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©T. I. PYATKOVSKYY[1]
pyatkovskyy@tdmu.edu.ua; ORCID: https://orcid.org/0000-0003-1240-1680

©O. V. POKRYSHKO[1]
pokryshko@tdmu.edu.ua; ORCID: https://orcid.org/0000-0001-9640-0786

©O. V. BILYK[2]
oleksandrbilyk900@gmail.com; ORCID: https://orcid.org/0009-0004-3018-4194

©S. O. DANYLKOV[3]
danylkiv@gmail.com; ORCID: https://orcid.org/0009-0008-7273-4296

I. Horbachevsky Ternopil National Medical University, Ternopil, Ukraine[1]
Swedish-Ukrainian Medical Center "Angelholm", Chernivtsi, Ukraine[2]
Bogomolets National Medical University, Kyiv, Ukraine[3]

Ozonated water as an adjunct in the management of combat-induced wounds: a prospective study

The aim of the work: to evaluate the effectiveness of electrolytically generated ozonated water as an adjunctive therapy in the treatment of complicated, infected combat-induced wounds.

Materials and Methods. This prospective study included 27 patients with combat-related injuries treated at the Swedish-Ukrainian Medical Center "Angelholm" (Chernivtsi, Ukraine). Wound management included debridement, necrectomy, stabilization of fractures, partial wound closure, and application of negative pressure wound therapy (NPWT) in deep or complex wounds. Electrolytically ozonated water (up to 4 mg/L) was used for wound irrigation during each dressing and NPWT system change. Microbiological cultures were collected routinely, and bacterial identification and antibiotic susceptibility were performed using the Vitek-2 Compact system.

Results. A total of 45 microbial isolates were identified from 27 patients, with *Acinetobacter baumannii* (28.89 %) and *Staphylococcus aureus* (20.00 %) being the most common pathogens. Some patients had mixed infections, and most of the isolates were multidrug-resistant. The use of ozonated water resulted in improved wound conditions, including fresh granulation tissue formation and absence of graft rejection following skin grafting. Patients experienced reduced edema, intoxication symptoms, and pain, contributing to improved quality of life during the healing period.

Conclusions. Electrolytically generated ozonated water proved to be a safe and effective adjunctive therapy in the management of complex combat-related injuries. Its antimicrobial activity, clinical safety, and ease of application make it a valuable addition to wound care protocols, especially in settings with high rates of multidrug-resistant infections.

Key words: antibiotic-resistant bacteria; aqueous ozone; combat-related wounds; inactivation of microorganisms; negative pressure wound therapy; wound infection.

Problem Statement and Analysis of Recent Research and Publications. Due to the ongoing war in Ukraine, which has continued for more than three years after the aggressor state's attack, the problem of combat-related wounds is extremely pertinent today. The fact that our country has large tracts of mined land and locations with unexploded ordnance further emphasizes the relevance of this study. Combat-related wounds can vary in nature and may include gunshot injuries, mine-blast trauma, burns, frostbite, and lacerations, all of which carry a risk of subsequent complications such as wound infection and osteomyelitis. According to Belmont et al., who studied musculoskeletal combat wounds in Iraq and Afghanistan, 77 % of all casualties sustained a musculoskeletal injury, most of which were caused by explosive blasts [1]. Mine explosions are devastating events that result in multiple injuries, and according to Osmanov et al., they represent the most frequent type of combat injury observed during the Russian-Ukrainian war [2].

As reported by Trutyak et al., mine blast and gunshot wounds are heavily contaminated with various Gram-negative and Gram-positive pathogens [3]. Additionally, Loban' et al. revealed that since the onset of the full-scale war in Ukraine, the rate of multidrug resistance among wound pathogens has increased to as high as 80 % [4]. These findings highlight that traditional wound management methods may be insufficient to address the complex nature of combat-related injuries [5]. The high risk of infection, particularly with multidrug-resistant pathogens, requires innovative approaches to wound treatment that can effectively control microbial colonization and promote tissue regeneration.

Ozonated water, known for its potent antimicrobial properties, has emerged as a promising therapeutic option in wound management. The strong antimicrobial action of ozone is primarily attributed to its powerful oxidative properties [6, 7]. As a highly reactive molecule, ozone rapidly interacts with microbial cell components, particularly cell membranes, proteins, and nucleic acids, leading to structural damage and functional disruption. This oxidative stress compromises membrane integrity, causes leakage of intracellular contents, and ultimately results in microbial cell death [8– 10]. Unlike antibiotics, ozone does not target specific metabolic pathways, making it effective against a wide range of microorganisms, including antibiotic-resistant strains [11,12]. Moreover, its non-selective mechanism of action significantly reduces the risk of resistance development. These properties, combined with its safety and ease of use in aqueous form, highlight ozonated water as a valuable tool in modern wound care.

Despite the growing interest in ozone-based therapies, there remains a notable lack of studies specifically addressing the use of ozonated water in the treatment of combat-related injuries. This prospective study contributes to filling this gap by demonstrating the effectiveness of ozonated water, produced via electrolysis, as an adjunctive therapy. When used in combination with negative pressure wound therapy (NPWT), ozonated water showed promising results in managing complicated, infected combat-induced injuries, supporting its potential role in modern wound care protocols.

The aim of the work: to evaluate the effectiveness of electrolytically generated ozonated water as an adjunctive therapy in the treatment of complicated, infected combat-induced wounds.

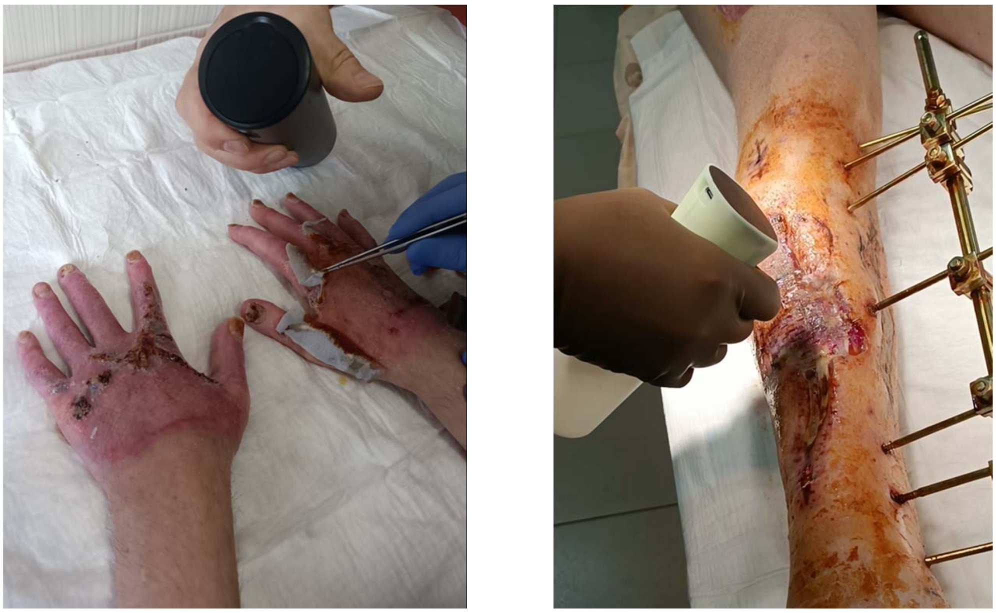
Materials and Methods. This prospective study was conducted at the Swedish-Ukrainian Medical Center "Angelholm", Chernivtsi, Ukraine, in January and February 2024. The sample size consisted of 27 patients with combat-induced wounds treated with NPWT and ozonated water as an adjunct. The study was conducted in accordance with the ethical principles outlined in the Declaration of Helsinki and the UNESCO Universal Declaration on Bioethics and Human Rights. The study design and procedures were approved by the Committee on Bioethics of I. Horbachevsky Ternopil National Medical University (Protocol No. 81, April 3, 2025), and informed consent was secured from all participants. The patient's personal information was handled confidentially. Patients were included based on the following criteria: mine blast injuries with soft tissue defects with and without fractures, burn injuries and frostbite injuries. Exclusion criteria encompassed gunshot wounds, intra-articular injuries, injuries complicated with osteomyelitis as well as chronic, non-combat-related wounds. In-hospital wound management included debridement, partial wound suturing, installation of a negative pressure wound therapy system (Confort C300, Eskişehir, Turkey) if necessary, and topical application of ozonated water during each dressing change. Ozonated water was produced on demand using a custom-made pre-production prototype that utilized water electrolysis with a diamond-coated anode. It was sprayed over the wounds during dressing changes (Fig).

Ozone concentration in water was assessed photometrically using a PoolLab 1.0 photometer (Water-i.d., Eggenstein, Germany), based on the intensity of color change upon reaction with N, N-diethyl-p-phenylenediamine sulfate. The concentration of ozone was determined by comparing the absorption of colored light (at wavelengths of 530 and 620 nm) by the sample to that of the untreated sample, utilizing calibration data programmed into the instrument. Tablet-based reagents were used for measuring ozone concentration. For microbiological testing, wound discharge samples were collected using sterile cotton swabs (Jiangsu Huida Medical Instruments Co., Ltd, Yancheng, China) and transported to the laboratory in Amies transport medium. Upon 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, followed by incubation at 37 °C for 24–48 hours. Microbial cultures were identified, and their antibiotic susceptibility profiles were determined using the automated Vitek-2 Compact system (bioMérieux, Marcy-l'Étoile, France).

Data were collected and tabulated using MS Excel version 2013, and were checked for normality before analysis. Qualitative data were presented as percentages and proportions.

Results. Among 27 patients, all males, predominant age group was 31–35 years (29.63 %) with an average age of (35±6.5) years. The most common cause of injury was mine blast injuries with fractures at 51.85 % (Table 1).

Upon hospitalization, patients with mine blast injuries (with and without fractures), burns, and frostbite underwent standard treatment procedures, including surgical debridement, necrectomy, fracture stabilization using external fixation, and partial wound closure with sterile Vaseline gauze or skin grafts when feasible. In cases of deep or complex wounds, negative



A B

Fig. Application of ozonated water using a portable electrolysis device during a dressing change: A – for burns; B – for mine-blast injuries.

Table 1. Clinical and demographic characteristics of the enrolled patients (n=27)

Clinico-demographics characteristics	Number	Percentage, %
Age (years)		
18–25	2	7.41
26–30	4	14.81
31–35	8	29.63
36–40	9	33.33
41–45	3	11.11
46–50	1	3.71
Gender		
Male	27	100
Female	0	0
Nature of injury		
Mine blast injuries with fractures	14	51.85
Mine blast injuries without fractures	7	25.93
Burn injuries	4	14.81
Frostbite injuries	2	7.41

pressure wound therapy (NPWT) was applied to support wound drainage and tissue repair.

Microbiological testing was performed for all 27 patients enrolled in the study, yielding a total of 45 microbial isolates. The majority of patients (n=16) presented with monoculture infections, while mixed infections involving two or three pathogens were identified in several cases (n=11). Among the Gram-negative isolates, *Acinetobacter baumannii* was the most frequently detected pathogen, accounting for 28.89 % of all cultured strains. *Staphylococcus aureus* was the most prevalent Gram-positive organism, representing 20.00 % of the isolates. The full distribution of pathogens is presented in Table 2. A portion of the isolated strains exhibited multidrug resistance (n=42).

Table 2. Distribution of isolated pathogens from wound discharge

Pathogen	Number	Percentage, %
Acinetobacter baumannii	13	28.89
Bacillus cereus	2	4.45
Candida albicans	1	2.22
Candida glabrata	1	2.22
Klebsiella oxytoca	1	2.22
Klebsiella pneumoniae	7	15.56
Pseudomonas aeruginosa	6	13.33
Pseudomonas fluorescens	1	2.22
Pseudomonas putida	1	2.22
Staphylococcus aureus ss. aureus	9	20.00
Staphylococcus spp.	3	6.67
Total	45	100

As part of the wound management protocol, electrolytically ozonated water was used for wound irrigation during each dressing change, or during NPWT system changes in cases of deep or complex wounds. Its application during NPWT changes is particularly important, as it helps prevent the development of anaerobic microflora in the oxygen-deprived environment created by negative pressure therapy. In addition to standard irrigation and washing, we employed high-pressure pulse lavage in selected cases. This method proved particularly effective in managing deep, narrow wound channels, allowing for thorough decontamination and reducing the need for prolonged drainage of blind wound sacs.

Over the course of treatment, clinical improvement in wound condition was consistently observed. The continuous application of ozonated water played a supportive role in maintaining a clean wound environment, reducing the risk of secondary infection, and promoting the formation of healthy granulation tissue. The presence of fresh granulating tissue in treated wounds indicated a favorable healing trajectory.

In patients who underwent skin grafting, no cases of graft rejection were recorded. The grafts demonstrated full integration with the surrounding tissues, further supporting the suitability of ozonated water as part of the wound care regimen. Upon discharge, all patients showed significant clinical improvement. Those with fractures were advised to continue outpatient follow-up, including radiographic control one month post-discharge, to monitor bone healing and overall recovery.

Discussion. In our protocol, the concentration of ozone in the aqueous solution did not exceed 4 mg/L, a level chosen to ensure safety while maintaining antimicrobial efficacy. This is notably lower than the concentration used in the study by Hu et al. [13], where higher levels of ozonated water were applied (10 mg/L). By maintaining a lower concentration, we minimized the risk of potential cytotoxic effects while still achieving favorable clinical outcomes. High-pressure pulse lavage use contributed to accelerated healing in these otherwise challenging wound types. Our experience aligns with findings reported by Bath et al. [14], who demonstrated that pulsed lavage significantly reduced the risk of surgical site infections. The application of ozonated water continued even after definitive wound closure with skin grafts to minimize the risk of postoperative infection and support optimal healing conditions.

Systemic antibiotic therapy was administered to all patients in accordance with clinical guidelines and microbial sensitivity testing. Ozonated water was employed exclusively as an adjunctive treatment, aimed at enhancing local antimicrobial control and complementing systemic antibiotic therapy, rather than replacing it. In this study, we selected aqueous ozone generated by electrolysis as the form of ozone application for several practical and clinical reasons. Electrolysis represents the most economically accessible and scalable method for producing active ozone, allowing for its generation ex tempore from ordinary drinking water in virtually unlimited quantities. In contrast to traditional ozonation by gas bubbling, electrolytically generated ozone remains stable in aqueous solution for a longer period, sufficient for clinical use during wound irrigation and dressing procedures (as demonstrated in our previous work [15]). The use of aqueous ozone eliminates the need to manage ozone in the gas phase, which poses toxicity risks to healthcare personnel [6, 16]. It also avoids the requirement for thermal or catalytic destructors to neutralize residual ozone gas and reduces the demand for specialized ventilation systems in clinical settings.

Combat-related injuries present unique challenges in wound management due to the severity and complexity of tissue damage, which often includes extensive soft tissue loss, contamination, and associated fractures. Our observations align with previous findings by Trutyak et al., who reported heavy microbial contamination, particularly with *Pseudomonas aeruginosa* and various enteric bacteria, in mine blast and gunshot wounds [3]. Similarly, Loban' et al. demonstrated a concerning rise in multidrug resistance among wound pathogens since the onset of the full-scale war in Ukraine, with resistance rates reaching 75.0 % for *Acinetobacter baumannii* and 80.0 % for *Klebsiella pneumonia* [4]. These findings underscore the growing need for adjunctive strategies in infection control, especially in cases involving highly resistant microorganisms.

Electrolytic ozonation is an emerging approach that enables on-demand generation of ozonated water with promising antimicrobial properties. Unlike traditional ozonation methods that rely on external ozone gas sources, electrolytic systems offer a more compact, cost-effective, and safer alternative for clinical and field applications. Epelle et al. successfully demonstrated the antimicrobial efficacy of electrolytically generated ozonated water against a range of microorganisms, including *Escherichia coli*, *Staphylococcus aureus*, *Candida albicans*, and *Aspergillus fumigatus* [17]. Their findings support the broad-spectrum activity of aqueous ozone and highlight its potential role in managing both bacterial and fungal contamination.

In addition to its local antimicrobial and healing effects, the use of ozonated water was associated with noticeable improvements in patients' overall quality of life during the recovery period, including reduction of edema, signs of systemic intoxication, and pain syndrome.

The successful utilization of ozonated water in the treatment of combat-induced wounds observed in our study is consistent with previous reports describing its clinical efficacy across various conditions. For instance, Hu et al. effectively combined negative pressure wound therapy using vacuum-assisted closure with ozonated water flushing for the management of diabetic foot ulcers, achieving favorable wound healing outcomes [13]. Similarly, T. Yasheng et al. reported positive results using ozonated water lavage at a concentration of 10 mg/L in combination with vacuum-sealed drainage for the treatment of chronic osteomyelitis [18]. These studies further support the role of ozonated water as a valuable adjunctive therapy in the management of complex and infected wounds.

Study limitations. This study has several limitations that should be acknowledged. Due to the severity and extensive nature of the combat-related wounds, as well as the high risk of septic complications, it was not ethically feasible to conduct a randomized trial with a placebo or untreated control group. Additionally, the microbial flora varied between patients, further complicating the possibility of standardizing treatment conditions. For these reasons, ozonated water was applied as an adjuvant rather than as a standalone treatment. While this approach reflects real-world clinical practice and prioritizes patient safety, it also limits the ability to draw definitive conclusions about the isolated efficacy of ozonated water. Future controlled studies with stratified patient groups may help to further clarify its independent therapeutic value.

Conclusions. This prospective study demonstrates that electrolytically generated aqueous ozone is a valuable adjunct in the management of various combat-related injuries, including mine blast wounds, burns, and frostbite. When used alongside standard surgical care, systemic antibiotics, and negative pressure wound therapy, ozonated water contributed to improved wound cleanliness, reduction in microbial contamination, including multidrug-resistant organisms, and supported the development of healthy granulation tissue. No adverse effects, including graft rejection, were observed with its use. The patients were discharged from the hospital with healed skin and soft tissue wounds. These findings suggest that aqueous ozone is a safe, accessible, and effective supportive therapy in complex wound management, particularly in high-risk and resource-limited settings. The antibacterial properties of ozonated water may help reduce the reliance on systemic antibiotics, which is essential for preventing antibiotic resistance and minimizing the adverse effects associated with long-term antibiotic use.

Conflict of interest. The authors declare no conflict of interests.

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Authors' contribution. Pyatkovskyy T. I. – Conceptualization, Methodology. Bilyk O. V. – Conceptualization, Patient care. Pokryshko O. V. – Methodology, Writing – Review & Editing. Danylkov S. O. – Writing – Original Draft Preparation.

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Email for correspondence: pyatkovskyy@tdmu.edu.ua

Т. І. П'ЯТКОВСЬКИЙ[1], О. В. ПОКРИШКО[1], О. В. БІЛИК[2], С. О. ДАНИЛКОВ[3]

Тернопільський національний медичний університет імені І. Я. Горбачевського МОЗ України, Тернопіль, Україна[1]
Шведсько-український медичний центр «Angelholm», Чернівці, Україна[2]
Національний медичний університет імені О. О. Богомольця, Київ, Україна[3]

ОЗОНОВАНА ВОДА ЯК ДОПОМІЖНИЙ ЗАСІБ ПРИ ЛІКУВАННІ ПОРАНЕНЬ, СПРИЧИНЕНИХ БОЙОВИМИ ДІЯМИ: ПРОСПЕКТИВНЕ ДОСЛІДЖЕННЯ

Мета роботи: оцінити ефективність озонованої води, створеної шляхом електролізу, як ад'ювантної терапії при лікуванні ускладнених, інфікованих ран, спричинених бойовими діями.
Матеріали і методи. Проспективне дослідження включало 27 пацієнтів із бойовими пораненнями, які перебували на лікуванні у Шведсько-українському медичному центрі «Angelholm» (м. Чернівці, Україна). Лікування ран включало очищення, некректомію, стабілізацію переломів, часткове закриття ран та застосування терапії ран негативним тиском (NPWT) при глибоких або складних

ранах. Електролітично озоновану воду (до 4 мг/л) використовували для зрошення ран під час кожної перев'язки та зміни системи негативного тиску. Проводили мікробіологічні дослідження, ідентифікацію бактерій та визначення чутливості до антибіотиків проводили за допомогою системи Vitek-2 Compact.
Результати. Загалом у 27 пацієнтів було виділено 45 штамів, найпоширенішими збудниками були *Acinetobacter baumannii* (28,89 %) та *Staphylococcus aureus* (20,00 %). Частина їх мала змішану інфекцію, більшість штамів була полірезистентною. Використання озонованої води привело до поліпшення стану ран, включаючи утворення свіжої грануляційної тканини та відсутність відторгнення трансплантата після пересадки шкіри. Пацієнти відчували зменшення набряку, симптомів інтоксикації та болю, що сприяло покращенню якості життя у період загоєння.
Висновки. Електролітично згенерована озонована вода виявилася безпечною та ефективною допоміжною терапією при лікуванні складних травм, пов'язаних із бойовими діями. Її антимікробна активність, клінічна безпека і простота застосування роблять її цінним доповненням до протоколів лікування ран, особливо в умовах високого рівня поширеності мультирезистентних інфекцій.

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