Peng 0000-0003-1667-4125; Ke Ma 0000-0002-5603-9321; Zhen-Yu Cai 0000-0002-1326-123X; Zhi-Jian Fu 0000-0002-9387-9768; Guang-Zhao Liu 0000-0001-7415-6292; Jin-Feng Liu 0000-0002-3459-3962; Wen-Tao Liu 0000-0001-8732-7927; Xiao-Hong Li 0000-0001-8610-6233; Tao Song 0000-0001-7929-2074; Da-Sheng Wu 0000-0002-9803-6209; Jing Yao 0000-0001-7483-1913; Peng Yao 0000-0002-8386-7848; Jian-She Yu 0000-0002-4274-2462; Yan-Qing Liu 0000-0002-7347-0789.

Author contributions: Zhuang ZG, Lu LJ, Peng BG, Liu YQ and Liu WT drafted the section “Pharmacological Mechanisms;” Zhuang ZG, Liu YQ and Ma K drafted the section “Indications and contraindications;” Zhuang ZG, Lu LJ, Liu YQ, Liu GZ, Liu JF, Cai ZY and Fu ZJ drafted some other parts of the manuscript; Zhuang ZG, Lu LJ, Liu YQ, Li XF, Song T, Wu DS, Yao J, Yao P and Yu JS drafted the section “Prevention and treatment of adverse reactions and complications” and “Expert consensus statement;” All authors participated in the revision of the consensus.

Conflict-of-interest statement: The

Zhi-Gang Zhuang, Department of Algology, The Second Affiliated Hospital of Zhengzhou University, Zhengzhou 450014, Henan Province, China
Li-Juan Lu, Department of Algology, Nanjing Drum Tower Hospital, The Affiliated Hospital of Nanjing University Medical School, Nanjing 210008, Jiangsu Province, China
Bao-Gan Peng, Department of Orthopedics, The Third Medical Center, General Hospital of the Chinese People’s Liberation Army, Beijing 100039, China
Ke Ma, Department of Algology, Xinhua Hospital Affiliated to Shanghai Jiaotong University School of Medicine, Shanghai 200000, China
Zhen-Yu Cai, Department of Algology, The First Affiliated Hospital of Xiamen University, Xiamen 361005, Fujian Province, China
Zhi-Jian Fu, Department of Algology, Shandong Provincial Hospital, Jinan 250021, Shandong Province, China
Guang-Zhao Liu, Department of Algology, The Second Hospital of Hebei Medical University, Shijiazhuang 050000, Hebei Province, China
Jin-Feng Liu, Department of Algology, The Second Affiliated Hospital of Harbin Medical University, Harbin 150001, Heilongjiang Province, China
Wen-Tao Liu, Department of Pharmacology, Nanjing Medical University, Nanjing 210029, Jiangsu Province, China
Xiao-Hong Li, Department of Algology, Foshan First People’s Hospital, Foshan 528000, Guangdong Province, China
Tao Song, Department of Algology, The First Affiliated Hospital of China Medical University, Shenyang 110001, Liaoning Province, China
Da-Sheng Wu, Department of Algology, Jilin Provincial People’s Hospital, Changchun 130499, Jilin Province, China
Jing Yao, Department of Algology, The Affiliated Hospital of Guizhou Medical University, Guiyang 550025, Guizhou Province, China
Peng Yao, Department of Algology, Sheng Jing Hospital of China Medical University, Shenyang 110004, Liaoning Province, China
INTRODUCTION

Ozone is a strong oxidant and can be used in the treatment of pain disorders due to a variety of significant biological effects in the body. Since 2002 in the Pain Department of hospitals in China, ozone has been used in the therapy of some disorders, including degenerative spinal diseases, musculoskeletal disorders and diseases, rheumatic immune diseases, vascular diseases, metabolic diseases, neuropathic pain, etc. There is a lack of uniform norms and guidelines in clinical practice due to the unreasonable application of ozone from time to time. Some adverse reactions and complications, such as injections into blood vessels or cerebrospinal fluid, have been observed. In order to standardize the rational application of ozone treatment technology in the pain clinic, improve the therapeutic effect and prevent and reduce the occurrence of adverse reactions, first-line clinical experts in the field of pain medicine in China were organized by the Chinese Association for the Study of Pain to assess and discuss the mechanism, indications, contraindications, operating norms and adverse reactions of ozone iatrotreatment in the treatment of pain disorders. We also referred to related previous preclinical and clinical studies published in recent years worldwide. The purpose of this consensus is to standardize the rational application of ozone iatrotechnique in pain treatment, to improve its efficacy and safety and to reduce and prevent adverse reactions and complications in this process.
ozone in the therapy of pain disorders. The China Expert Consensus on the Application of Ozone Therapy in the Pain Department was reached by assessing the topics discussed below.

**PHARMACOLOGICAL MECHANISMS**

Under the appropriate ozone concentration, biochemical reactions similar to preadaptation are produced in the body’s cells\(^8\). There are no ozone receptors in the human body. Therefore, the pharmacological mechanism of ozone is indirectly realized through other factors.

Ozone has a well-known analgesic effect. Subcutaneous injection of ozone at painful area quickly inactivates the inflammatory factors, reduces the stimulation of inflammatory factors to sensory nerve endings and inhibits peripheral sensitization thereby producing its analgesic effects\(^9\). In addition, direct stimulation to sensory nerve endings by ozone can induce the activation of endorphins in the nervous system thus inhibiting the transmission of peripheral injurious stimulation signals to the advanced center\(^10\). Ozone administered via the transforaminal route also stimulates inhibitory interneurons to release enkephalin and other substances thereby achieving central analgesia\(^11\). This type of analgesia occurs quickly after injection. This may be the molecular mechanism of rapid analgesia following ozone administration.

An endogenous antioxidant system can be initiated by ozone therapy (ozone autohemotherapy or tissue injection) by a variety of methods. The expression of heme oxygenase-1 in the local microenvironment is increased, and antioxidation is activated through heme oxygenase-1-mediated signaling to downstream targets\(^12\). The expression of superoxide dismutase is stimulated further decomposing excess peroxidation free radicals. Catalase is generated, and hydrogen peroxide is decomposed. The synthesis of glutathione peroxidase is increased, and organic peroxides are decomposed. Furthermore, the level of glucose-6-phosphate-dehydrogenase is increased in pentose phosphate bypass metabolism enhancing the antioxidant reduction ability of nicotinamide adenine dinucleotide phosphate\(^13\). Due to the effect of ozone, the body’s active removal of free radicals and peroxides generated in the microenvironment as a result of physiological and pathological processes is accelerated.

Ozone has immunomodulatory and anti-inflammatory effects\(^14\). Peripheral sensitization caused by inflammation is the core factor in pain\(^15\). Ozone autohemotherapy or tissue injection have immune enhancement effects, such as enhancing phagocytic function of granulocytes and macrophages and improving the body’s removal of pathogenic microorganisms or metabolic waste. On the one hand, it inhibits the synthesis of proinflammatory cytokines by inhibiting nuclear transcription factors, such as nuclear factor kappa-B\(^16\). On the other hand, it increases the synthesis and release of anti-inflammatory cytokines leading to the rapid elimination of inflammation\(^17\). Local tissue injection of ozone can increase oxygen supply, improve tissue hypoxia and act as a free radical scavenger. Ozone can be quickly reduced to oxygen after exposure to the surface of reductive cells creating an oxygen-rich environment in the local area. Stimulation of ozone affects vascular endothelial cells, which can release nitric oxide and other substances, dilate blood vessels to improve local microcirculation and thus stimulate tissue repair\(^18\).

The effect of ozone on bidirectional regulation of immunity is also manifested in the induction of immune cells to produce massive amounts of cytokines. Ozone autohemotherapy can lead to a small release of interferon-γ, interferon-β, tumor necrosis factor α and granulocyte–monocyte colony stimulating factor in human blood\(^19\). These cytokines have multiple effects, such as immunostimulation or immunosuppression. The excessive use of ozone has a bidirectional regulation effect even on immunosuppression\(^20\). Therefore, different ozone concentrations and courses have different regulation methods and effects on the immunologic function of the body.

It should be noted that taken together, the functions of immunoregulation and antioxidation are achieved by triggering the body’s endogenous protective mechanism. However, the buffering ability of the body’s endogenous protection mechanism is limited. There are also great differences in the buffer capacity and repair capacity of different tissues and cell types. Ozone overdose within a short time may exceed the body’s buffer capacity leading to reduced immune function, oxidative damage and adverse reactions. Therefore, it is necessary to strictly control the application of ozone concentration and total capacity. Indications and contra-
indications should be strictly observed.

**INDICATIONS AND CONTRAINDICATIONS**

Indications for ozone therapy are as follows: (1) Neuropathic pain: Herpes, postherpetic neuralgia and central pain\(^2\), syringomyelia, diabetic peripheral neuropathy\(^2\) and central and peripheral nerve injury pain; (2) Vasogenic pain: diabetes and peripheral vascular disease\(^2\), thrombotic ischemic pain\(^1,2,7\), Raynaud’s disease, erythromelalgia and vasculitis\(^6-8\); (3) Metabolic immune diseases: Ankylosing spondylitis, rheumatoid arthritis\(^9\), allergic diseases and gout\(^10\); (4) Infectious diseases: Necrotizing ulcers, hard to heal wounds\(^14,15\) and burns; (5) Physiological pain: Dysmenorrhea; (6) Tumor pain: Tumor pain during adjuvant therapy, radiotherapy and chemotherapy side effects, tumor consumption treatment and cancerous neuralgia\(^16\); and (7) Degenerative spinal diseases and joint and skeletal muscle diseases: Discogenic low back pain, lumbar disc herniation, cervical spondylosis, knee osteoarthritis, hip osteoarthritis and pain caused by chronic muscle, tendon, ligament, fascia and joint capsule strain\(^17\).

Contraindications to ozone therapy are as follows\(^18\): (1) Ozone allergy; (2) Favism (glucose-6-phosphate-dehydrogenase deficiency); (3) Pregnant women; (4) Hyperthyroidism; (5) Sickle cell anemia; (6) Patients receiving kinase anticoagulant drugs; (7) Severe arrhythmia, hypertensive crisis and other cardiovascular diseases; (8) Hemochromatosis and patients receiving copper or iron therapy; and (9) Other relative contraindications (myocardial infarction, hypotension, hypocalcemia, hypoglycemia, internal hemorrhage, thrombocytopenia, coagulopathy, acute alcoholism and citrus allergy).

**COMMONLY USED INJECTION CONCENTRATIONS, CAPACITY, TREATMENT AND OPERATION SPECIFICATIONS**

Several forms of ozone are used in the treatment of pain. Ozone gas is easily decomposed at room temperature and pressure, is very unstable and can decompose into oxygen. It cannot be stored. On-site production is commonly used for immediate application. Ozone water is an ozone gas under a saturated state dissolved in distilled water. Different to ozone gas, it is still a strong oxidation agent. In clinical medicine, ozone water is mainly used for local anti-inflammatory treatment, infection wound treatment and pelvic inflammatory disease treatment. Ozone oil is a clinical medicine, ozone water is mainly used for local anti-inflammatory treatment, and ozone oil is an ozone gas under an air bath therapy, high-concentration therapy is not recommended.

The capacity of ozone injection is related to the therapeutic target, as described below: (1) Intra-articular injection\(^19\): It is recommended that intra-articular injection should be performed under X-ray/ultrasound positioning to ensure the injection of ozone into the joint cavity. According to the capacity, the joint cavity of the human body can be divided into large joints (shoulders, knees, hips), medium joints (skull) and small joints (elbows and wrists). The recommended standard for intra-articular injection of ozone is shown in Table 1; (2) Injection around the joint: Ozone is accurately injected into the pain points around lesions, the tendons and ligaments. The recommended ozone injection concentration is no greater than 30 μg/mL, and capacity is 1-5 mL/site. The total amount during a course of treatment is no greater than 30 mL with a frequency of 1-3 times/wk. The treatment course is 2-4 wk. Commonly used joint injection sites are around shoulder joints including coracoid sites, large and small
Table 1 Intra-articular injection and treatment

| Target       | Concentration, μg/mL | Capacity, mL | Frequency, times/wk | Course of treatment, wk |
|--------------|----------------------|--------------|---------------------|-------------------------|
| Large joint  | < 30                 | 10-20        | 1-2                 | 2-4                     |
| Medium joint | < 30                 | 5-10         | 1-2                 | 2-4                     |
| Small joint  | < 30                 | 1-5          | 1-2                 | 2-4                     |

nodules of the humerus, intertubercular sulcus, sites below the acromion, the insertion point of the triangular muscle, the superior angle and inner corner of the scapula, the upper part of spinae scapulae and the lower part of spinae scapulae. Usually, 3-5 injection points are selected for each injection and include the lateral collateral ligament attachment sites, the suprapatellar bursa and infrapatellar bursa, fat pad sites, tubercles of the tibia and other painful areas; (3) Injection of soft tissue at pain points: The most obvious area of tenderness is selected for injection. It is recommended that the myofascial trigger points are located under the guidance of B-scan ultrasonography. The recommended ozone injection concentration is no greater than 30 μg/mL, capacity of 1-5 mL/site at a frequency of 1-3 times/wk and a treatment course of 2-4 wk. The total amount during treatment is no greater than 30 mL; (4) Injection around the nerve roots: Transforaminal injection, epidural steroid injection and interlaminar epidural injection are widely used to treat nerve root pain caused by diseases such as disc herniation. Bonetti et al.[41] used 25 μg/mL ozone for transforaminal injection into the epidural space and achieved good results in the treatment of low back pain. In addition, other studies have confirmed the effectiveness of concentrations of 10 μg/mL and 20 μg/mL. Therefore, the recommended concentration for ozone injection of the epidural space is 10-30 μg/mL through various access points. The recommended volume is 3-5 mL for the cervical segment, 5-10 mL for the thoracic segment and 10-20 mL for the lumbar segment with a frequency of 1-3 times/wk. The course of treatment is 2-4 wk. It is recommended that this should be performed under the guidance of X-ray, nerve stimulator and ultrasound. If necessary, angiography can be performed to locate the puncture site. Local anesthetic testing should be performed to ensure the integrity of the dura mater before injection. The injection speed should be slow with the aim of obtaining a more precise curative effect and to ensure safety; and (5) Intradermal injection: This is mainly used for the treatment of herpes zoster and postherpetic neuralgia. The specific operation is as follows. An injection point on the skin in the painful area is selected. The ozone concentration for injection is 20 μg/mL. After injection, an orange peel-like ridge of less than 1 cm is formed at each point. The point-to-point distance is approximately 1 cm, forming a network arrangement. Injection is performed once every other day, 2-3 times a week.

Operation specifications are as follows. The injection should be implemented according to the relevant operation specifications shown in the Clinical Practices-Pain Science Volume published by the Chinese Medical Association. The injection should be performed under strict aseptic conditions. It is recommended that the accurate position should be achieved under imaging guidance. If necessary, angiography can be performed to locate the puncture position. Vital signs should be monitored to prevent the occurrence of adverse reactions.

**OZONE INJECTION ABLATION FOR THE TREATMENT OF INTER-VERTEBRAL DISC DISEASES**

Ozone has been proven to cause dehydration of the nucleus pulposus[42,43]. Therefore, the injection of ozone into the intervertebral disc can reduce the lesion volume of the intervertebral disc and help alleviate the compression on nerve roots[44,45]. More importantly, ozone has a good anti-inflammatory effect, which is conducive to reducing inflammation of the intervertebral disc, nerve roots, ganglia and surrounding tissues.

Indications include patients with disc herniation who have similar clinical symptoms, signs and imaging findings. Contraindications include issues with ozone application and in patients receiving lumbar puncture.

Ozone ablation in the treatment of herniated lumbar intervertebral disc should be performed in a sterile environment and monitored by imaging. Patients should be
informed of all the potential risks and benefits of treatment and have signed an informed consent before treatment.

The concentration for ozone ablation in the treatment of herniated lumbar intervertebral disc is usually 40 μg/mL [46,47]. The capacity of each lumbar spine disc is 4–5 mL [46], and the capacity of each cervical spine disc is 2–3 mL. The injection rate should be slow, and the patient’s response should be observed throughout. Although intervertebral disc ablation is effective after only one treatment, it can be repeated several weeks or months later.

**PREVENTION AND TREATMENT OF ADVERSE REACTIONS AND COMPLICATIONS**

Allergic reaction is a common side effect. If patients suffer diffuse erythema, rash and itching, it is usually considered an allergic reaction. No further risks and complications have been noted.

**OZONE AUTOLOGOUS BLOOD THERAPY**

Ozone autologous blood transfusion therapy (hereafter referred to as “autologous blood”), also known as ozone immunotherapy, involves an appropriate concentration and volume of ozone used to treat a certain amount of blood extracted from the patient’s body. Then the blood is reinfused into the patient’s body in an effort to obtain clinical efficacy. It includes large autologous blood therapy and small autologous blood therapy [1]. In large autologous blood therapy, a total of 100-150 mL of blood is obtained each time. It is then reinfused into venous blood vessels after treatment with an appropriate volume of ozone. In small autologous blood therapy, only 5-10 mL blood is obtained. After ozone treatment, intramuscular injection is performed, generally into the gluteus muscle [48,49]. Large autologous blood therapy is mostly used during surgery.

**Mechanism of the action of large autologous blood therapy**

The mechanism of the action of large autologous blood therapy is unclear at present. Some studies have shown that ozone binding to blood effects the following aspects: (1) Activates erythrocyte metabolism, increases the oxygen saturation of hemoglobin and enhances the application of oxygen and adenosine triphosphate in tissues. It improves oxygen supply, promotes blood circulation, enhances cell vitality and repairs tissue cells; (2) Regulates the body’s immune system. It enhances the phagocytic function of granulocytes and macrophages, improves the body’s ability to remove metabolic waste and accelerates the removal of germs, viruses, etc.; and (3) Activates the antioxidant enzyme system, removes lipids from the blood and metabolic waste, enhances the activity of antioxidant enzymes in the body and reduces the damage caused by free radicals in the body [14]. It improves blood viscosity, reduces blood glucose, uric acid, bilirubin, lactic acid and pyruvate, strengthens the decomposition of cholesterol and triglycerides, improves the status of vascular walls and prevents systemic atherosclerosis and neurological lesions [50].

**Equipment and large autologous blood therapy procedure**

The large autologous blood treatment room should be a well-ventilated, air-disinfected independent treatment space with an average area greater than 20 square meters per treatment bed.

**Preparation before treatment**

The windows (doors) of the treatment room are opened for ventilation. The power supply, oxygen cylinder and interface connection are checked. The oxygen cylinder switch is opened. The oxygen pressure is checked to make sure there is no air leakage. The power switch of the ozone generator is turned on. The supplies are checked: Special package for basic autologous blood therapy, a bottle of 150 mL saline and treatment vehicle (tourniquet, disinfection cotton swab and disinfectant).

**Treatment**

The patient is placed in the supine position. The middle vein of the patient’s elbow is
selected for blood collection. The blood collected should be shaken slowly and evenly clockwise during blood collection, thereby blood and anticoagulants are fully mixed. A volume of 100-150 mL is often used for blood collection. The maximum volume is 200 mL. After the completion of blood collection, a certain concentration of ozone gas at the same volume is injected under aseptic collections. At the same time as ozone injection, the blood collected is slowly and evenly shaken clockwise, so that ozone and blood are fully mixed. The mixing time is approximately 3-4 min from the time of ozone injection, and then the blood is reinfused into the patient’s body. Attention is paid to monitoring the patient at the time of reinfusion.

**Courses and concentrations of large autologous blood therapy**

In large autologous blood therapy, the course of treatment is generally 10-15 times. The treatment can be performed once a day or every other day. A course interval of more than 6 mo is recommended.

The concentration of ozone during large autologous blood treatment is usually increased from a low dose. The initial concentration is 20-30 μg/mL with an increment of 5 μg/mL. The concentration can be increased between the first and second therapy. The maximum concentration is no more than 45 μg/mL. The patient’s treatment outcome and side effects need to be assessed before each increase in concentration to ensure safety.

**Precautions**

The whole process should be carried out under sterile conditions. Every operator should have a set of consumables (blood collector, blood harvesting and infusion tubes, ozone collectors, normal saline). The patient’s condition should be closely observed during the operation process. If there is a problem, then it should be solved immediately. The amount of blood collected, ozone injection and ozone concentration should not be increased without authorization. Blood reinfusion should be slow. It is usually completed within 10-15 min. During the first treatment, it should be slowed down further to prevent complications, especially in elderly patients.

**Side effects of large autologous blood therapy**

There are few side effects of large autologous blood therapy. Patients may have a rash or other allergic reactions, which can be easily resolved. If necessary, symptomatic treatment can be performed. Some patients faint during venous puncture due to emotional stress. Anticoagulant allergy is manifested as a mild numbness of the lip and tip of the tongue, which can be relieved spontaneously. In addition, it can be solved by changing the anticoagulant. Some patients feel nauseous, and flatulence or mouth odor can occur. These symptoms can be relieved spontaneously.

In addition to the general introduction of ozone treatment indications, large autologous blood therapy is widely used in other treatments, including respiratory, digestive, neurological, endocrine and metabolic systemic diseases\[51-54]\.

**EXPERT CONSENSUS STATEMENT**

Ozone is a gaseous molecule with strong oxidation characteristics, which is widely used in the treatment of pain and related diseases.

The effects of ozone include analgesia, anti-inflammation, oxygen supply increase and bidirectional regulation of immunologic function.

Local ozone injection can be used in intradermal sites, skeletal muscle pain points, sites around the joint cavity, nerve roots, etc. The local injection concentration is no greater than 30 μg/mL. The total amount during each treatment is no greater than 30 mL. The course of treatment is determined according to the location.

Ozone injection can be used for the treatment of intervertebral disc diseases. For ozone injection ablation for the treatment of intervertebral disc diseases, 40 μg/mL is considered the commonly used concentration. The capacity of each lumbar spine disc is 4-5 mL, and the capacity of each cervical spine disc is 2-3 mL.

Indications, contraindications and operating norms should be strictly observed in ozone autologous blood therapy. Generally, 100-150 mL of blood is extracted, up to a maximum of 200 mL. The maximum ozone concentration is 45 μg/mL.

Ozone can also be used for adjuvant treatment of pain-related diseases.

Operators should comply with the consensus on ozone application indications, contraindications and use norms to ensure the safe application of ozone treatment technology.
CONCLUSION

The purpose of this consensus is to standardize the rational application of ozone iatrotechnique in pain treatment, to improve its efficacy and safety and to reduce and prevent adverse reactions and complications due to this process.

REFERENCES

1. Bocci V, Borrelli E, Travagli V, Zanardi I. The ozone paradox: ozone is a strong oxidant as well as a medical drug. Med Res Rev 2009; 29: 646-682 [PMID: 19260079 DOI: 10.1002/med.20150]
2. Yu B, Chen HQ, Lu CH, Lin QR, Wang BW, Qin CH. [Effect of intra-articular ozone injection on serum and synovial TNF-α, TNFR I, and TNFR II contents in rats with rheumatoid arthritis]. Nan Fang Yi Ke Da Xue Xue Bao 2011; 31: 1055-1058 [PMID: 21690068]
3. Giunta R, Coppola A, Luongo C, Sammartino A, Guastafierro S, Grassia A, Giunta L, Mascolo L, Tirelli A, Coppola L. Ozonized autohemotransfusion improves hemorheological parameters and oxygen delivery to tissues in patients with peripheral occlusive arterial disease. Ann Hematol 2001; 80: 745-748 [PMID: 11797116 DOI: 10.1007/s002770100377]
4. Bocci V, Zanardi I, Huijberts MS, Travagli V. It is time to integrate conventional therapy by ozone therapy in type-2 diabetes patients. Ann Transl Med 2014; 2: 117 [PMID: 25568870 DOI: 10.3978/j.issn.2305-5839.2014.07.07]
5. León OS, Menéndez S, Merino N, Castillo R, Sam S, Pérez L, Cruz E, Bocci V. Ozone oxidative preconditioning: a protection against cellular damage by free radicals. Mediators Inflamm 1998; 7: 289-294 [PMID: 9792340 DOI: 10.1080/0962935989098983]
6. Re L, Sanchez GM, Mawouf N. Clinical evidence of ozone interaction with pain mediators. Saudi Med J 2010; 31: 1363-1367 [PMID: 21136002]
7. Ozturk O, Ozcan AH, Adali Y, Yildirim CH, Aksoy O, Yagmurdu H, Bilge A. Effect of ozone and methylprednisolone treatment following crush type sciatic nerve injury. Acta Cir Bras 2016; 31: 730-735 [PMID: 27982260 DOI: 10.1590/s0102-85602016010000005]
8. Ma K, Zhuang ZG, Wang L, Liu XG, Lu LJ, Yang XQ, Lu Y, Fu ZJ, Song T, Huang D, Liu H, Huang YQ, Peng BG, Liu YQ. The Chinese Association for the Study of Pain (CASP): Consensus on the Assessment and Management of Chronic Nonspecific Low Back Pain. Pain Res Manag 2019; 2019: 8957847 [PMID: 31511784 DOI: 10.1155/2019/8957847]
9. Takahashi Y, Takahashi S, Yoshimi T, Miura T, Mochitate K, Kobayashi T. Increases in the mRNA levels of gamma-glutamyltransferase and heme oxidease-1 in the rat lung after ozone exposure. Biochem Pharmacol 1997; 53: 1061-1064 [PMID: 9174121 DOI: 10.1016/0006-2952(97)00104-4]
10. Martínez-Sánchez G, Al-Dalain SM, Menéndez S, Re L, Giuliani A, Candelario-Jalil E, Alvarez H, Fernández-Monquein JI, León OS. Therapeutic efficacy of ozone in patients with diabetic foot. Eur J Pharmocol 2005; 523: 151-161 [PMID: 16198334 DOI: 10.1016/j.ejphar.2005.08.020]
11. Sliwa K, Ansari AA. Immunosuppression as therapy for congestive heart failure. Lancet 2008; 371: 184-186 [PMID: 18207004 DOI: 10.1016/S0140-6736(08)60115-4]
12. Fildes JE, Shaw SM, Yonan N, Williams SG. Non-specific immunomodulation in chronic heart failure. Lancet 2008; 361: 2083; author reply 2084 [PMID: 18572075 DOI: 10.1016/S0140-6736(08)60991-3]
13. Pinho-Ribeiro FA, Verri WA Jr, Chiu IM. Nociceptor Sensory Neuron-Immune Interactions in Pain and Inflammation. Trends Immunol 2017; 38: 5-19 [PMID: 27793571 DOI: 10.1016/j.it.2016.10.001]
14. Sagai M, Bocci V. Mechanisms of Action Involved in Ozone Therapy: Is healing induced via a mild oxidative stress? Med Gas Res 2011; 1: 29 [PMID: 22185664 DOI: 10.1186/2045-9912-1-29]
15. Lu L, Pan C, Chen L, Hu L, Wang C, Han Y, Yang Y, Cheng Z, Liu WT. AMPK activation by peri-arterial ozone treatment reduces oxidant stress and necrosis factor (TNF-α) in the sciatic nerve administration of ozone attenuates CCI-induced neuropathic pain in rats. J Mol Cell Biol 2017; 9: 132-143 [PMID: 27744376 DOI: 10.1093/jmcb/mjw043]
16. Bocci V. The clinical application of ozonotherapy. Netherlands, Springer 2010
17. Bocci V, Paulus L. Studies on the biological effects of ozone 1. Induction of interferon gamma on human leucocytes. Haematologica 1990; 75: 510-515 [PMID: 2129118]
18. Paulus L, Luzzi E, Bocci V. Studies on the biological effects of ozone: 2. Induction of tumor necrosis factor (TNF-alpha) on human leucocytes. Lymphokine Cytokine Res 1991; 10: 409-412 [PMID: 1768744]
19. Bocci V, Luzzi E, Corradi E, Paulus L, Rossi R, Cardaoli E, Di Simplicio P. Studies on the biological effects of ozone: 4. Cytokine production and glutathione levels in human erythrocytes. J Biol Regul Homeost Agents 1993; 7: 133-138 [PMID: 8023701]
20. Zamora ZB, Borrego A, López OY, Delgado R, González R, Menéndez S, Hernández F, Schulz S. Effects of ozone oxidative preconditioning on TNF-alpha release and antioxidant-prooxidant intracellular balance in mice during endotoxic shock. Mediators Inflamm 2005; 2005: 16-22 [PMID: 15779062 DOI: 10.1155/MI.2005.16]
21. Bocci V. Does ozone therapy normalize the cellular redox balance? Med Hypotheses 1996; 46: 150-154 [PMID: 8692040 DOI: 10.1016/s0306-9877(96)90016-x]
22. Molinari F, Rimini D, Liboni W, Acharya UR, Franzini M, Pandolfi S, Ricevuti G, Vaiano F,
Valdenassi L, Simontetti V. Cerebrovascular pattern improved by ozone autohemotherapy: an entropy-based study on multiple sclerosis patients. Med Biol Eng Comput 2017; 55: 1163-1175 [PMID: 27734309 DOI: 10.1007/s11517-016-1580-z]

23 Borrelli E, Diadori A, Zalaffi A, Bocci V. Effects of major ozonated autohemotherapy in the treatment of dry age related macular degeneration: a randomized controlled clinical study. Int J Ophthalmol 2012; 5: 708-713 [PMID: 23275905 DOI: 10.3980/j.issn.2222-3959.2012.06.11]

24 Bocci V, Zanardi I, Huijberts MS, Travagli V. Diabetes and chronic oxidative stress. A perspective based on the possible usefulness of ozone therapy. Diabetes Metab Syndr 2011; 5: 45-49 [PMID: 22814842 DOI: 10.1016/j.dsx.2010.05.014]

25 Bocci V, Zanardi I, Huijberts MS, Travagli V. An integrated medical treatment for type-2 diabetes. Diabetes Metab Syndr 2014; 8: 57-61 [PMID: 24661761 DOI: 10.1016/j.dsx.2013.10.004]

26 Biedunkiewicz B, Lizardowski S, Tylicki L, Skiboecka A, Nieweglowski T, Charnienia A, Debeka-Sliuzien A, Rutkowski B. Blood coagulation unaffected by ozonated autohemotherapy in patients on maintenance hemodialysis. Arch Med Res 2006; 37: 1034-1037 [PMID: 17045123 DOI: 10.1016/j.armed.2006.06.006]

27 Coppola L, Lettieri B, Cozolino D, Luongo C, Sammartino A, Guastafierro S, Coppola A, Mastrolorenzo L, Gombos G. Ozonized autohaemotransfusion and fibrinolytic balance in peripheral arterial occlusive disease. Blood Coagul Fibrinolysis 2002; 13: 671-681 [PMID: 12441905 DOI: 10.1097/00001721-200212000-00002]

28 Tylicki L, Niew glowski T, Biedunkiewicz B, Burakowski S, Rutkowski B. Beneficial clinical effects of ozonated autohemotherapy in chronically dialysed patients with atherosclerotic ischemia of the lower limbs—pilot study. Int J Artif Organs 2001; 24: 79-82 [PMID: 11256512]

29 Masiennikov OV, Sharov IG, Potbchina IP, Dushkina NG, Kryzhansovskaia NA, Maslenkova NO, Bolgov VF, Pavlovskaya EE, Zheglova LV, Chalkina SN. Effect of ozone therapy on hemostatic changes in patients with vascular atherosclerosis. Klin Med (Mosk) 1997; 75: 35-37 [PMID: 9490335]

30 Tylicki L, Nieweglowski T, Biedunkiewicz B, Charnienia A, Debeka-Sliuzien A, Aleksandrowicz E, Lysiak-Szydlowska W, Rutkowski B. The influence of ozonated autohemotherapy on oxidative stress in hemodialysed patients with atherosclerotic ischemia of lower limbs. Int J Artif Organs 2003; 26: 297-303 [PMID: 12757028 DOI: 0.1177/039939880326004004]

31 Ohtsuka H, Ogata A, Terasaki N, Koiwa M, Kawanura S. Changes in leukocyte population after ozonated autohemotherapy in cows with inflammatory diseases. J Vet Med Sci 2006; 68: 175-178 [PMID: 16520542 DOI: 10.1292/jvms.68.175]

32 Li LY, Ni JX. Efficacy and safety of ozonated autohemotherapy in patients with hyperuricemia and gout: a Phase I pilot study. Exp Ther Med 2014; 8: 1423-1427 [PMID: 25289033 DOI: 10.3892/etm.2014.1951]

33 Li LY, Ma RL, Du L, Wu AS. Ozonated autohemotherapy modulates the serum levels of inflammatory cytokines in gouty patients. Open Access Rheumatol 2017; 9: 159-165 [PMID: 28860878 DOI: 10.2147/OARRR.S117949]

34 Shah P, Shyam AK, Shah S. Adjuvant combined ozone therapy for extensive wound over tibia. Indian J Orthop 2011; 45: 376-379 [PMID: 21772635 DOI: 10.4103/0019-5413.80332]

35 Degli Agosti I, Ginelli E, Mazzacane B, Peroni G, Bianco S, Guerriero F, Ricevuti G, Perna S, Rondanelli M. Effectiveness of a Short-term Treatment of Oxygen-Ozone Therapy into Healing in a Posttraumatic Wound. Case Rep Med 2016; 2016: 9528572 [DOI: 10.1155/2016/9528572]

36 Clavo B, Pérez JL, López L, Suárez G, Lloret V, Macías D, Santana M, Hernández MA, Martin-Óliva R, Robaina F. Ozone Therapy for Tumor Oxygenation: a Pilot Study. Evid Based Complement Alternat Med 2004; 1: 93-98 [PMID: 15257330 DOI: 10.1093/ebcm/neh009]

37 Çarlı AB, İncedaiy M. Oxygen-ozone autohemotherapy in sacroilitis. Acta Reumatol Port 2017; 42: 334-335 [PMID: 29017176]

38 Tollefsen J. First sun-dimming experiment will test a way to cool Earth. Nature 2018; 563: 613-615 [PMID: 30479388 DOI: 10.1038/s41586-018-07533-4]

39 Alexandre A, Borrelli E, Alexandre A, Liliakis E. Disc Herniation and Knee Arthritis as Chronic Oxidative Stress Diseases: The Therapeutic Role of Oxygen Ozone Therapy: J Arthritis 2015; 4: 161 [DOI: 10.4172/2167-7921.1000161]

40 Manoto SL, Meca MJ, Motaung SK. Medical ozone therapy as a potential treatment modality for regeneration of damaged articular cartilage in osteoarthritis. Saudi J Biol Sci 2018; 25: 672-679 [PMID: 29736142 DOI: 10.1016/j.sjbs.2016.02.002]

41 Bonetti M, Fontana A, Cotticelli B, Volta GD, Guindani M, Leonardi M. Intraforaminal O2-(O3) vs periradicular steroidal infiltrations in lower back pain: randomized controlled study. AJNR Am J Neuroradiol 2005; 26: 996-1000 [PMID: 15891150]

42 Ezeldin M, Leonardi M, Princootta C, Dall’olio M, Tharwat M, Zaki M, Abdel-Wanis ME, Cirillo L. Percutaneous ozone nucleolysis for lumbar disc herniation. Neuroendocrinology 2018; 60: 1231-1241 [PMID: 30206674 DOI: 10.1007/s00334-018-2083-4]

43 Liliakis E, Valadakis V, Vynios DH, Tsiganos CP, Agapitos E. Rationalization of the activity of medical ozone on intervertebral disc-A histological and biochemical study. Rivista Di Neuroradiologia 2001; 14: 23-30 [DOI: 10.1177/19714009010140S105]

44 Dall’Olio M, Princootta C, Cirillo L, Budai C, de Santos F, Bartolini S, Serchi E, Leonardi M. Oxygen-ozone therapy for herniated lumbar disc in patients with subacute partial motor weakness due
to nerve root compression. *Interv Neuroradiol* 2014; **20**: 547-554 [PMID: 25363257 DOI: 10.15274/INR-2014-10078]

45 Bocci V. Ozone: A New Medical Drug. Springer Netherlands 2011 [DOI: 10.1007/1-4020-3140-8]

46 Niu T, Lv C, Yi G, Tang H, Gong C, Niu S. Therapeutic Effect of Medical Ozone on Lumbar Disc Herniation. *Med Sci Monit* 2018; **24**: 1962-1969 [PMID: 29611536 DOI: 10.12659/msm.903243]

47 Beyaz SG, Sayhan H. Six-Month Results of Cervical Intradiscal Oxygen-Ozone Mixture Therapy on Patients with Neck Pain: Preliminary Findings. *Pain Physician* 2018; **21**: E449-E456 [PMID: 30045611]

48 Bocci V. Biological and clinical effects of ozone. Has ozone therapy a future in medicine? *Br J Biomed Sci* 1999; **56**: 270-279 [PMID: 10795372]

49 Bocci V. The case for oxygen-ozonetherapy. *Br J Biomed Sci* 2007; **64**: 44-49 [PMID: 17444419 DOI: 10.1080/09674845.2007.11732755]

50 Tsuzuki N, Endo Y, Kikkawa K, Korosue K, Kaneko Y, Kitauchi A, Katamoto H, Hidaka Y, Hagi M, Torisu S. Effects of ozonated autohemotherapy on the antioxidant capacity of Thoroughbred horses. *J Vet Med Sci* 2016; **77**: 1647-1650 [PMID: 26166812 DOI: 10.1292/jvms.15-0225]

51 Biedunkiewicz B, Tylicki L, Nieweglowski T, Burakowski S, Rutkowski B. Clinical efficacy of ozonated autohemotherapy in hemodialyzed patients with intermittent claudication: an oxygen-controlled study. *Int J Artif Organs* 2004; **27**: 29-34 [PMID: 14984181 DOI: 10.1177/039139880402700107]

52 Tylicki L, Lizakowski S, Biedunkiewicz B, Skibowska A, Nieweglowski T, Chamiensia A, Debska-Slisien A, Rutkowski B. Platelet function unaffected by ozonated autohaemotherapy in chronically haemodialysed patients. *Blood Coagul Fibrinolysis* 2004; **15**: 619-622 [PMID: 15389131 DOI: 10.1097/00001721-200410000-00014]

53 Lintas G, Molinari F, Simonetti V, Franzini M, Liboni W. Time and time-frequency analysis of near-infrared signals for the assessment of ozone autohaemotherapy long-term effects in multiple sclerosis. *Annu Int Conf IEEE Eng Med Biol Soc* 2013; **2013**: 6171-6174 [PMID: 24111149 DOI: 10.1109/EMBC.2013.6610962]

54 Ciborowski M, Lipska A, Godzien J, Ferrarini A, Korsak J, Radziwon P, Tomasiak M, Barbas C. Combination of LC-MS- and GC-MS-based metabolomics to study the effect of ozonated autohemotherapy on human blood. *J Proteome Res* 2012; **11**: 6231-6241 [PMID: 23148940 DOI: 10.1021/pr3008946]
