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# The use of aqueous ozone solution in the treatment of mine blast injury with extensive soft tissue defects: A case study

**Taras Pyatkovskyy***
PhD in Medical Sciences, Associate Professor
I. Horbachevsky Ternopil National Medical University
46001, 1 Maidan Voli, Ternopil, Ukraine
https://orcid.org/0000-0003-1240-1680

**Olena Pokryshko**
PhD in Medical Sciences, Associate Professor
I. Horbachevsky Ternopil National Medical University
46001, 1 Maidan Voli, Ternopil, Ukraine
https://orcid.org/0000-0001-9640-0786

**Oleksandr Bilyk**
PhD in Medical Sciences, Plastic and Reconstructive Surgeon
Swedish-Ukrainian Medical Center "Angelholm"
58026, 185-V Ruska Str., Chernivtsi, Ukraine
https://orcid.org/0009-0004-3018-4194

**Serhii Danylkov**
PhD in Medical Sciences, Assistant Professor
Bogomolets National Medical University
01601, 13 Taras Shevchenko Blvd., Kyiv, Ukraine
https://orcid.org/0009-0008-7273-4296

**Abstract.** Mine explosions frequently result in severe and complex injuries, presenting challenges in wound management and infection control. This study aimed to examine the efficacy, safety, and practical implications of ozonated water in wound care for mine explosion injuries. A 37-year-old male soldier presented with extensive lacerations of both legs sustained during a mine explosion. Following initial stabilisation, foreign bodies removal and debridement, the patient was hospitalised where he underwent surgical intervention, and negative pressure wound therapy to repair tissue damage. Microbial cultures obtained from wound samples revealed the presence of multi-drug resistant strains of *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Antibiotic susceptibility testing indicated limited treatment options due to resistance of the isolated strains. Given the severity of the injuries and microbial colonisation, ozonated water was introduced as part of the wound care regimen. Topical application of ozonated water was initiated on admission and repeated with each dressing change. Over the course of treatment, the patient demonstrated significant improvement in wound healing, reduction in microbial burden, and resolution of infection signs. The use of ozonated water facilitated expedited wound closure and minimised the need for systemic antibiotics. This case highlights the potential of ozonated water as an effective adjunct therapy in the management of mine explosion wounds, particularly

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*Corresponding author

in cases of multi-drug resistant microbial colonisation. The findings underscore the practical value of ozonated water in improving wound care outcomes and reducing reliance on systemic antibiotics in complex trauma cases

**Keywords:** aqueous ozone; wound infection; antibiotic-resistant bacteria; inactivation of microorganisms; negative pressure wound therapy

## Introduction

The issue of injuries caused by mines and explosive devices is highly relevant today, affecting both military personnel and civilians due to the ongoing warfare in Ukraine, which have persisted for over two years following the attack by the aggressor state. The relevance of this study is further underscored by the extensive areas of mined territory in our country, as well as regions containing unexploded ordnance.

Mine explosions are devastating events that result in a multitude of injuries, including burns, lacerations, and infections, presenting significant challenges for medical personnel tasked with providing effective wound care in the aftermath [1]. Traditional methods of wound management may be insufficient in addressing the complex nature of injuries sustained in mine explosions, particularly due to the risk of infection and delayed healing [2]. In the recent study, I. Trutyak *et al.* [3] reported that mine blast and gunshot wounds were heavily contaminated with *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Enterobacter aerogenes*, *Proteus vulgaris*, and *Enterococcus faecalis*. Studies conducted in conflict zones and military settings additionally have reported varying rates of multidrug resistance among microbial isolates from blast injuries. G. Loban' *et al.* [4] revealed that, since the onset of the full-scale war in Ukraine, the rates of multidrug resistance in *A. baumannii* and *K. pneumoniae* isolated from infected wounds have reached 75.0% and 80.0%, respectively. Similarly, K. Moussally *et al.* [5] reported that approximately 70% of positive cultures, primarily from patients with osteomyelitis in Gaza hospitals, exhibited multidrug resistance. The researchers found that about 65% of *Staphylococcus aureus* isolates were resistant to methicillin, while approximately 35% of *Pseudomonas aeruginosa* isolates showed resistance to ceftazidime and imipenem. Among Gram-negative isolates, 30% exhibited extended-spectrum beta-lactamases, and nearly 25% of resistant Enterobacteriaceae strains were resistant to carbapenems. Therefore, there is a critical need for innovative approaches to wound treatment that can effectively combat microbial colonisation and promote tissue regeneration.

Ozonated water, produced by dissolving gaseous ozone in water or through water electrolysis, has emerged as a promising therapeutic option for wound management due to its potent antimicrobial properties and ability to enhance tissue oxygenation and wound healing processes. According to L. Mascarenhas *et al.* [6], ozonated water can be easily distributed over surfaces or wounds using portable spray disinfection devices. Previous studies have reported the successful using ozonated water and gaseous ozone, and in some cases ozonated oil in the treatment of various types of wounds. S. Dhamnaskar *et al.* [7] observed improved healing and reduction in microbial load of diabetic foot ulcers in patients who received topical application of gaseous ozone for 30 days, 30 minutes per session comparing to the group with conventional wound management. Comparably, X. Hu *et al.* [8] successfully utilised negative pressure wound therapy (NPWT) using vacuum-assisted closure (VAC) and ozonated water flushing for treatment of diabetic foot ulcers. A. Roth *et al.* [9] demonstrated the possibility to utilise gaseous ozone to treat different infected dermal wounds. In their study on the antimicrobial activity of ozone, X. Wang *et al.* [10] discovered that gaseous ozone, and ozonated water and oil exhibited a bactericidal effect in vitro against forty strains of multidrug-resistant pathogens isolated from burn wounds. The analysis of available studies indicates that the use of ozonated water for treating mine blast injuries remains relatively unexplored. This clinical case study aimed to demonstrate the effectiveness of ozonated water, obtained by electrolysis, as an adjunctive therapy in treating complicated, infected mine blast injuries in combination with NPWT.

## Materials and Methods

***Patient.*** The patient, a 37-year-old male observed in early 2024, experienced a mine blast injury resulting in open comminuted fractures of both right and left tibia and fibula, accompanied by extensive bone and soft tissue defects with displacement of fragments. Before hospitalisation, the patient underwent shrapnel removal, wounds debridement, and external fixation. Upon hospitalisation at the Swedish-Ukrainian Medical Centre "Angelholm" on January 16, 2024, the patient complained of pain and purulent haemorrhagic discharge from the wounds of both legs. He also experienced restriction of passive and active movements, inability to bear weight on the limbs, malaise, and general weakness.

***Wound management.*** In-hospital management of wounds included debridement, partial suturing of the wounds, installation of negative pressure wound therapy system (Confort C300, Eskişehir, Turkey), and topical application of ozonated water with each dressing change. Ozonated water was produced on demand using a custom-made pre-production prototype that utilises water electrolysis on a diamond-coated anode (Fig. 1), a recently developed technology [11].

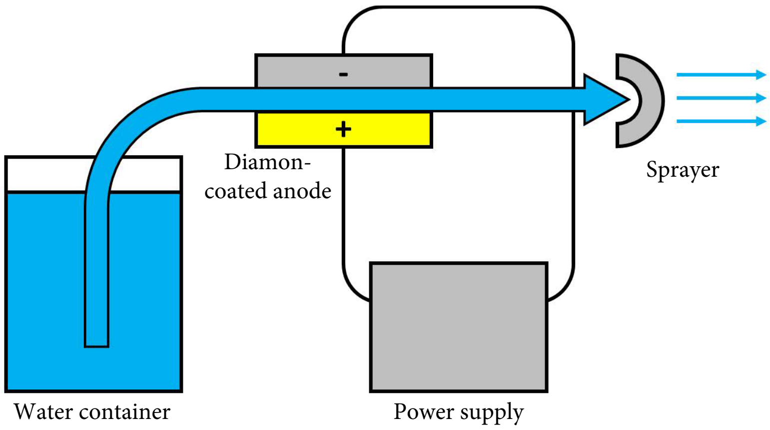


**Figure 1.** Principle of operation of the ozonator
**Source:** compiled by the authors of this study

It was sprayed over the wounds during dressing changes (Fig. 2). In cases where wounds exhibited skin defects, Vaseline gauze (Sumbow Medical Instruments Co., Ltd, Ningbo, China) was used to provide coverage and protection.

**Figure 2.** Application of ozonated water during the dressing change
**Source:** photographed by the authors of this study

Ozone concentration in water was assessed photometrically using a PoolLab 1.0 photometer (Water-i.d., Eggenstein, Germany), based on the intensity of colour change upon reaction with N,N-diethyl-p-phenylenediamine sulfate. The concentration of ozone was determined by comparing the absorption of coloured light (at wavelengths of 530 and 620 nm) by the sample to that of the untreated sample, utilising calibration data programmed into the instrument. Tablet-based reagents were used for measuring ozone concentration.

***Wound cultures and antibiotic susceptibilities.*** The samples of wound discharge were collected on sterile cotton swabs (Jiangsu Huida Medical Instruments Co., Ltd, Yancheng, China) and transported to the laboratory using Amies transport medium. Upon the delivery the samples were inoculated onto blood agar, yolk-salt agar (Sanimed-M, LLC, Kharkiv, Ukraine), and MacConkey agar (bioMérieux, Marcy-l'Étoile, France) plates with subsequent incubation at 37°C for 24-48 hours. Initial identification of bacteria was based on their cultural and morphological properties. Gram-negative rods were identified by performing a series of tests: fermentation in Kliger Iron Agar, Simon's citrate agar (Farmaktiv, LLC, Kyiv, Ukraine), indole production, catalase production, and motility test. Gram-positive cocci were identified based on their catalase, lecithinase and coagulase (Biolik Pharma LLC, Kharkiv, Ukraine) test results.

The antibiotic susceptibility of the isolated strains was determined using the Kirby-Bauer disk diffusion method. Inocula were prepared from isolated pure cultures and diluted in sterile isotonic saline solution (0.9% NaCl). The density of the suspensions was adjusted to 0.5 McFarland units using the Biosan DEN-1 densitometer (BioSan SIA, Riga, Latvia). These suspensions were then uniformly inoculated onto Mueller-Hinton agar (Farmaktiv, LLC, Kyiv, Ukraine) plates. Antibiotic-impregnated paper disks (Farmaktiv, LLC, Kyiv, Ukraine) were placed onto the agar surface, followed by incubation at 37°C for 24 hours to allow for antibiotic diffusion and bacterial growth assessment. The diameters of the inhibition zones around the discs were measured to the nearest millimetre using a ruler. Subsequently, they were categorised as sensitive, intermediate, or resistant based on the guidelines chart provided by CLSI 2020 [12]. The antibacterials used in the test for both Gram-negative and Gram-positive strains of bacteria were ciprofloxacin (5 µg) and levofloxacin (5 µg). Gram-negative rods were tested for susceptibility to piperacillin/tazobactam (100/10 µg), ceftazidime (30 µg), cefepime (30 µg), gentamicin (10 µg), amikacin (30 µg), imipenem (10 µg), and meropenem (10 µg). Oxacillin

(1 μg), vancomycin (30 μg), and linezolid (30 μg) were used for Gram-positive bacterial isolates.

***Ethics statement.*** The research protocol for this study was conducted in accordance with the principles outlined in Declaration of Helsinki "Ethical Principles for Medical Research Involving Human Subjects" [13] and the UNESCO Universal Declaration on Bioethics and Human Rights [14]. The study design and procedures were approved by the Committee on Bioethics of I. Horbachevsky Ternopil National Medical University (Protocol No. 77, April 18, 2024), and the patient provided informed consent prior to his involvement in the study. The patient's personal information was handled confidentially in accordance with ethical guidelines.

## Results and Discussion

Upon hospitalisation, the patient presented with multiple wounds on both legs, exhibiting massive soft tissue defects and comminuted fractures of shin bones with purulent and haemorrhagic discharge. The fractures were stabilised with external fixators, and partial wound closure using sterile Vaseline gauze was performed (Fig. 3).

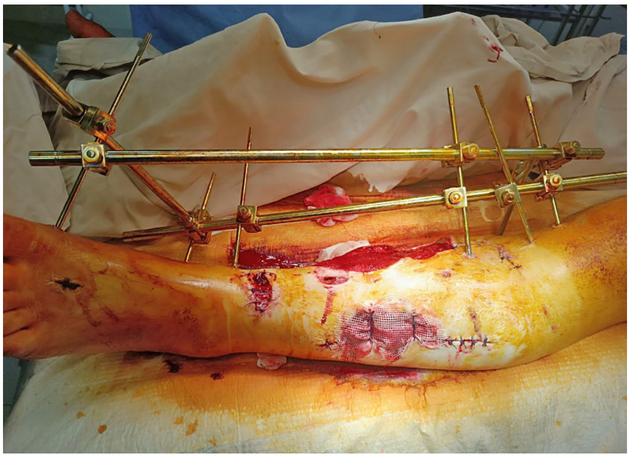

**Figure 3.** Angular external fixator. Partial wound closure using sterile Vaseline gauze
**Source:** photographed by the authors of this study

Sampling of the wound discharge revealed cultures of *Pseudomonas aeruginosa* and *Staphylococcus aureus*. The strains were resistant to almost all antibiotics tested. The *P. aeruginosa* strain was sensitive to ciprofloxacin and levofloxacin only. Similarly, *S. aureus* demonstrated susceptibility to these antibiotics as well as to vancomycin. Wound management included debridement and negative pressure wound therapy (Fig. 4 and Fig. 5). After necrectomy and the application of a negative pressure wound therapy dressing, the condition of the wounds improved and fresh granulating tissue was observed.

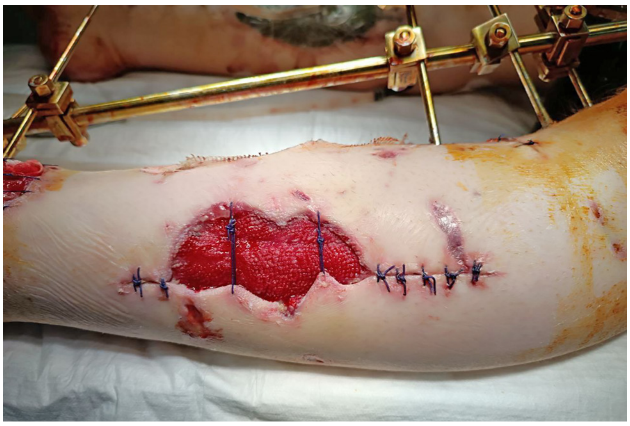

**Figure 4.** Granulating wound surface
**Source:** photographed by the authors of this study

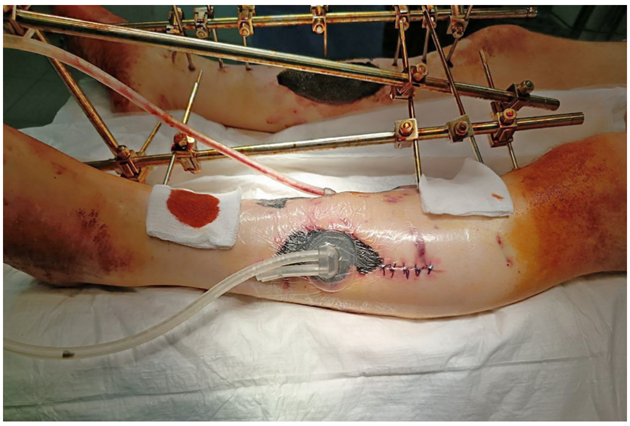

**Figure 5.** Negative pressure wound therapy system in situ
**Source:** photographed by the authors of this study

To combat antibiotic-resistant bacteria, an aqueous solution of ozone was topically applied during each wound dressing procedure. Measurements of ozone concentration revealed variations ranging from 3.10 to 4.13 mg/L. The utilisation of NPWT in combination with the application of ozonated water created favourable conditions for wound healing. As the wounds continued to heal and granulation tissue formed, the wounds were covered with skin autografts harvested from the patient's hips to minimise the risk of rejection. The autografts provided a viable means of covering the extensive soft tissue defects, promoting further healing, and ultimately aiding in the restoration of the skin's integrity and function. (Fig. 6 and Fig. 7).

**Figure 6.** Granulating wound surface prepared for autologous skin grafting
**Source:** photographed by the authors of this study

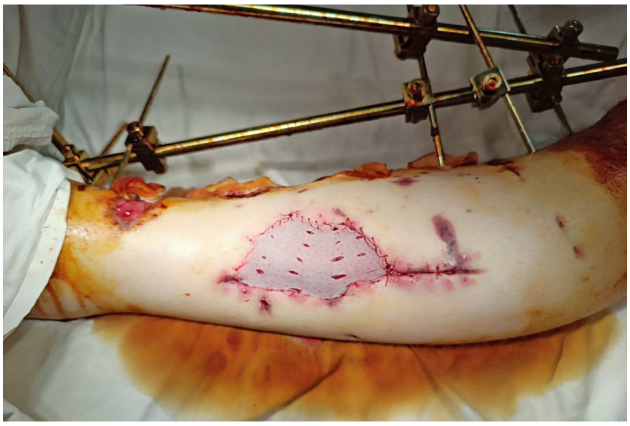

**Figure 7.** The wound is covered with the skin autograf
**Source:** photographed by the authors of this study

Following the grafting procedures, the use of ozonated water was maintained during all dressing changes and throughout the patient's hospitalisation until discharge. This continuous application played a crucial role in the successful engraftment of the grafts. The ozonated water helped keep the wound environment clean, reducing the risk of infection and promoting optimal conditions for healing.

On the fifth day after the grafting procedure, the skin flaps showed successful engraftment, which was a positive indication of the treatment's efficacy (Fig. 8). The absence of graft rejection throughout the healing process underscored the benefits of using ozonated water as part of the wound care regimen.

**Figure 8.** Day 5 after skin flap transplantation procedure
**Source:** photographed by the authors of this study

By day 10, complete revascularisation of the flaps was observed, indicating that the blood supply had been effectively restored and the grafts were fully integrating with the surrounding tissue (Fig. 9). The patient's wounds remained free from signs of infection, and there was a noticeable reduction in inflammation and exudate, which are common challenges in managing complex wounds. The patient's recovery was closely monitored, and the consistent use of ozonated water was maintained.

**Figure 9.** Day 10 after skin flap transplantation procedure
**Source:** photographed by the authors of this study

Throughout the course of treatment, the patient demonstrated improvement in wound healing, accompanied by the resolution of infection. The application of ozonated water for wound sanitation and treatment, in conjunction with surgical interventions and NPWT, significantly contributed to the successful management of the mine blast injuries in this patient. Upon discharge from the hospital on March 5, 2024, the patient's skin and soft tissue wounds had healed, albeit with ongoing bone healing requiring the continued use of external fixators on both legs for stability and support.

Mine blast injuries present unique challenges in wound management due to the severity and complexity of tissue damage, including fractures or other bone injuries. Since the introduction of the first commercial vacuum-assisted closure device in 1995, subsequent research has substantiated the beneficial physiological impact of negative pressure on wound healing. Initially employed to enhance the healing process of open nonsurgical wounds by secondary intention, the clinical utility of NPWT has expanded considerably. S. Poteet *et al.* [15] reported that NPWT is now utilised not only in open surgical wounds and closed surgical incisions but also in skin graft surgery. Furthermore, advancements in device technology have led to the integration of additional functionalities and features such as instillation, antimicrobial sponges, and enhanced portability.

While NPWT creates a controlled environment that can help reduce bacterial load and promote wound healing, it may not completely eliminate the risk of infection, especially in wounds that are already contaminated or infected. In the clinical case report described, multi-drug resistant strains of *Pseudomonas aeruginosa* and *Staphylococcus aureus* were isolated from the patient's wounds. This mirrors the findings of I. Trutyak *et al.* [3] who observed heavy contamination with *Pseudomonas aeruginosa* and enteric bacteria in mine blast and gunshot wounds. Similarly, R. Staruch & S. Hettiaratchy [16] found that *Staphylococcus aureus* and *Pseudomonas* were the causative agents for osteomyelitis in a group of 84 patients with open tibial fractures resulting from gunshot wounds and lower velocity metal fragments.

The portable custom-made ozonator considered in this study produced ozone concentrations ranging from 3.10 to 4.13 mg/L in aqueous solution. In the study by H. Li *et al.* [17], higher ozone production levels were achieved, up to 7.686 mg/L, using boron-doped diamond as the electrode material in the electrochemical ozone production process. Furthermore, they demonstrated that an ozone concentration of 4.86 mg/L in water effectively inactivated *Escherichia coli* with inoculum concentrations ranging from $1\times10^3$ to $3\times10^9$ CFU/mL. Similarly, B. Schorr *et al.* [18] reported the use of boron-doped diamond electrodes to generate reactive oxygen species for in-situ electrochemical oxidation. This method effectively eliminated *Escherichia coli*, *Pseudomonas fluorescens*, *Pseudomonas aeruginosa*, and *Bacillus subtilis* spores from water samples.

The successful utilisation of ozonated water in the treatment of mine explosion wounds presents a prospect for improving wound care outcomes in challenging environments. This clinical case demonstrates significant improvements in wound healing and a reduction in microbial burden following the topical application of ozonated water. These results are consistent with previous studies that have investigated the antimicrobial properties of ozone in wound management. Several studies have reported similar outcomes when using ozonated water for the treatment of various types of wounds. For instance, Hu *et al.* [8] effectively employed NPWT with VAC along with ozonated water flushing to treat diabetic foot ulcers. Their study revealed that patients who received ozonated water (10 μg/mL) administered into the VAC system twice daily experienced accelerated wound healing and lower infection rates compared to those treated with VAC alone. Similarly, A.N. Murakami *et al.* [19] investigated the use of ozonated water as an adjunct therapy for surgical site infections following pediatric cardiovascular surgery. Their results showed a significant reduction in microbial colonisation and improved wound healing outcomes in patients treated with ozonated water (0.2-0.6 mg/L) compared to standard wound care protocols. Additionally, T. Yasheng *et al.* [20] demonstrated the clinical efficacy of ozonated water (10 mg/L) lavage combined with vacuum-sealed drainage in the treatment of eighteen patients with chronic osteomyelitis in the limbs.

A. Roth *et al.* [9] described utilisation of ozone as an adjunct therapy. According to the authors while ozone therapy has drawbacks due to its toxicity at high concentrations, combining it with antibiotics could enhance treatment effectiveness without relying on high doses of ozone or antimicrobials. Ozone treatment weakens microbial cell walls, making them more susceptible to antibiotics, thus improving their effectiveness.

While the use of ozonated water did not shorten the overall treatment duration due to the inherent nature of the healing process, which requires adequate time [personal observation of O. Bilyk], it significantly improved the treatment course. The use of ozonated water reduced the dependence on antibiotics, which are often associated with various side effects. By maintaining a clean and conducive wound environment, ozonated water helped to control infection and promote healing, thereby minimising the need for systemic antibiotics. This approach not only lessened the potential adverse effects of prolonged antibiotic use but also addressed the challenge of antibiotic resistance, making the treatment more sustainable and patient-friendly.

The authors of the discussed publications utilised various forms of ozone for wound treatment. However, electrolytic ozonation, which involves generating ozone through electrolysis of water, is generally considered safer compared to conventional ozonation methods. According to E. Grignani *et al.* [21], traditional methods usually involve bubbling gaseous ozone through water, which can cause irritation to the eyes, skin, and mucous membranes upon contact. Additionally, M. Alimohammadi & M. Naderi [22] reported that gaseous ozone is toxic to humans at high concentrations and is corrosive to certain materials, such as natural rubber. As a result, electrolytic ozonation, which was utilised in this particular case, offers a safer alternative, reducing the need for expensive thermal or catalytic destructors to neutralise residual gaseous ozone.

## Conclusions

This clinical case report provided compelling evidence of the successful utilisation of negative pressure wound therapy in combination with ozonated water for the effective management of mine blast injuries with extensive soft tissue defects complicated by wound infection caused by multi-drug resistant strains of *Pseudomonas aeruginosa* and *Staphylococcus aureus*. The combination of NPWT with ozonated water treatment provided a controlled environment that promotes wound healing by removing excess exudate, reducing edema, and enhancing blood flow to the wound bed, which accelerates tissue regeneration. Throughout the treatment course, the patient demonstrated significant improvement in wound healing, accompanied by the resolution of infection. The application of ozonated water contributed to wound sanitation and treatment. The antimicrobial action of ozonated water reduces the reliance on systemic antibiotics, which is crucial in combating antibiotic resistance and minimising the adverse effects associated with prolonged antibiotic use. Additionally, the successful integration of skin autografting further enhanced the healing process, resulting in the successful closure of soft tissue wounds. The patient was discharged from the hospital with healed skin and soft tissue wounds. He continued to wear an external fixator as his bones were still healing. This study underscores the potential of aqueous ozone solution as an adjunctive therapy in wound management, offering positive outcomes for patients with complex soft tissue injuries. It represents a promising strategy for managing complex wounds, especially those contaminated with antibiotic-resistant bacteria, ultimately leading to improved clinical outcomes and enhanced patient well-being. Moving forward, further research and clinical trials are warranted to explore the full therapeutic potential of NPWT and ozonated water in the treatment of mine blast injuries and other traumatic wounds.

## Acknowledgements

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## Conflict of Interest

The authors declare no conflict of interest.

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# Використання водного розчину озону при лікуванні мінно-вибухової травми з великими дефектами м'яких тканин: клінічний випадок

**Тарас П'ятковський**
Кандидат медичних наук, доцент
Тернопільський національний медичний університет імені І. Я. Горбачевського
46001, Майдан Волі, 1, м. Тернопіль, Україна
https://orcid.org/0000-0003-1240-1680

**Олена Покришко**
Кандидат медичних наук, доцент
Тернопільський національний медичний університет імені І. Я. Горбачевського
46001, Майдан Волі, 1, м. Тернопіль, Україна
https://orcid.org/0000-0001-9640-0786

**Олександр Білик**
Кандидат медичних наук, пластичний і реконструктивний хірург
Шведсько-український медичний центр «Angelholm»
58026, вул. Руська, 185-В, м. Чернівці, Україна
https://orcid.org/0009-0004-3018-4194

**Сергій Данилков**
Кандидат медичних наук, асистент
Національний медичний університет ім. О. О. Богомольця
01601, бульв. Тараса Шевченка, 13, м. Київ, Україна
https://orcid.org/0009-0008-7273-4296

**Анотація.** Вибухи мін часто призводять до важких і складних травм, що створює проблеми з лікуванням та інфекційним контролем ран. Цей рукопис мав на меті дослідити ефективність, безпеку та практичні наслідки застосування озонованої води при лікуванні ран, отриманих від вибуху мін. Військовослужбовець, віком 37 років, отримав значні рвані рани обох ніг під час вибуху міни. Після первинної стабілізації, видалення сторонніх тіл і санації пацієнта було госпіталізовано, де йому було проведено хірургічне втручання та лікування низьким тиском для відновлення пошкоджених тканин. Мікробіологічні дослідження отриманих із зразків ран виявили наявність полірезистентних штамів *Pseudomonas aeruginosa* та *Staphylococcus aureus*. Тест на чутливість до антибіотиків показав обмежені можливості лікування через резистентність виділених штамів. Враховуючи тяжкість ушкоджень і мікробну колонізацію, озонована вода була введена в режим догляду за ранами. Місцеве застосування озонованої води було розпочато одразу при госпіталізації і повторювалося з кожною заміною пов'язки. Протягом курсу лікування пацієнт продемонстрував значне покращення загоєння ран, зменшення мікробного навантаження та зникнення ознак інфекції. Використання озонованої води сприяло швидкому загоєнню ран і мінімізувало потребу в системних антибіотиках. Цей випадок підкреслює потенціал озонованої води як ефективної допоміжної терапії при лікуванні ран від вибуху мін, особливо у випадках мікробної колонізації, стійкої до багатьох лікарських засобів. Отримані дані підкреслюють практичну цінність озонованої води для покращення результатів лікування ран і зменшення залежності від системних антибіотиків у випадках складних травм

**Ключові слова:** водний озон; ранова інфекція; антибіотикорезистентні бактерії; інактивація мікроорганізмів; лікування ран негативним тиском