



Original Contribution

TREATMENT OF COMPLICATED DEEP SOFT TISSUE INFECTIONS BY A COMBINATION OF NEGATIVE PRESSURE WOUND THERAPY AND INTRALESIONAL TOPICAL APPLICATION WITH STABLE OZONIDES OZOILE® FIRST CASES IN BULGARIA

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ABSTRACT

Introduction. Severe necrotizing soft tissue infections (SNSTI) are life-threatening conditions that require timely diagnosis and immediate surgical treatment. As part of the comprehensive treatment of severe fasciitis, the application of a new method of treatment, combined permanent negative pressure wound therapy (NPWT) and Ozoile®, stable ozonides from organic extra- virgin olive oil +OIL®, may be considered.

The **aim** is a retrospective analysis of patients with severe soft tissue infections treated at the First Surgical Clinic of UMHAT "Dr. G. Stranski" Ltd - Pleven with NPWT negative pressure and topical applications of Ozoile®, stable ozonides from organic extra- virgin olive oil +OIL® and to compare the final results and outcome with group patients treated by conventional methods and NPWT alone.

Materials and methods. A retrospective, randomized study over 1 year (March 2022- March 2023), including 84 patients with SNSTI treated surgically with NPWT and local ozone therapy; 35 with NPWT and 170 by fasciotomy, wound debridement, and wound lavages.

Results. A total of 232 patients with SNSTI were divided into three subgroups according to treatment strategy. NPWT with local ozone therapy has a significantly shorter hospital stay ($p < 0,05$), time to sterilization ($p < 0,05$), and faster epithelization rate ($p < 0,05$), compared with the conventional treatment strategy. **Conclusion** The NPWT combines with topica application of Ozoile® and stable ozonides is a novel and effective method for the treatment of SNSTI.

Key words: fasciitis, negative pressure wound therapy, local ozone therapy, stable ozonides, Ozoile

INTRODUCTION

Soft tissue infections (STI) can occur as an inflammatory process that progresses from the superficial to the deep layers of the soft tissues or develop based on a hospital-acquired infection in the area of the surgical site. The complicated forms of STI may be classified into two main groups- without necrosis of adjacent fascia and with necrosis. Delay in recognition and surgical treatment of necrotizing cellulitis, fasciitis, and myositis leads to a high mortality rate because of

multiorgan failure due to sepsis. (1) The treatment of severe soft tissue infections remains a challenge because of the increasing antibiotic multi-resistance of the leading bacterial agents of surgical site infections, leading to increased treatment costs and prolonged hospital stays. (2) The first step of treatment of necrotizing deep STI is radical surgical debridement with necrosectomy and decompressive fasciotomy, followed by wound lavage with antiseptics. One of the contemporary options for improvement of the wound healing process, after aggressive surgical debridement, is negative pressure wound therapy (NPWT). This method was implemented in practice in 1995 by Dr Louis Argenta and bioengineer Michael Morykwas, through their Vacuum-Assisted Closure (V.A.C.) device. (3)

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Its action combines physical and physicochemical processes that affect tissue changes in the inflammatory stages of alteration, exudation, and tissue complex pathway proliferation. (4) Classical design of NPWT is changed with the possibility for the instillation of topical antiseptic solution in the system during sub-atmospheric pressure was sustained. The result of that modification of the NPWT method was better than the final effect of the original method- without solution instillation. The solution that was used was Prontosan® (Product of B Broun) in two different time regimens of instillation- 6 and 20 minutes dwell time (the period of absence of negative pressure and the solution contained in the foam absorber.(5) Another variant of a combination of NPWT is with the topical, intralesional application of epidermal growth factor (EGF) (Haberprot-P, Has Biotec) for stimulation of fibroblast proliferation, migration of epithelial cells into the wound surface and its edges retraction. The EGF was administered three times per week after diluting with 5 ml saline solution, on the base and edges of the wound, followed by packing the NPWT system The preferred negative pressure is 100- 125 mmHg in the continuous application. In intervals of 48- 72 hours, the device was de-packed and wound lavage was performed with EGF. (6) The contemporary variant of the instillation of drugs, that have both antiseptic and anti-inflammatory effects, is the application of ozonated water with a concentration of ozone of 10 mg/l. The ozonated solution was performed ex- tempore by an ozone generator and double distilled water, at room temperature, and administered in the wound immediately. After irrigation of ozonated water, the vacuum aspiration was performed by a portable vacuum device and the final clinical effect was good and that method was recommended for the treatment of chronic osteomyelitis. (7) The combination of the classical method of NPWT with topical application of Ozoile® stable ozonides from organic extra virgin olive oil +OIL®_has not yet been described in the literature.

AIM

This study aims to make a retrospective analysis of the patients with severe necrotizing deep soft tissue infections treated at First Surgical Clinic of UMHAT "Dr. Georgi Stranski" Ltd –Pleven, Bulgaria with standard methods and –_topical

application of Ozoile® year period and to compare the results with the effect of conventional methods of surgical treatment of necrotizing STI.

MATERIALS AND METHODS

Study design: a retrospective, randomized study over 1-year period (March 2022-March 2023) at the First Surgical Clinic, including patients with severe necrotizing STI treated surgically with performed initial surgical source infection control by emergency fasciotomy and wound debridement. The wound lavage was performed and topical application of Ozoile® was applied. Negative pressure wound therapy (NPWT) was used, combined with the instillation of Ozoile®, stable ozonides in the wound. A study was carried out, and the patient's data were extracted by Gamma code master hospital database according to the ICD-10 code system, related to the treatment of moderate and severe soft tissue infections. We reviewed all surgical protocols and microbiological and histological data were collected. The research was improved by Medical University Hospital - Pleven Ethical Commission and every patient has written informed consent. Postoperatively, a vacuum system was used for treatment with a permanent negative pressure of -100 to 125 mmHg, performing continuous aspiration of the wound exudate. We used a system consisting of a microporous sponge, an adhesive non-absorbable folio, and a system for permanent aspiration whit a manometer for negative pressure control (A NPWT set of Smith and Nephiew®). The replacement of the microporous absorber happened in scheduled intervals of 48- 72 hours. At the time of the replacement of the absorber, we performed a wound lavage with detergent, containing Ozoile® - Idrozoil. The local treatment was carried out with applications of medical devices for topical use based on Ozoile® in the form of rinse cleanser, oily spray, and cream. The trade name of these products is Idrozoil® Detergent, Rigenoma cream®, and Rigenoma spray® - for shock therapy of severely contaminated wounds. Both products are trademarked by Erbagil, holder of the Italian patent 202020000001738, perfected by the Ministry of economic development - Italian Patent and Trademark Office. Ozoile® is produced, through a patented process, by 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 +OIL® organic extra virgin olive oil forming stable ozonides. The process is monitored in the temperature and pressure parameters to ensure the stability of the molecules involved in the reaction. The products are distributed in Bulgaria by Salvis Pharma Ltd. We used the following algorithm for local administration of Ozoile®, stable ozonides from organic olive oil +OIL®: after surgical necrosectomy and wound debridement, we performed wound washing with Idrozoil liquid detergent®, containing decyl glucoside, a non-irritating, delicate and tissue-friendly surfactant with 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 solution with saline 0,9% sodium chloride in proportion 1:1 in the next washing procedures. The washing out of detergent was with hydro peroxide H₂O₂, that combination lead to foam formation and evacuation of all secrets and wound debris out of the wound. The second step was the application of Rigenoma® spray and finally, on the periphery of the wound- the application of Rigenoma® crème. The surrounding skin was cleaned with Jodo povidone and it was not administered over the wound surface, where stable ozonides act, because of the possibility of inactivity. The procedure was finalized by a new microporous absorber placement in the wound cavity and the system for negative pressure was re-activated. During 48- 72 hours intervals, we performed a total of 1 or 2 lavages with a solution of Idrozoil® with saline chloride 0,9% in concentration rate 1:1, instilled through the drain, connected with the vacuum aspiration device. The vacuum was stopped for 30- 40 min and during that time interval, Idrosoil solution was instilled into the wound, and the drain was clamped. After the NPWT regimen was stopped, the wound cares continued with secondary suture and topical application of Ozoile®, stable ozonides from organic olive oil +OIL®. The standard method of surgical treatment includes surgical debridement, necrosectomy, drainages placement and lavages

with hydroperoxide and povidone-iodine (Betadine®, Braunol®), followed by standard sterile dressing with povidone-iodine or variants of local application of silver alginate, on a microporous absorbent base for chemical necrosectomy. This method requires daily changes of sterile dressings, sometimes two or three times per day, depending on the quantity of exudate, discharged by the inflamed wound. All patients with necrotizing fasciitis underwent microbiological monitoring before starting treatment and at the end of therapy, despite the applied method. We analysed patient comorbidity profiles, duration of hospital stay, rates of wound retraction and granulation and time for its sterilization, time to reach secondary suture procedure, and time to reach the full epithelisation stage. The effect of a new method of combined NPWT topical application of Ozoile®, stable ozonides from organic olive oil +OIL® was compared with conventional methods of treatment of severe necrotizing STI. We performed univariate, multivariate, and correlation analysis, mean values and standard error of the mean, Chi-square test, and Student's test for comparison between the two groups. We used IBM SPSS version 21 for Windows for statistical data analysis and $p < 0.05$ was accepted as a statistically significant result. From the research were excluded all cases with superficial STI, patients with burns and dermatological skin disorders.

RESULTS

For the first time, the topical application of stable ozonides at First Surgical Clinic of UMHAT "Dr. G. Stranski" Ltd - the city of Pleven, Bulgaria started from the beginning of March 2022. Since then at the beginning of March 2023, at First Surgical Clinic were admitted 380 patients for soft tissue infections of the body and extremities, of which 148 (30.8%) had superficial inflammation of the soft tissues -skin and subcutaneous tissue, and the remaining 232 (69.2%) were with deep and severe necrotizing soft tissue infections (SNSTI) affecting the underlying fascia and muscle groups. We separate the patients in the SNSTI group into three subgroups, according to the leading method of treatment. Subgroup 1 – SNSTI consists of patients treated with conventional methods- 170 patients (73,3%); Subgroup 2 – consists of 35 patients (15,1%) with NPWT as an independent

method and subgroup 3 – patients who received NPWT with topical application of Ozoile® stable ozonides from organic olive oil +OIL® - 27 patients (11,6%). The demographic characteristics of each subgroup are Subgroup 1: 87 males, 83 females, mean age 63±12,4; subgroup 2: 21 males, 14 females, mean age 64±11,3; subgroup 3: 17 males and 10 females, mean age 65±9,8. The main characteristics of wound treatment in each subgroup are visible in **Table 1**. The microbiological results showed the following distribution: the most frequent isolates belong to the Gram-negative bacteria of the

family Enterobacteriaceae, the first three places are E.coli - 54.5%, Klebsiella spp., -43.6%, and Serratia spp. and of Gram-positive Staphylococcus aureus-10.5%, Staphylococcus haemolyticus 8.7%, Enterococcus faecalis are 6.7%, and multi-resistant in-hospital strains are most often isolated Acinetobacter baumannii and Pseudomonas aeruginosa - 3.6%.

We have shown stages of the treatment of severe fasciitis with NPWT topical application of Ozoile®, stable ozonides from organic olive oil +OIL® in the **Figures 1-13**

Table 1. Main characteristics of patients, treated for SNSTI*

Variables	Subgroup 1 Standard surgical treatment	Subgroup 2 NPWT** - alone	Subgroup 3 NPWT + LOT***	P value
Number of patients (n)	170	35	27	
Patients with Diabetes mellitus (n)	48	18	14	
Patients with systemic corticosteroid therapy	8	4	2	
Days to reach total wound sterilization (mean)	8±2,5	6±1,2	4±0,5	p<0,05
Days to reach wound retraction with 25 % of initial size (mean)	8±2,6	4±2,1	3±1,4	p<0,04
Mean hospital stay (days)	18±2,7	12±2,1	10±1,8	p<0,05
Treatment in ICU (mean duration in days)	5±3,4	2,7±2,1	0,8±0,8	p<0,05
Numbers of procedures over wound (mean)	8±2,3	5±1,2	4±1,5	p<0,04
Days with wound exudation	23±4,6	12±2,3	10±2,8	P<0,04
Days to secondary suture (mean)	16±2,1	10±2,8	9±2,4	P<0,05
Days to reach full epithelization (mean)	35±3,5	19±2,6	15±1,8	p<0,04
Complications	Sepsis with MOD	Wound bleeding	Hipergranulation Capillary bleeding	
Death cases (n)	1	0	0	
30 days follow up complains (n= number of patients)	Local wound discomfort (n= 58), redness (n=79), edema (n=73) or hypo sensation (n=35)	Itching (n=12) Moderate hyperpigmentation (n=16) Epithelial desquamation (n= 14)	Mild hyperpigmentation (n=5)	

*SNSTI- Severe Necrotizing Soft tissue infections

**NPWT- Negative Pressure Wound Therapy

***LOT- Local ozone therapy with Ozoile®, stable ozonides from organic olive oil +OIL®

****ICU- Intensive Care Unite

*****MOD –Multi Organ Dysfunction



Figure 1. Wound washing with Idrosoil detergent



Figure 4. Micropore sponge for negative pressure wound therapy



Figure 2. Topical application of Rigenoma® spray



Figure 5. Wound packing with sterile folio



Figure 3. Application of Rigenoma® crème



Figure 6. Wound with a clean surface and granulation tissue- 6th day after NPWT and intralesional application of Ozoile®



Figure 7. Secondary wound suture on 6th day after NPWT and intralesional application of Ozoile®



Figure 8. Necrectomy and fasciotomy stage with wound packing for negative pressure wound therapy in the perianal area



Figure 9. Negative pressure wound therapy with local ozone therapy with Ozoile®- 7-th day of treatment in perianal region.



Figure 10. Negative pressure wound therapy with local ozone therapy with Ozoile®- 14 th day of treatment in the perianal region.



Figure 11. Negative pressure wound therapy with local ozone therapy with Ozoile®- 6th day of treatment of fasciitis of left pectoral area.



Figure 12. Negative pressure wound therapy with local ozone therapy with Ozoile®- 6th day of treatment in the left pectoral area – the wound is clean and with granulation.



Figure 13. Secondary suture after Negative pressure wound therapy with local ozone therapy with Ozoile®- 6th day of treatment in the left pectoral area.

DISCUSSION

NPWT is based on the effect of low-dosed negative pressure, which creates a local microclimate leading to the following four phenomena: macro deformation of the wound, micro deformation, permanent evacuation of exudate and creation of a beneficial environment for recovery. Macrodeformation consists of the contraction of the wound surface and a significant reduction in the size of the wound defect. Microdeformation is a set of processes that

maintain homeostasis at the tissue level by stimulating cell differentiation, and local thermogenesis and favoring the balance of the inflammatory reaction in the direction of reducing endothelial dysfunction and hypermobility. The system permanently evacuates the inflammatory exudate and makes it possible to maintain a low microbial count of the bacteria that caused the infection. (8) **Figure 14.**

The effect of negative pressure in the soft tissues provides good and constant conditions for regeneration- by keeping the wound moist, with a constant local temperature, controlling the amount of extracellular fluid, and reducing around the wound edema. This modulates the internal architectonics of the soft tissues, and the "cupping" effect leads to passive local hyperemia with improved tissue oxygen utilization, fibroblast proliferation, and collagen matrix construction. (9) According to Scalise A. et al. /2016/ the effect of tissue repair begins 48 hours after starting treatment under negative pressure, as a result of activation of the cell-mediated immune response and release of fibroblast growth factor responsible for fibroblast proliferation and neoangiogenesis. (10) Over that effect of NPWT is superponed the local action of stable ozonides, so the final effect over the wound healing is additive, according to the results in our research. The topical application of Ozoile®, stable ozonides from organic olive oil +OIL® in the alterative stage, first 2- 3 weeks of complex treatment of inflammation, results in stimulation of autolytic wound debridement and subsequent proliferation and formation of new capillaries. The tissue effect of Ozoile® is associated with its antibacterial and local antioxidant effect, stimulation of expression, and synthesis of epidermal, vascular, and fibroblast growth factors. (11-13) Antibacterial and antifungal effects of ozonides are realized by hydroperoxides- active oxygen radicals that destruct the microbial wall due to oxidative stress. The antibacterial effect of stable ozonides without any tissue toxicity or evidence for allergy reactions, make them preferred for local advanced and enhanced treatment strategy with the possibility for shortening the antibiotic course duration. The olive oil component in Ozoile® ozonides prevents wound dehydration and has a therapeutic effect on irritated skin- it reduces the local discomfort, protects the epidermis, and

preserves the elasticity of the skin. (14) In our research we have statistically significant results, comparing the NPWT + topical applications with Ozoile®, stable ozonides from organic olive oil +OIL® and standard methods.

In the application of the novel method the wound is free of any microbial cells in mean $4\pm 0,5$ days, compared with standard method that requires mean $8\pm 2,5$ days for full sterilization. ($p<0,05$) The tested method needs mean of $3\pm 1,4$ days to reduce the wound size by 25% from baseline size; for a standard method that happened for mean of $8\pm 2,6$ days ($p<0,04$). This accelerated wound healing process is remarkable in a group with local Ozoile® application and our results support data in the literature. (1, 4, 5) The secondary wound suture in subgroup 3 is performed after the mean $9\pm 2,4$ days from the beginning of initial treatment, but in subgroup 1 wound closure was after the mean $16\pm 2,1$ days. ($p<0,05$). That explains the shorter hospital stay for subgroup 3 versus subgroup 1: mean $10\pm 1,8$ days versus $18\pm 2,7$ days, respectively and this result is significant. ($p<0,05$). In a systemic contemporary review of the effect of ozonated oils in wound healing, a meta-analysis over 22 studies of human and mammalian animal wound treatment options was done. (15) The authors conclude that topical ozone therapy with ozone solutions quickly reduces wound size and reaches complete epithelization in a shorter time, compared with the controls, treated with conventional methods. Our results show the beneficial and additive effect of combined methods of NPWT with Ozoile®. As a side effect of researched treatment method, we registered hyper granulation and moderate capillary bleeding from the wound surface, which was more expressed after more than 14 days treatment schedule. Such side effects were not reported in other articles for local ozone therapy, which may be because of some limitations of study designs, and limitations on used methodologies without randomization of patients population. We suppose, that “side effects” are part of the local effects of ozone molecules – their tissue effects lead to vasodilatation, the proliferation of new capillaries, and fibroblastic cell in the wound bed. (15) According to the Consensus Statement of the

American college of surgeons, 2016 for the treatment of soft tissue infections and surgical site infections, the factors that influence wound healing are clearly defined. These factors are divided into two groups - internal (related to the patient), which can be variable and static, and external factors determined by the surgical procedure and the conditions and quality of health care for patients. Of the potentially modifiable factors on the part of patients, these are glycaemic control in diabetes mellitus, dyspnoea and hypoxia in COPD, alcohol intake and smoking, anemia, preoperative albumin levels below 3.5 mg/dl and total bilirubin above 1 mg /dl, immune suppression and overweight. This is the risk profile of patients who are expected to have problematic wound healing in the postoperative period and who may develop nosocomial surgical site infections. (16) Our report supports these claims and confirms the risk profile of patients who, due to severe soft tissue infection, need NPWT with or without stable ozonides. In the conventional treatment of fasciitis, aggressive debridement and evacuation of the exudate is achieved through frequent procedures in the operating room, which increases the risk for patients of frequent anaesthesia, as well as the risk of developing a hospital-acquired infection.

In standard treatment, excessive exudation from the wound surface requires changing dressings more than 4 times per day. (17) Standard wound treatment involves more healthcare professionals in caring for these patients than negative pressure treatment. The cost of treating patients with fasciitis by the conventional method is more expensive compared to NPWT based on increased hospital stay and cost of treatment materials and amounts to just over \$54,000 per person per stay. (17) NPWT patient care requires special technical supplies but also creates a higher quality of patient care. Contamination of the hospital environment is reduced as much as possible, due to the drainage of exudate from the wound directly into an individual canister collector. The patients with sepsis originated from SNSTI, the inhibition of sepsis and its complication by local ozone therapy has a faster effect than conventional treatment. (18)

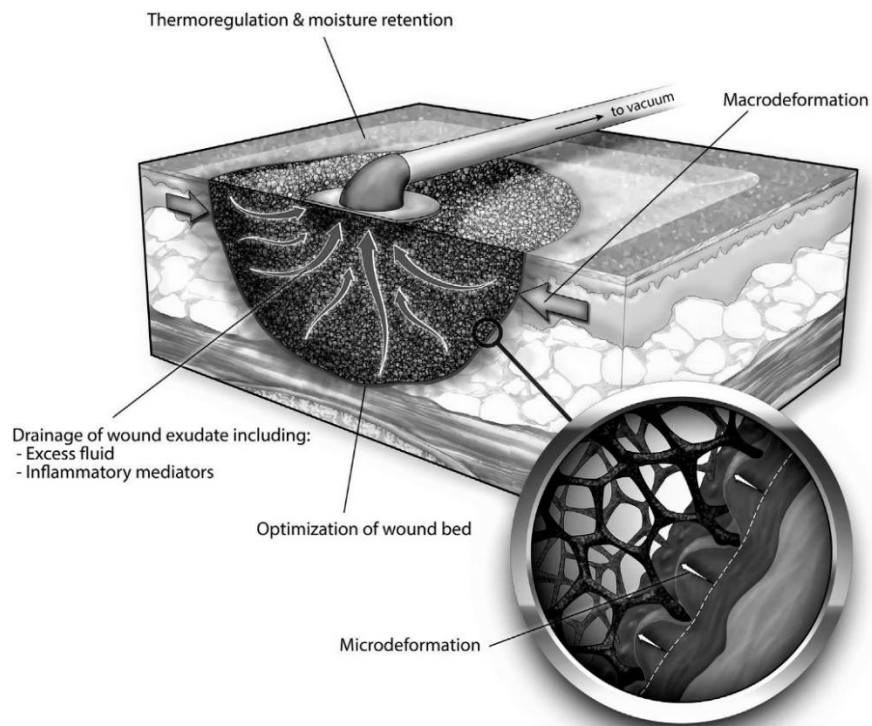


Figure 14. Effect of negative pressure wound therapy on wound healing. (8)

CONCLUSION

The treatment of SNSTI with NPWT +_with Ozoile[®] and stable ozonides is a new approach to the management of complicated soft tissue infections. Till that moment there are no any articles that describe the combination of NPWT with topical administration of stable ozonides – Ozoile[®] and compare the results of the treatment with the conventional methods of treatment. We do believe that research is a new vision of the possibilities of fast wound healing, effective tissue reparation, and accelerated epithelization. The quality of life after NPWT_ with Ozoile[®] remains good and in 30-ty – day follow up there were no specific wound-related complaints. This innovative method may be useful and effective in the complex management of severe soft tissue infections and have advantages compared with standard methods of STI treatment strategies. These pioneering and preliminary results may be helpful in future detailed investigations of treatment regimens options in a combination of NPWT and topical stable ozonides as Ozoile[®].

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