Effectiveness of Ozone Therapy as an Adjunct Treatment for Lower-Limb Ulcers: A Systematic Review

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ABSTRACT

OBJECTIVE: To evaluate the effectiveness of topical ozone therapy as an adjuvant treatment in the healing of lower limb ulcers through a systematic literature review.

DATA SOURCES: Three databases were used to search for studies conducted in the period up to and including September 2020: PubMed, Scopus, and the Web of Science.

STUDY SELECTION: The search identified 44 studies, 7 of which met the eligibility criteria and were evaluated.

DATA EXTRACTION: Study design, study location, number of patients, patient age, type of control, wound type, intervention type, equipment used to generate ozone (ozone generation), evaluation methodology, and main results were extracted from each study.

DATA SYNTHESIS: A total of 506 patients 18 years or older with chronic wounds, such as venous or diabetic ulcers, on the lower limbs were enrolled. The majority of studies addressed diabetic foot ulcers.

CONCLUSIONS: The ozone therapy protocols demonstrated a healing effect in all included studies, and none reported adverse effects. This reinforces the need for more controlled and randomized clinical trials to determine the effectiveness of this treatment and establish clinical criteria for its use.

KEYWORDS: adjunct therapy, diabetic foot, leg ulcer, ozone therapy, systematic review, ulcer, wound healing

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INTRODUCTION

Chronic wounds of the lower limbs represent a significant burden for the health system and for the patient and their family; they can lead to serious physical consequences, such as limb amputations, and serious social, psychological, and economic implications.[1,2] The prevalence of chronic wounds in the general population is 2.21 per 1,000 population, and chronic lower limb ulcers are estimated at 1.51 per 1,000 population.[3] In Europe, it is estimated that between 1.5 and 2 million people suffer from acute or chronic wounds.[4]

Costs related to wound care are a major burden on healthcare budgets; the main costs do not relate to dressings, but time spent by nursing staff providing care and hospital costs, which together account for about 80% to 85% of the total wound management costs. The prolonged period required for treatment and injury closure is also a significant factor in relation to costs, as are the frequent dressing changes and possible complications.[4]

Internal or external factors can produce wounds that are difficult to heal,[1] including aging, the use of systemic drugs, hormones, poor nutrition, obesity, trauma, vascular disease, and diabetes. Moreover, local factors, such as edema, dryness, local infection, necrosis, the presence of foreign bodies, inadequate pressure and dressing techniques, the extent and location of the wound, and the use of inappropriate topical agents, can also have significant effects.[2,5]

Accordingly, a number of new wound care practices using modern and advanced technology are being explored to try to reduce the time to wound closure, the frequency of dressing changes, wound complications, and (consequently) costs. This literature review examines one specific treatment—the use of ozone therapy for difficult-to-heal lower-extremity ulcers, particularly those with the highest prevalence and impact (eg, venous ulcers).

Ozone is a gas formed by three oxygen atoms and was discovered in the middle of the 19th century. It is currently being used in several countries, such as Italy, Germany, Spain, Portugal, Russia, Cuba, China, and Brazil, for the treatment of wounds. Ozone therapy has been proposed

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as an adjuvant treatment because of its potential therapeutic effects (immunologic, bactericidal, fungicidal, and virucidal), which can optimize cellular metabolism and therefore promote healing. Further, the gas potentially acts to trigger controlled oxidative stress when administered in precise therapeutic doses. Studies indicate that there is a significant benefit to healing using this therapy, and this has encouraged its application in a number of different ways.[6–13]

Objective

The present study aimed to synthesize the available evidence on the therapeutic use of ozone in patients with venous, arterial, mixed, and/or diabetic lower-limb ulcers through a systematic review of the literature to enhance decision-making regarding this treatment, advance wound healing processes, and promote patient safety.

METHODS

Search Strategy

The study followed the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-analyses) recommendations and was registered on the PROSPERO database (no. CRD42021232825). Three internet databases were used to search for appropriate articles that met study criteria: the National Library of Medicine (MEDLINE-PubMed), Scopus, and the Web of Science. Investigators used the following search strategy with MeSH (Medical Subject Headings) terms in Portuguese and English: ((skin ulcer) OR (leg ulcer) OR (venous ulcer) OR (venous ulcers) OR (varicose ulcers) OR (venous stasis ulcers) OR (stasis ulcers) OR (diabetic foot) OR (foot ulcer) OR (peripheral arterial disease)) AND ((ozone) OR (ozone gas) OR (ozone therapy)) AND ((healing, wound) OR (healings, wound) OR (wound healings)), as well as different combinations of the following keywords: skin ulcer, leg ulcer, venous ulcers, diabetic foot, foot ulcer, peripheral arterial disease, ozone, ozone gas, ozone therapy, and wound healing.

The databases were searched for studies published up to and including September 2020. The structured search strategy was designed to identify any published document that evaluated the administration of topical ozone as an adjunct treatment in the healing process of chronic lower-limb ulcers. The authors did not contact individual study investigators, nor did they try to identify unpublished data or gray literature.

Study Selection

All electronic search titles, selected abstracts, and full-text articles were independently reviewed by a minimum of two reviewers. Disagreements over whether studies met the inclusion/exclusion criteria were resolved through the involvement of a third reviewer. The following inclusion criteria were applied: clinical controlled trials in humans that evaluated the administration of topical ozone as an adjunct treatment in the healing process of chronic lower-limb ulcers. Animal studies, review articles, meta-analyses, abstracts, conference proceedings, editorials/letters, and case reports were excluded.

Data Extraction

Data were extracted by one reviewer using standardized forms and checked by a second reviewer. The following information was extracted from all studies: study design, study location, number of patients, patient age, type of control, wound type, intervention type, equipment used to generate the ozone (ozone generation), evaluation methodology, and main results.

Risk of Bias Assessment

Risk of bias (RoB) was assessed using the validated Cochrane Risk of Bias Assessment tool for Non-randomized Studies, which evaluates six different domains: (1) selection of participants (selection bias), (2) confounding variables (selection bias), (3) measurement of exposure (performance bias), (4) blinding of outcome assessment (detection bias), (5) incomplete outcome data (attrition bias), and (6) selective reporting (reporting bias). Each domain was evaluated as having a low, unclear, or high RoB.[14]

RESULTS

The initial search identified 44 studies that were evaluated according to the eligibility criteria; 7 of these met the inclusion criteria (Figure 1). The studies were carried out in five countries, with a total of 506 patients 18 years or older with chronic wounds, such as venous or diabetic ulcers, on the lower limbs. The majority addressed diabetic foot ulcers (Tables 1 and 2).

Figure 1. STUDY SELECTION FLOW DIAGRAM

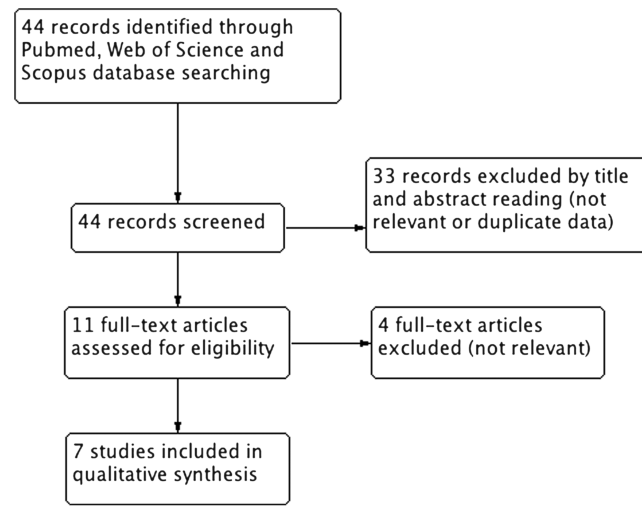


Table 1. CHARACTERISTICS OF INCLUDED STUDIES

Authors, Year, Country	Design, Location, Ulcer Type	Control	Intervention	Ozone Generation	Evaluation Methodology	Main Results
Solovăstru et al,[21] 2015, Ukraine	RCT; not reported; venous ulcer	N = 14; standard epithelial cream containing vitamin A, vitamin E, talc, and zinc oxide	N = 15; local ozone (oil). Ozonized spray formulation and α-bisabolol daily use	Did not use	Patients were evaluated on days 0, 7, 14, and 30. At each visit, wound surface was measured via ruler, and the lesion was covered with protective gauze. Routine care (cleaning and debridement) given in both groups	Patients with complete ulcer healing: OG, 25%; CG, 0%. Changes in ulcer surface area were significant for OG, with a significant mean reduction ($P < .05$) at 7, 14, and 30 d (34%, 59%, and 73%, respectively). The CG reductions in mean wound surface were 5%, 8%, and 13%, respectively
Izadi et al,[15] 2019, Iran	RCT; hospital; DFUs Wagner grades 2 and 3	N = 100; medical and surgical treatments (antibiotics, regular sterile dressing, and debridement) performed for all patients	N = 100; local and systemic ozone twice a week until the wound closure/ epithelialization Location: bag (30 min) Ozonized gel applied to the wounds every 12 h, occluded with sterile gauze. Subcutaneous ozone injection used around the wound Systemic ozone rectal or IV administration (minor and major ozone therapy) after necessary preparations. Patients took vitamin C tablets immediately after each systemic procedure	Not reported	Wounds graded with Wagner criteria and wound surface measured via ruler at the longest and widest width of the wound. Laboratory tests such as complete blood count, ESR, CRP, FBS, prothrombin time, partial thromboplastin time, and creatinine were undertaken. ESR, CRP, and FBS were checked again after the ulcer healed. In addition, the healing time and ozone therapy and amputation surgery sessions were recorded	All wounds healed in the OG; average healing time, 69.44 ± 36.055 d (range, 15–180 d). In the CG after 180 d, 25% of the patients did not heal completely. The average cure time in the OG was less than CG ($P = .012$). More patients had amputations in the CG (57%) than the OG (19.1%; $P < .05$). In the OG, not only did all variables decrease, but also after treatment, the values were lower than in the CG
Rosul and Patskan,[17] 2016, Ukraine	CCT; hospital; DFUs	N = 24; traditional therapy including blood glucose correction (intermittent dosing insulin), antibacterial therapy, antiaggregating agents, anticoagulants, rheologic preparation infusion, and detoxifying agents Local treatment included daily dressing with antiseptics. Cytologic examination of wound secretion was performed	N = 23 Systemic: EV 200 mL of ozonated SF (ozone concentration 1,000 – 1,300 μg/L) Topical: 0.9% ozonated SF and ozonized oil Time: 12–14 d, one session per day Patients also received traditional therapy and cytologic wound examination	Ozone UM-80	The state of lipid peroxidation and the state of antioxidant protection were evaluated. Wound healing progress was determined by type of tissue present in the wound bed: necrosis, granulation, epithelialization, or healed wound	OG saw improvement and a positive evolution of the clinical signs of the main disease and its complications. Use of local and general therapy reduced swelling and hyperemia around the wound by 10.17 ± 0.74 d, accompanied by considerable reduction or disappearance of pain on palpation. Granulation occurred in a similar timeframe (14.46 ± 0.40 d). Granulation and initial epithelialization were observed at 19.83 ± 0.21 d from hospitalization. Duration of hospitalization: CG, 23.42 ± 0.45 d; OG, 17.09 ± 0.27 ($P < .05$)

(continues)

Table 1. CHARACTERISTICS OF INCLUDED STUDIES, CONTINUED

Authors, Year, Country	Design, Location, Ulcer Type	Control	Intervention	Ozone Generation	Evaluation Methodology	Main Results
Zhou et al,[20] 2016, China	RCT; hospital; venous ulcers	N = 42; EVL	N = 50; topic: ozone by bag combined with EVL, 60 mg/L for 1 h, once a day until necrosis and infection in the ulcer area improved and was suitable for puncture	Haslerrail Medical Ozone Therapy System, Germany	Patients scheduled for outpatient evaluation within 1 wk, then monthly until ulcer healing, then at 3-mo intervals. The pressure bandage was removed in the first outpatient assessment to visualize ulcers. All patients monitored for a minimum of 12 mo	No significant difference in venous occlusion between groups. The proportion of ulcer healing was significantly higher in OG (92%) than CG (76.19%) over 12 mo ($P < .05$). The OG showed better satisfaction and a lower recurrence rate (6.25%) than the CG (25%; $P < .05$). No serious complications or adverse effects in either group
Wainstein et al,[16] 2011, Israel	RCT; specialized clinic; DFUs	N = 29; patients received sham treatments (ozone device circulated ambient air) in addition to usual treatment for DFUs; treatment sessions lasted 26 min	Topical: active ozone treatment in two phases: sessions 4×/wk for a maximum of 4 wk, or until 50% of wound area was granulated, whichever comes first. Intervals between treatments not to exceed 1 d, or 5 d/wk; gas concentrations were 96% oxygen and 4% (80 lg/mL) ozone. In second treatment period, frequency reduced to 2×/wk until 12 wk; gas concentration changed to 98% oxygen and 2% (40 lg/mL) ozone	Ozoter 101	Demographic and medical data included age, sex, medical history, laboratory values, and wound assessments. Laboratory tests included complete blood count, ESR, liver function, HbA_{1c}, and urine analysis. Wound surface area was measured by applying a transparent grid to the wound. Infections were assessed clinically and via bacterial cultures collected on the first day of treatment, every fourth treatment, and at week 24	The between-group difference in the proportion of patients with full wound closure was not significant in the ITT group. The wound size and the proportion of patients who had a reduction in wound size did not differ between treatment groups in either the ITT or per protocol. The 16-patient OG had a significantly greater proportion of complete wound closure than controls (81% vs 44%, $P = .03$). When this comparison was repeated in the subgroup of patients with a baseline wound size of 5 cm^2, 100% of OG patients vs 50% of CG exhibited complete wound closure ($P = .006$)
Zhang et al,[18] 2014, China	RCT; hospital; DFU Wagner degrees 2, 3, and 4	CG (n = 25) received standard treatment including debridement every 2 d and wound dressings appropriate for exudate and moisture	Topic: noninvasive oxygen-ozone treatments with 52 μg/mL ozone (total volume: 20–50 mL) in a special bag for 30 min/d for 20 d using ozone generator device in addition to standard treatment	Humazon Promedic	Study visits were performed at baseline, day 11, and day 20. At each visit, ulcer photographs were taken at a distance of 20–30 cm in the same light; wound condition, length, width, depth, healing progression, infection, and the need for debridement were assessed. Ulcer areas calculated from tracings using grid paper	At day 20, wound size in both groups was significantly smaller than at start ($P < .001$ and .022, respectively). In the OG, wound size reduction was significantly more than in CG (6.84 ± 0.62 vs 3.19 ± 0.65 cm^2, $P < .001$). After treatment in the OG, there were more collagen fibers than in the CG (4.48 ± 0.43 vs 3.07 ± 0.23, $P = .012$)

(continues)

Table 1. CHARACTERISTICS OF INCLUDED STUDIES, CONTINUED

Authors, Year, Country	Design, Location, Ulcer Type	Control	Intervention	Ozone Generation	Evaluation Methodology	Main Results
Kadir et al,[19] 2020, Indonesia	CCT; home care; DFUs Wagner grades 2 and 3	N = 13; standard wound care with antimicrobial dressings once every 3 d for 21 d	Topical: dressing plus oxygen-ozone therapy at 70 μg/mL in a special ozone-resistant plastic bag for 10 min using an ozone generator once every 3 d for 21 d	MOG003	Bacterial colonies were examined on days 0 and 21; wound healing was measured using a digital caliper and the DFU Assessment score questionnaire on days 0, 6, 12, and 21	The number of bacterial colonies in OG significantly decreased ($P = .001$). The mean number of bacteria was significant after the intervention ($P = .037$) in the OG. The dominant type of bacteria found in all participants was *Proteus mirabilis* (n = 10, 25.0%). In the OG, the frequency of Gram-negative and -positive bacteria decreased from 20 to 15. In the CG, the frequency of these bacteria did not decrease. For wound healing, OG and CG showed a significant difference in daily DFU Assessment score ($P < .05$)

Abbreviations: CCT, controlled clinical trial; CG, control group; CRP, C-reactive protein; DFU, diabetic foot ulcer; ESR, erythrocyte sedimentation rate; EVL, endovenous laser; FBS, fasting blood glucose; ITT, intention to treat; LL, lower limbs; OG, ozone group; PF, physiologic solution; RCT, randomized clinical trial.

Studies from various parts of the world show that the practice of ozone therapy seems to be effective, safe, and low risk as an adjunct therapy in the treatment of lower-limb ulcers, whether applied topically through water, oil, or gas; subcutaneously (perilesional); systemically (rectally, intravenously); and/or in combination.[6–13]

Regarding diabetic ulcers, the studies indicated that treatment with topical and/or systemic ozone associated with conventional treatment was superior to conventional treatment alone and reduced the chances of infection and amputation.[15,16] Further, ozone therapy had significant positive effects on the wound healing process, promoting an improvement in lipid peroxidation rates and antioxidant protection, thereby reducing treatment time and length of hospitalization (Table 1).[17]

A study by Zhang et al[18] demonstrated that reduction in wound size was significantly greater in the group that received ozone than in the control group ($P < .001$). The authors reported that ozone therapy promoted the healing of diabetic foot wounds through the induction of vascular endothelial growth factor, transforming growth factor β, and platelet-derived growth factor at the beginning of the treatment stage (Table 1).[18]

Regarding ozone application, topical application using the bagging method was the most common method (n = 5).[15,16,18–20] In this method, the gas is applied directly to the lesion using a sealed plastic bag that remains in contact with the wound for a specified amount of time. The second most common method was ozonized oil (n = 3; Table 1).[15,17,21] Ozonized oil is produced by means of industrial generators that add ozone gas to sunflower oil or olive oil; it is an important option in the treatment of wounds because of the stability of the gas in the product, which enhances antimicrobial activity.

Four of the studies used only topical ozone therapy (Table 1). Systemic therapy was used in two studies,[15,17] with one using the rectal method or IV solution (minor and major ozone therapy), and the other using IV ozonized saline.[15] These two studies also used topical treatments: the first used the oil and bagging methods, as well as subcutaneous application around the lesion, and the second used ozonized serum with ozonized oil.

Rectal ozone insufflation is a systemic route, in which the gas is applied through a probe and is quickly dissolved in the luminal contents of the intestine, where mucoproteins and other secretory products with antioxidant activity react readily with ozone to produce reactive oxygen species and lipid peroxidation products. These compounds penetrate the muscular mucosa and enter the circulation of the venous and lymphatic capillaries.[22]

Ozonized saline solution is made with very low concentrations of ozone, calculated based on the patient's weight, and is prepared by bubbling the gas into the

Table 2. SOCIODEMOGRAPHIC CHARACTERISTICS

Authors, Year	Country	N	n, OG	n, CG	Age, y, Mean (Min-Max)
Solovăstru et al,[21] 2015	Ukraine	29	15	14	≥18
Izadi et al,[15] 2019	Iran	200	100	100	18-85
Rosul and Patskan,[17] 2016	Ukraine	47	50	24	60.06 ± 1.28
Zhou et al,[20] 2016	China	92	50	42	≥18
Wainstein et al,[16] 2011	Israel	32			CG, 62.6 ± 9.5 (46–62); OG, 62.6 ± 10.2 (40–81)
Zhang et al,[18] 2014	China	25			CG, 61.12 ± 10.90; OG, 59.72 ± 12.20
Kadir et al,[19] 2020	Indonesia	14			CG, 53.8 ± 7.3; OG, 58.4 ± 6.8

Abbreviations: CG, control group; max, maximum; min, minimum; OG, ozone group.

solution by one of four methods, depending on the equipment available and the preparer's knowledge. Using this method, ozone can be delivered in a low, average, or high proportion of 1, 2 or 5 μg/kg, respectively. The volume of solution used for a procedure is between 200 and 400 mL, and is applied daily or on alternate days. The number of procedures per treatment cycle is 6 to 10.[22]

In regard to concentration, frequency, session time, and duration of ozone therapy in studies that used local applications (bag and/or ozonized oil), ozone concentrations ranged from 40 to 80 mg/L, with a frequency ranging from 24 to 72 hours, with the duration of the procedure varying from 10 to 60 minutes over a period of 12 to 21 days. In studies that examined systemic applications, 24-hour intervals between sessions were used, with doses ranging from 1,000 to 1,300 mg/L for 14 days of treatment or until complete healing of the lesion (Table 1).

No study reported any adverse effects, and the results of the ozone therapy protocols demonstrated some curative effect in all studies included in this review; five reported a significant reduction in the lesion surface area. Two studies[16,19] did not show any significant difference in healing between groups; however, in one of the studies, the results were produced by an intention-to-treat[16] analysis, and the other[19] demonstrated different beneficial effects of ozone treatment. In the first of these two studies, investigators reported that the proportion of individuals with total wound closure did not differ significantly by treatment assignment[16] (41% vs 33%, $P = .34$). However, of the 61 patients who started the study, only 34 individuals completed data collection (16 in the ozone group, 18 in the placebo group), and a significantly higher rate of complete wound closure was observed in the ozone group (81% vs 44%, $P = .03$). In the second study,[19] although the authors reported that ozone did not have a significant effect on wound healing, the combination of wound care and ozone therapy reduced the number of bacteria in ulcers (Table 1).

Thus, despite several biases identified in the studies selected for this review (see the following section), overall the results support the effectiveness of ozone therapy as a complementary treatment for lower-limb ulcers, especially diabetic foot ulcers, and indicate a potential accompanying reduction in rates of hospitalization, infection, amputation, and venous ulcer recurrence.

Risk of Bias

Figures 2 and 3 summarize RoB data from the seven included studies. Most studies adequately randomized, presenting a low RoB, with only two studies presenting a high risk. Domains, such as attrition bias, reporting bias, and other bias, were also considered adequate, with

Figure 2. RISK OF BIAS GRAPH

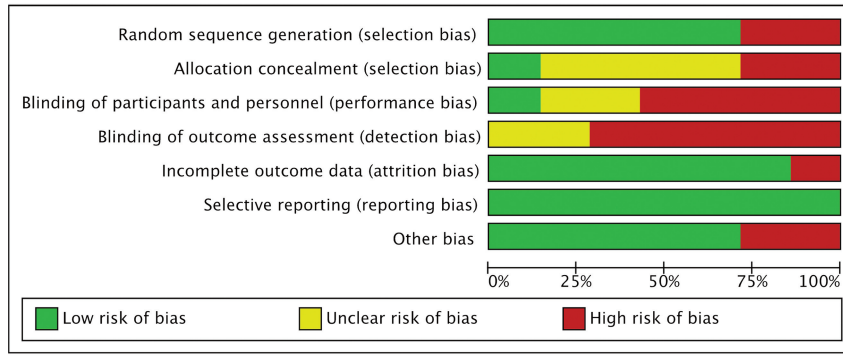
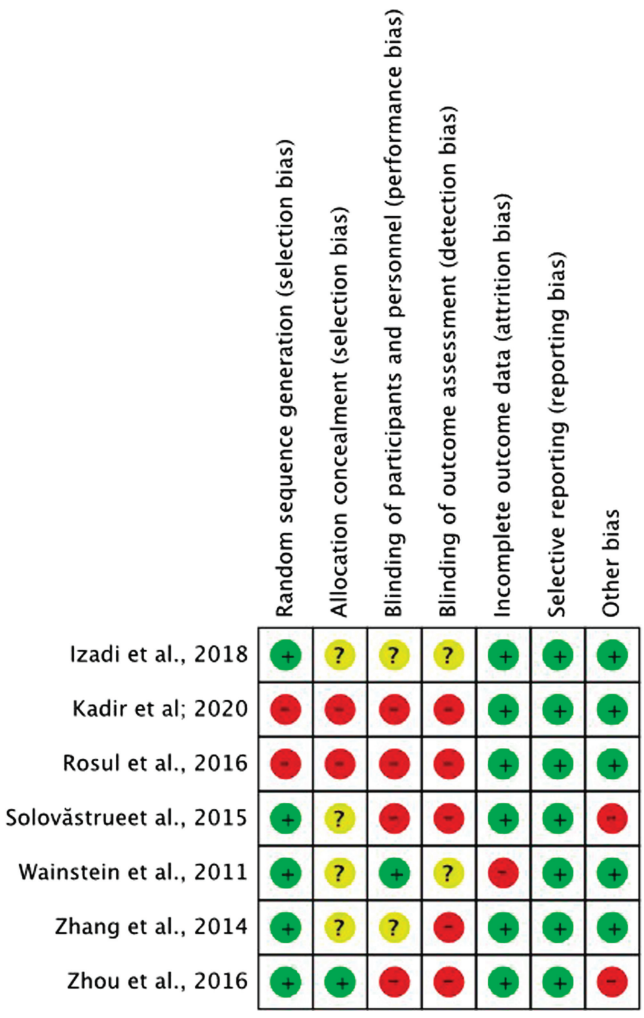


Figure 3. RISK OF BIAS SUMMARY



almost all studies showing low-risk results. However, the selection bias and performance bias criteria based on allocation concealment and blinding of participants/personnel were the most problematic aspects. Most of the included studies were open-label because of the characteristics of ozone in the treatment of wounds (eg, a gas bath applied directly to the lesion, which has a characteristic odor). In addition, in some studies, it is not clear whether data collection was carried out by third parties or by the research authors themselves, making it even more difficult to evaluate whether the tests were double-blind.

DISCUSSION

Ozone is notable for its high oxidizing power, reactivity, and instability, characteristics that are responsible for its therapeutic effects in the body associated with "controlled" oxidative stress when administered in low doses (without any toxicity or adverse effects). Essentially, it causes an antioxidant reaction, known as the "ozone paradox."[22–24] This process must be precisely controlled with a calculated dosage of ozone. Used in this way, ozone is not harmful and is capable of provoking a multitude of useful biologic responses and possibly reversing chronic oxidative stress from aging, chronic infections, and various diseases.[22–24]

When human blood is exposed to the gas mixture (O_2-O_3), both gases dissolve in the plasma water, depending on its solubility, partial pressure, and temperature. Although oxygen readily equilibrates, ozone cannot balance itself because it reacts with biomolecules (antioxidants, proteins, carbohydrates, and polyunsaturated fatty acids) present in the plasma, generating reactive oxygen species, lipid oxidation products, and controlled oxidative stress. These reactions activate antioxidant enzymes, such as superoxide dismutase, catalase, and glutathione.[25]

It is worth noting that oxidative stress plays an important role in the development of diabetes complications, and that ozone therapy has been indicated as an emerging treatment method because it can activate the antioxidant system, which is of potential benefit for the closure of diabetic ulcers. In addition, it can inactivate pathogens, modulate vascular and endogenous growth factors, and activate the immune system. Ozone therapy can also improve blood glucose levels by influencing some markers of damage to endothelial cells in patients with diabetes.[10,23,24]

However, the study identified in this review by Kadir et al[19] found that ozone therapy combined with standard treatment in patients with diabetic foot ulcers did not have a significant effect on the healing process, although the authors did report that the intervention reduced the number of bacteria in this type of injury.

In the study by Solovăstru et al[21] in patients with venous lower limb ulcers who received an innovative topical formulation in the form of an ozonized oil spray combined with α-bisabolol, the proportion with complete ulcer healing was higher than in a control group who were treated with a cream containing vitamin A, vitamin E, and oxide of zinc (control; 25% vs 0%). In addition, the reduction in ulcer surface area was greater in the ozone group. This study showed that the innovative formulation was an effective therapeutic option in the adjuvant treatment of venous ulcers.[21]

Leite Rodrigues et al[26] highlighted the healing power and antibacterial properties of ozonized oils and their usefulness as a complementary treatment in infections, in particular those caused by multidrug-resistant strains of bacteria, because of their high capacity to remove adhered cells and eradicate biofilms within 24 hours.[26]

Regarding the administration of topical gas (bagging) in patients with venous ulcers, the proportion of ulcer healing in the group who received ozone therapy through

an ozone gas bath combined with an IV laser was significantly higher than in the control group (only IV laser) in a study with a follow-up time of at least 12 months. The patients in the ozone group showed better satisfaction and a lower rate of recurrence than those in the control group. The authors also emphasized the feasibility and safety of this treatment.[20]

No controlled clinical trials were found that addressed the topical administration of ozone to wounds from peripheral arterial disease; however, review articles, case reports, and a clinical trial of patients undergoing extracorporeal blood oxygenation and ozonation were found. However, because these types of reports did not meet the exclusion criteria, they were not included in this review. It is worth noting that these studies reported that systemic ozone therapy improves peripheral arterial disease and patient quality of life. They also reported that the treatment promotes healing and proved to be an effective adjuvant therapy in preventing complications of the disease, such as cardiovascular events, amputations, or other extreme surgical solutions. The treatment also stands out for not having any adverse events or effects, and as an economic solution, given that the costs of standard treatment can be reduced by up to 25% with the addition of ozone.[27–29]

Regarding complications, a case study[30] of a patient with a diabetic foot ulcer reported necrosis and severe foot infection after receiving intralesional ozone injections, and the authors of the study concluded that the method was unsafe. However, the patient had already used topical antibiotics for almost 2 weeks with no success in treatment, as well as topical ozone therapy and main autohemotherapy for 7 days, both of which were also unsuccessful. Further, the patient's glycemic control was poor, with hemoglobin A_{1c} of 11% and fasting blood glucose of 299 mg/dL. Accordingly, it is not possible to state conclusively that the poor clinical outcome was caused by the intralesional ozone therapy, and again, ozone therapy was considered safe and low risk in all the articles cited in this review.

It should be noted that although the studies showed the effectiveness of ozone therapy, there was no standardization of the interventions performed, even in studies where the wounds had the same etiology. Further, the concentrations used and the duration and frequency of treatment were quite divergent. In addition, the environments in which the studies were conducted varied widely. Data were obtained in patients' homes, outpatient clinics, hospitals, and specialized ozone treatment centers.

One of the studies that used the bagging technique did not mention the gas generator used. This information is important, because ozone must be produced using medicinal oxygen with a reliable nontoxic generator that allows the measurement of accurate ozone concentrations (1–100 μg/mL) using a photometer frequently controlled by iodometric titration. The total ozone dose is equivalent to the volume of gas (mL) multiplied by the ozone concentration (μg/mL).[23] For different applications, the healthcare practitioner must know the ideal gas doses. Moreover, it is of fundamental importance to use a good generator, equipped with a vacuum and connected to a gas destroyer for the safety of the patient and providers.

Providers working in this area must have sufficient theoretical background and knowledge of the various forms of ozone administration applied in these clinical studies to adapt the method for each patient, considering not only the etiology of the injury, but also the entire patient context (therapeutic indications, contraindications, and clinical condition) and wound assessment (extent, infection, location, tissue type, borders, and healing, among other factors), as well as the social and general factors involved (ability to attend consultations, cognitive deficit, hygiene conditions, malnutrition, etc) and the patient's adherence to treatment in general.

Given this context, institutional protocols based on available evidence should be created to standardize treatments and improve the results achieved in a process as complex as wound healing. Because ozone is an adjuvant treatment, routine practices in the care of patients with lower-limb ulcers (cleaning, debridement, use of antiseptics, etc) should not be neglected, and the underlying disease must be concomitantly targeted alongside wound healing. Further, all treatment must involve good management and an interprofessional team, as well as the full engagement of the patient and their family in the search for the safest, most appropriate, and most effective treatment possible.

CONCLUSIONS

Although the outcomes of the studies were positive, there is still a need for further controlled studies and randomized clinical trials to prove the efficacy of this technology for the healing of lower-limb ulcers and explore in more detail its use as an adjuvant therapy in a safe and effective way. Moreover, clear clinical criteria and standardized protocols for the use of ozone therapy need to be established. Future studies need to detail the protocol that was used for each type of wound (dosage, frequency and duration of administration, and the ozone-generating equipment used) to support and strengthen the performance of the health professionals who wish to use this therapy to assist patients with difficult-to-heal ulcers on the lower extremities. ●

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