



Original Contribution

THE EFFECT OF TOPICAL APPLICATION WITH OZOILE® AS A NOVEL METHOD IN THE COMPLEX TREATMENT OF SOFT TISSUE INFECTIONS AND CHRONIC WOUNDS

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ABSTRACT

The treatment of severe and deep soft tissue infections, especially those combined with fascial necrosis is complex and involves a multidisciplinary team. The aim is to research the effect of topical application of Ozoile®, stable ozonides from organic extra-virgin olive oil +OIL in the complex treatment of soft tissue infections and chronic wounds and to optimize the algorithm of these applications. Results: We applied LOT to a total of 84 patients (22.1%) of all 380 patients with soft tissue infections treated for the period March 2022-March 2023 at the First surgical clinic of Medical University – Pleven, Bulgaria. At Department “Surgical diseases” there were 49 (58,3%) patients with the superficial inflammatory process, 6 (7,1%) with chronic wounds, and 27 (34,6%) with deep soft tissue infection and necrotizing fasciitis who received topical applications of Ozoile®, stable ozonides from organic extra-virgin olive oil +OIL®OT. We compared the results with a group of 296 patients treated with conventional methods and routinely used antiseptics – hydrogen peroxide, povidone-iodine, and chlorhexidine gluconate. Statistically significant results in the Ozoile® group were: wound size reduction by 25% on the 5th day of treatment ($p < 0.04$), reduction by 50% of baseline C-reactive protein levels on day 5th of treatment ($p < 0.05$), the local status of the wound on the 30th day after discharge - in the Ozoile® group we did not observe any complaints of redness, itching or other local discomforts ($p < 0.05$). We optimized an algorithm for the application of ozone therapy with Ozoile®

Key words: soft tissue infections, Ozoile®, topical ozone therapy, algorithm

INTRODUCTION

Soft tissue infections are serious surgical diseases that accompanied human life since age. In Ancient Greece, Hippocrates shared in his encyclopedic book “Materia Medica” different recipes for wound treatment. In his articles on wound care, the wounds were termed “Ulcers”. (1) Hippocrates applied different methods to prepare a variety of herbal extracts and dressings that can be used to treat “ulcers”. The treatment options depended on the degree of wound curative stage and the presence of purulent exudate. To clean them and keep them moist, he

recommended using fig leaves, olive oil, and different herbal extracts with a local antiseptic effect. Today, the modern manual for the diagnosis and treatment of superficial and deep soft tissue infections was designed after an expert meeting conference, held on 28th June 2018 in Bertinoro, Italy. The expert board created a consensus, the content of which is accepted and improved by the World Society of Emergency Surgery (WSES) and the Society of Surgical Infections- Europe (SIS-E). (2) The social impact of soft tissue infections is high. They can lead to a long period of temporary invalidation, patient disability, and long periods with reduced quality of life. Treatment of severe and deep soft tissue infections, mostly those combined with fascial necrosis, is complex and requires multidisciplinary teamwork. The consensus suggests appropriate the application of

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hyperbaric oxygenation in cases with anaerobic flora that caused an infection. (3) The effect of oxygen in the treatment of necrotizing soft tissue infection is clearly beneficial. The biological effect on the tissues is a modulator of tissue regeneration processes. It stimulates the deposition of collagen in the wound and favors the process of epithelization and the formation of new blood vessels (4). The application of new methods of wound treatment was researched with the aim to ensure a greater amount of oxygen in soft tissues in the condition of an active inflammatory process. The search for new formulas for the treatment of wounds, with a positive effect on their healing and supporting their regeneration, has led to the application of new methods to ensure a greater amount of oxygen in soft tissues in the condition of an active inflammatory process. This is achieved by applying stable ozonides, ozone derivatives topically to the wound. Ozone is a molecule made up of three oxygen atoms, which very quickly decay into an oxygen molecule and a single oxygen atom. The action of the latter is like a strong oxidizer on the bacterial cell and leads to its destruction. Liyao Liu in 2022 presented a review article on the current possibilities and effects of applying local ozone therapy (LOT) in soft tissue lesions (5). There are different medicinal forms that can be administered both systemically (ozone autohemotherapy) and locally through ozonated water, ozonated oils and emulsions. New variants includes ozonated

microemulsions and nanoemulsions, ozonated oil microcapsules that help to separate these microcapsules only at the site of application. The challenge in working with ozone comes from its physical properties. Its structure is unstable and rapidly breaks down into molecular oxygen and atomic oxygen (6). In order to ensure the long-term stability of the molecule, methods have been developed for the ozonation of natural oils – olive oil, sunflower oil, such as at a special temperature and pressure. Even Hippocrates noticed the beneficial effect of olive oil on wounds. Olive oil exhibits antibacterial properties against Gram-positive bacteria, including against *Staphylococcus aureus*, Gram-negative bacteria and *Candida* species (7). Due to these properties, olive oil has been used as a vehicle for medicinal substances used to treat inflammatory skin diseases of bacterial and fungal origin. Improvement of the antibacterial characteristics of olive oil is obtained through the possibility that the double bonds of fatty acids included in its composition can react with ozone molecules forming - stable ozonides that can be used in the complex treatment of soft tissue infections. (8) The treatment of complicated soft tissue infections with innovative dressings based on Ozoile®, stable ozonides from organic olive oil +OIL® is an innovative method for wound sterilization and healing. The molecule mechanisms of reaching the unique structure of stable ozonides are shown on **Figure 1** (9).

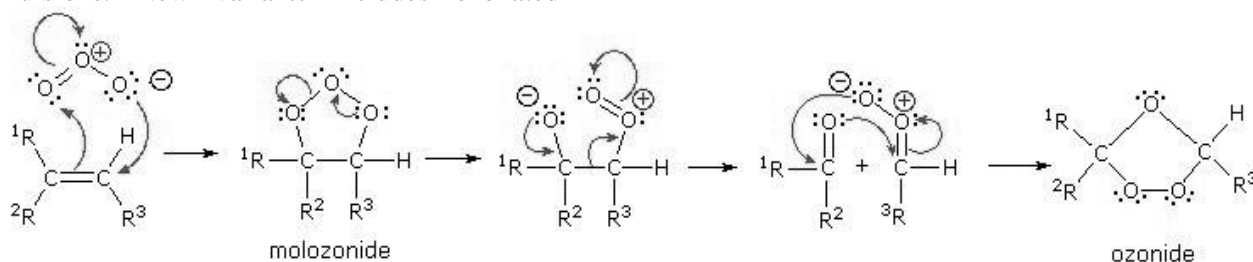


Figure 1. Molecular structure of ozonides (9)

AIM

The purpose of the present study is to perform a retrospective analysis of the effect of topical applications of Ozoile®, stable ozonides from organic olive oil +OIL® to patients with soft tissue infections and chronic wounds. To clarify the algorithm and principles of application of local ozone therapy with Ozoile®, stable ozonides from organic olive oil +OIL® as an

element of the complex treatment of surgical wounds in the postoperative period.

MATERIALS AND METHODS

We performed a retrospective analysis of all patients treated for soft tissue infections and chronic wounds for the period March 2022-March 2023 in the First Surgical Clinic of UMHAT "Dr G. Stranski" EAD - Department "Surgical Diseases" at Medical University -

Pleven. We analyzed patient demographics. We evaluated the topography and location of the infections, the severity of the inflammatory process, the laboratory markers of inflammation, and the microbiological results at the beginning and at the end of the topical treatment with stable ozonides. We compared the results with conventional methods of treating soft tissue infections in terms of rates of infection resolution and progression of the regenerative phase. We performed laboratory monitoring of serum markers of inflammation - leukocytes, granulocytes, and C-reactive protein levels. We followed the duration in days of antibiotic treatment, length of hospital stay, and quality of life after the first month of treatment in patients treated with Ozoile®, stable ozonides from organic olive oil +OIL® and in those treated with the conventional method. We evaluated the comorbidity profile of the patients regarding the manifestation of diabetes mellitus, peripheral vascular diseases based on systemic atherosclerotic process, arterial hypertension, hyperuricemia, and gout. Based on the obtained results, we optimized an algorithm for the treatment of infected wounds with Ozoile®, stable ozonides from organic olive oil +OIL® as part of the complex treatment of infections of the soft tissues and chronic wounds. All ethical requirements were improved for the application of with Ozoile®, stable ozonides from organic olive oil +OIL®, as each patient to whom it was applied signed an informed consent for this treatment before the start of therapy, and was informed about the indications, the steps of applying ozonides in the treatment of soft tissue infections and chronic wounds. The research was improved by the University Hospital Ethical committee. Patients with soft tissue defects based on thermal trauma, complicated dermatological diseases, or allergic conditions with skin rashes were excluded from the study. All patients with soft-tissue infections and chronic wounds underwent the standard operative techniques of incision, debridement, and lavage, and for deep infections involving the fascia, we performed fasciotomy. The systemic therapy was the same for both groups of patients - administration of broad-spectrum antibiotics from the group of 3rd generation cephalosporins, lincosamides, carbapenems, and metronidazole with activity against anaerobic flora. In patients who received topical applications of Ozoile®, stable ozonides

from organic olive oil +OIL®, standard iodine-containing antiseptics, or those containing chlorhexidine gluconate (Hibitane®) for lavage were not applied to the wound. We have used medical devices available in the pharmacy network for the topical treatment, containing as exclusive component Ozoile®, stable ozonides from organic extra-virgin olive oil +OIL®, obtained through a patented process of the Italian company Erbagil (patent number 20202000001738) with an official distributor in Bulgaria - Salvis Pharma Ltd. The products are approved for use in Bulgarian medical practice. Ozoile® is the result of the reaction of a defined oxygen-ozone mixture with the olefinic bonds of the fatty acids of the +OIL® organic olive oil produced by Erbagil Tenuta. The patented device for making Ozoile® consists of a generator that converts, through a powerful electric discharge, a high percentage of pure medical oxygen into ozone. The oxygen/ozone mixture is then bubbled in a reactor containing organic extra virgin olive oil leading to the formation of stable ozonides. The process is monitored in the temperature and pressure parameters to ensure the stability of the molecules involved in the reaction. We used – delicate liquid detergent (Idrosoil®), non-gas oily spray (Rigenoma®) - containing Ozoile® and natural substances rich in alpha lipoic acid with an antioxidant effect; cream-emulsion (Rigenoma creme®), stimulating the regeneration of the epidermis and the epithelization of skin defects. Idrosoil liquid detergent® contains decyl glucoside. It is a non-irritating, delicate and tissue-friendly surfactant. It has a cleansing action that promotes the opening of the biofilm meshes and facilitates the action of Ozoile® in normalizing secretions and local microflora. We used it concentrated in the first wound debridement and in solution with saline 0,9% sodium chloride in proportion 1:1 in the next surgical debridement procedures. The second step was an application of Rigenoma spray and on the periphery of the wound-application or Rigenoma crème. The surrounding skin was cleaned with povidone -iodine and it was not administered over the wound surface, where stable ozonides act, because of the possibility of inactivity. The final step was covering the wound surface with a sterile gauze dressing.

Patients treated with Ozoile® were randomly selected, and the treatment was applied to patients

with both superficial soft tissue infections and severe necrotizing fasciitis and chronic wounds. The anatomical location of the infections treated with topical applications of stable ozonides was also randomized to reduce the likelihood of bias and to minimize the effect of possible confounding factors in the interpretation of the final results. We have used a descriptive method and statistical methods - correlation, mean values, regression analysis for data processing, through the program IBM -SPSS 21 for Windows. We accepted $p < 0.05$ as a statistically significant value.

RESULTS

The first application of topical applications with Ozoile®, stable ozonides from organic olive oil +OIL®, in the First Surgical Clinic of UMHAT "Dr. G. Stranski" EAD - the city of Pleven dates back to the beginning of March 2022. From this period until the beginning of March 2023 in the First Surgical Clinic, a total of 380 patients were treated for soft tissue infections of the body, of which 148 (30.8%) had inflammation of the superficial soft tissues covering the skin and subcutaneous tissue, and the remaining 232 (69.2%) with deep and severe infections affecting the underlying fascia and muscle groups. We have presented the localization of infections by anatomical areas in **Figure 2**.

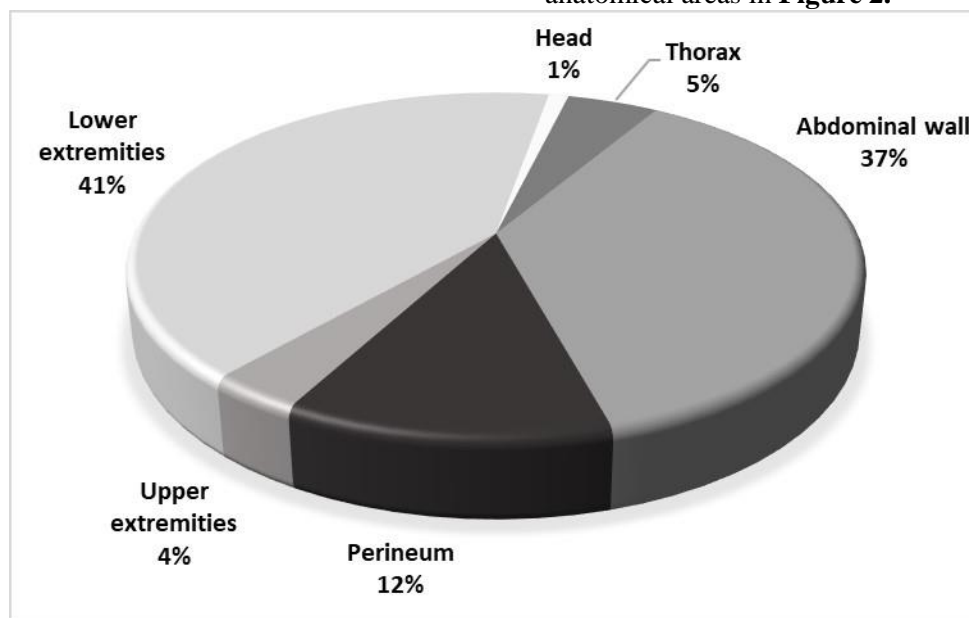


Figure 2. Localization of soft tissue infections by anatomical areas

The demographic profile is as follows: men 245 (64.5%), women 135 (35.5%), age from 18 to 89 years, average age 56.05 years \pm 13.5 years. We applied Ozoile® to a total of 84 patients (22.1%) of all patients with soft tissue infections. Patients with superficial inflammatory processes who have been treated with topical applications of Ozoile®, stable ozonides from organic extra-virgin olive oil +OIL® were 49, which is 58,3% of all who received this therapy, 6 (7,1%) with chronic wounds, and 27 (34,6%) patients in the ozone therapy group had deep tissue infections accompanied by necrosis and fasciitis. The comorbid profile in the studied group of patients includes arterial hypertension - 49 (58.3%), diabetes mellitus - 28 (33.3%), chronic arterial

insufficiency of the extremities 17 (20.2%), overweight 26 (30.1 %), dyslipidemia - 12 (14.3%), gout - 4 (4.7%), systemic treatment with corticosteroids - 7 (8.3%). Percentages are greater than 100 because some of the patients had more than one of the comorbidity at the same time. Regarding the time required for complete healing of the wound surface in the conventionally applied methods of treatment of soft tissue infections (lavages with hydroperoxide H₂O₂, povidone-iodine, chlorhexidine-gluconate, Hibitane®) and in the Ozoile® group, we did not find a statistically significant difference - an average of 5.3 days for the first group and 5.1 days for the second group ($p = 0.003$). We observed the reduction of the size of the wound

by 25% of its initial size in the group of conventionally treated patients on day 6-7 of treatment, while in the LOT group, this effect was achieved on day 3-4, which we note as a statistically significant result. ($p < 0.04$). Regarding the laboratory monitoring of inflammation, we reported a 50% reduction in baseline levels of C-reactive protein in the first group on day 8-9th of treatment, and in the Ozoile® group on day 4-5th of treatment, which was statistically significant in our study, ($p < 0.05$). Regarding the days of hospital stay for the first and second groups of patients, we did not find a statistically significant difference, the average hospital stay for the first group was 6 ± 1.3 days, and for the Ozoile® group 5 ± 1.5 days. We found a statistically significant difference

regarding the indicator of the local status of the wound in the 30-day period after discharge, as in the first group patients had persistence of serous secretion, presence of itching and redness of the wound edges, slow epithelization, while in the group treated with Ozoile®, stable ozonides from organic olive oil +OIL®, we did not observe similar complaints, $p < 0.05$. Stages of treatment with Ozoile® are shown in **Figures 3a, 3b, 3c; 4a, b, c; 5 a, b**. Based on the results obtained and the visible effect of the treatment, we optimized an algorithm for the topical application of Ozoile® in patients with soft tissue infections, training the dressing nurses in the clinic on the correct application of the methodology in the following sequence of steps, but always as part of the complex treatment, and not as monotherapy!



a **Figure 3a.** Stages of the treatment of soft tissue infection of an upper extremity with Ozoile® - on the 8th day of treatment.
b **Figure 3b.** Stages of the treatment of soft tissue infection of an upper extremity with Ozoile® - on - on 15th day of treatment.
c **Figure 3c.** Stages of the treatment of soft tissue infection of an upper extremity with Ozoile® - on 35th day of treatment.



a **Figure 4a.** Stages of the treatment of a soft tissue infection of a lower limb with Ozoile® - on the 8th day of treatment.

b **Figure 4b.** Stages of the treatment of a soft tissue infection of a lower limb with Ozoile® -15th day of treatment.

c **Figure 4c.** Stages of the treatment of a soft tissue infection of a lower limb with Ozoile® -35th day of treatment.



Figure 5a. Wound on the back- 8-th day of treatment with Ozoile®



Figure 5b. Wound on the back- 35-th day of treatment with Ozoile®

Initial and essential steps: Depending on the severity of the soft tissue infection, primary surgical treatment is performed in an operating room by surgeons. Incisions, contra-incisions are performed, followed by surgical wound

debridement; excisional cleaning of the wound, if indicated, necrosectomy and fasciotomy of a relieving nature are performed. The surgical procedures aim to clean, debride and mechanically to reduce the severe inflamed and

non-vital tissues. After these initial steps, the wound surface is ready to welcome the local ozone therapy to start acting!

First step –Washing. During the lavage of the soft tissues, the detergent Idrozoil® Erbagil is applied, and during the first surgical treatment, we applied it concentrated. For the subsequent wound cleansing procedures it is applied and dissolved in a physiological solution in a ratio of 1:1. When mixed with hydrogen peroxide, rich foam is produced, which absorbs and evacuates all debris from the wound surface. We have never mixed Idrozoil with povidone iodine or chlorhexidine gluconate, due to the risk of inactivation of ozone molecules in contact with iodine or chlorine-containing compounds. Idrozoil can be washed out with saline or sterile water for injections. This step may be repeated two or three times per dressing, depending on the degree of contamination of the wound. The wound surface is then dried with sterile gauze.

Second step – “Shock therapy” with the application of Rigenoma® spray, we spray the wound surface abundantly so that the fatty components of its composition cover the wound evenly. The application should be from a distance of at least 5 cm above the wound. The spray

reduces exudation from the wound and stimulates autolytic debridement. It reduces microbial contamination and inhibits inflammatory progression.

Third step - application of Rigenoma® cream on the edges of the wound, in order to stimulate epithelization, improve skin rehydration and prevent the formation of rough keloids and cicatrices.

Fourth step - covering the wound with gauze smeared with Rigenoma® cream.

Dressings can be done daily or at intervals of 48 hours, depending on the degree of contamination of the wound and the depth of the infection.

The soft tissue sanitation and regeneration were histologically verified during the healing process. In the initial, acute stages, it shows infiltration of neutrophils and huge areas of cellular detritus. **Figure 6.** On the 12-th day of treatment, in the proliferative stage, activated by Ozoile®, there are increasing of fibroblasts and new capillaries formation. Early fibrosis with hypercellular areas of fibroblastic proliferation, some inflammatory cells and accumulation of collagen bundles are typical local proliferative effects, triggered by Ozoile®. **Figures 7a, 7b**

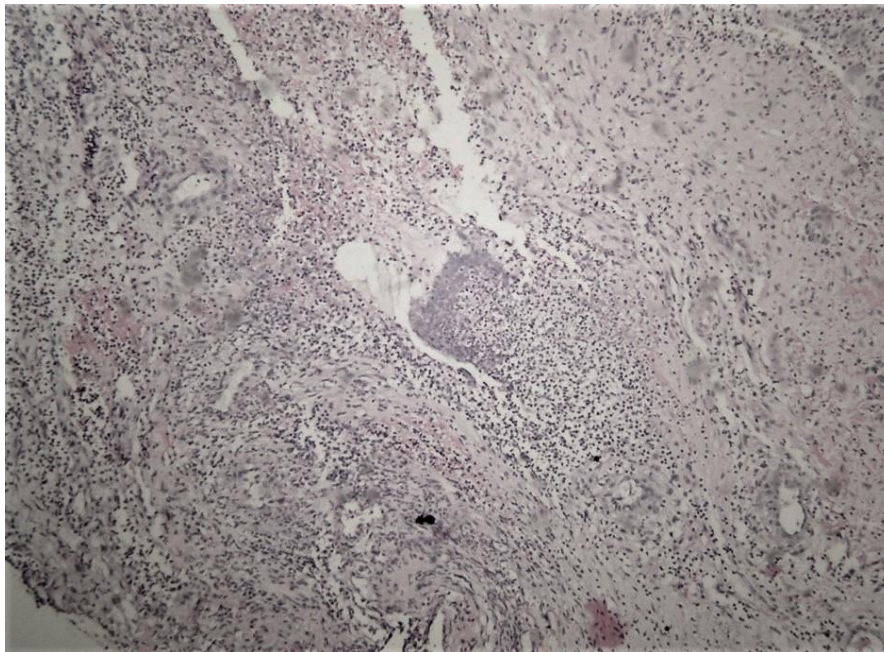


Figure 6. Areas with necrosis - acute inflammatory infiltrate and cellular detritus, hematoxylin and eosin staining [H& E], magnification x 200, 1-st day of treatment with Ozoile®

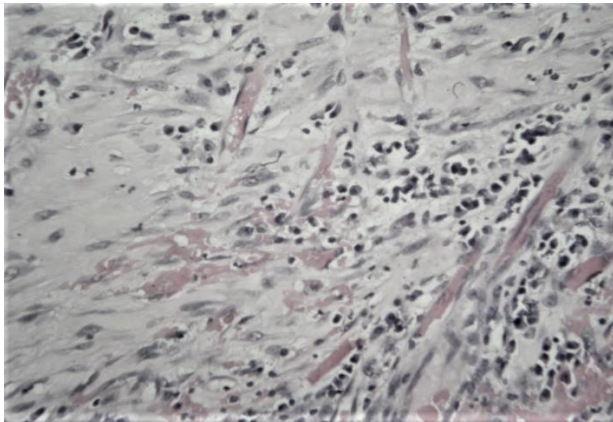


Figure 7a. Area with granulation tissue with focal haemorrhages and mixed inflammatory cell infiltrate of histiocytes, lymphocytes, plasma cells, hematoxylin and eosin staining [H & E], magnification x 400, on 35-th day of treatment with Ozoile®

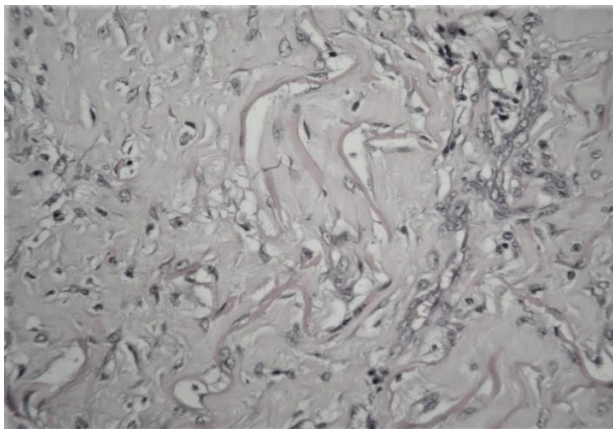


Figure 7b. Early fibrosis with hypercellular areas of fibroblastic proliferation, some inflammatory cells and accumulation of collagen bundles, hematoxylin and eosin staining [H & E], magnification x 400, on 35-th day of treatment with Ozoile®

DISCUSSION

The treatment of soft tissue infections is a challenge, even in the conditions of modern achievements of science and the pharmaceutical industry. A major problem is the ever-increasing resistance of microorganisms to the standard antibacterial preparations used in practice. The powerful antibacterial effect of ozonized oils is due to the oxidation of the cell wall of microorganisms and the release of bioperoxides involved in radical and oxidative processes. [9]. The topical application of stable ozonides is a way to achieve an antiseptic effect in the wound and its sanitation with comparatively shorter antibiotic courses of treatment. When comparing the antiseptic effect of ozonated oils with a

peroxide charge of 560/590 mmol- equiv/kg, compared to that of povidone-iodine 10% and chlorhexidine-gluconate 0.2%, by disc diffusion test, it was found that ozonated oils have more - large diameter of a zone in which the growth of colonies of the tested strains of *Staphylococcus aureus* and *Porphyromonas gingivalis* is not detected (10). The antibacterial effect of ozonides and Ozoile®, without any tissue toxicity or evidence of allergy reactions, makes them preferred for local advanced and enhanced treatment strategy with the possibility of shortening the antibiotic course duration. In the literature, however, there are several works on ozonated vegetable oils, their properties and topical applications and in particular on the use of Ozoile® in the topical treatment of inflammatory states of various tissues with activation of regeneration processes (11-13) and in the treatment of acute and chronic skin lesions of various etiologies. (14, 15) This unique structure determines the antibacterial and antifungal properties of Ozoile®, with a sparing and soothing effect on infected tissues (16). In addition to the antibacterial effects of Ozoile® has a beneficial effect on inflamed tissues by several mechanisms - they exert an immunomodulatory and anti-inflammatory effect, stimulate the proliferation of new capillaries and collagen synthesis, stimulate epidermal growth and the process of epithelization (17). The mechanism of action of ozone and its derivatives on tissues is not limited only to its antibacterial effects. Ozoile® promotes the gene expression and synthesis of several biologically active growth factors such as hypoxia-inducible factor (HIF), epidermal growth factor, and endothelial growth factor. (15) All these growth factors stimulate the proliferative stage of tissue regeneration with collagen fibers synthesis, neoangiogenesis, and epithelization. By this mechanism, it improves the trophic and regeneration of hypoxic tissues, which, against the background of severe inflammation and compromised capillaries, are in tissue hypoxia and local acidosis. Antibacterial and antifungal effects of ozonides are realized by hydroperoxides- active oxygen radicals that destruct the microbial wall due to oxidative stress. Our results support the conclusions of research on the effect of Ozoile in soft tissue infections and chronic wounds. On the initial stage of tissue alteration, we observe in

histological specimens a diffuse infiltration of polymorphonuclear cells and cells detritus. At the beginning of treatment with Idrosoil and Rigenoma Spray, the main idea is cleaning of the cell detrit materials and decreasing of the microbial cells in wound surface. With next wound washing procedures, the cell population changes to fibroblasts and proliferative stage just begin. Neoangiogenesis is running in progress and give possibility for growing of young granulating tissue. We have not observed side effects from the application of Ozoile®, and we have not encountered similar articles in the available Bulgarian literature with such a novel method of wound treatment till that moment. Even the opposite - more and more clinical studies abroad report the positive effect of ozone and ozonated oils in the treatment of difficult-to-heal wounds and chronic ulcers in the diabetic foot, peripheral vascular insufficiency and in various superficial dermatopathies.(18,19) The successful results are also due to properly technically performed dressings of the patients in the postoperative period, where the nurses in the surgical and intensive care units play a major role.

CONCLUSION

The proposed algorithm for the local application of Ozoile® in patients with soft tissue infections is innovative and presents a new look at their treatment. Ozoile® is gentle on tissues and supports their regeneration. Such treatment with a prepared algorithm has not been shared in scientific journals in Bulgaria till now; therefore we believe that our experience will be useful for both surgeons and nurses who take care of wounds in surgical and intensive care departments. Adherence to the indicated steps of the algorithm will support the complex treatment of patients with infected wounds to achieve a good end result and improved quality of life.

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