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A systematic review of ozone therapy for treating chronically refractory wounds and ulcers

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Abstract

This study aims at evaluating the efficacy and safety of ozone therapy for chronic wounds. The Cochrane Library, PubMed, Ovid Embase, Web of Science, and Chinese Biomedical Literature Database were searched. Randomised controlled trials (RCTs) about participants with chronic wounds were included. Risk of bias assessment was performed by the Cochrane risk-of-bias tool. A randomised-effects model was applied to pool results according to the types of wounds or ulcers. Among 12 included studies, ozone was implemented by topical application (ozone gas bath, ozonated oil, ozone water flushing) and systematic applications including autologous blood immunomodulation and rectal insufflation. The results indicated compared with standard control therapy for diabetic foot ulcers, ozone therapy regardless of monotherapy or combined control treatment markedly accelerated the improvement of the wound area(standardised mean difference(SMD) = 66.54%, 95% confidence interval (CI) = [46.18,86.90], $P < .00001$) and reduced the amputation rate (risk ration (RR) = 0.36, 95% CI = [0.24,0.54], $P < .00001$). But there is no improvement in the proportion of participants with completely healed wounds and length of hospital stay. No adverse events associated with ozone treatment have been reported. And the efficacy of ozone therapy for other wound types is still uncertain because of no sufficient studies. More high-quality randomised controlled trials are needed to confirm the efficacy and safety of ozone therapy for chronic wounds or ulcers.

KEYWORDS

chronic ulcers, chronic wounds, meta-analysis, ozone therapy, systematic review

Abbreviations: CI, confidence interval; CLI, critical limb ischemia; EVLT, endovenous laser therapy; IMT, autologous blood immunomodulation therapy; OEVLT, ozone gas bath and endovenous laser therapy; RCTs, randomised controlled trials; RR, risk ratio; SMD, standardised mean difference; the UK, the United Kingdom.

Key Messages

- many pathologies caused chronically refractory wounds where the results of the standard of treatment are not optimal
- ozone regardless of monotherapy or combined control treatment markedly accelerated the improvement of the wound area and reduced the amputation rate compared with standard control therapy for diabetic foot ulcers by meta-analysis with Revman 5.4
- the narrative analysis suggested ozone therapy significantly improved the wound area for chronic venous leg ulcers and digital ulcers in systemic sclerosis, whereas the proportion of participants with completely healed wounds is no significantly high
- all the general quality of the above evidence was not high
- other pre-specified outcomes are unclear on account of limited information and studies

1 | INTRODUCTION

Chronic wounds, often known as manifested any breach in the cutaneous continuity, need the length of time to heal more than 3 months, even does not heal and palindromia.[1,2] Wound healing generally conforms to an orderly and timely reparative process following inflammation, angiogenesis, matrix deposition, wound contraction, epithelialisation, and cicatrices generation with an appropriate healing time based on various detriments.[3,4] While chronically refractory wounds characterised by the interruption of typical progression to healing and delayed rehabilitation are incurred by fibrotic tissue, dead necrotic slough, and multiple infections,[4,5] many causative pathologies of chronic wounds include vascular insufficiency, rheumatoid arthritis, diabetes, tumours, chronic osteomyelitis, trauma, burns, hematologic diseases, vasculitis, infection, pressure, or oedema. The aged people and communities with multiple diseases tend to be vulnerable to suffer from these non-healing wounds. Only leg ulcers have been reported to impact about 0.45% to 3.33% of the population worldwide, and medical resource cost has been exceeded GBP (Great Britain Pound) 1 billion years in the UK.[6-8]

Standard treatment strategies include the management of the underlying pathology, local treatments for improving the wound environment such as debridement, dressing, and systematic treatment (eg, application of antibiotics, nutrition supplements),[9] and many others. However, such conservative methods were known to result in high rates of ulcer recurrence and amputations. Ozone, a gas composed of three atoms of oxygen with a cyclic structure, was initially discovered as an oxidant and a disinfectant in 1834, exerting medical effectivity firstly for gunshot gangrene.[10] Evidence supports ozone has been used for the treatment of cutaneous wounds with satisfactory healing results.[11] Ozone was mainly used for ozonised olive or sunflower oil, the mixture of ozone and oxygen mediated by compresses, tent, bag, even injection, and systematic applications referring to rectal insufflation (conveyed into the final portion of the gut/intestines) as well as autohemotherapy (blood withdrawn from the body is mixed with a combination of oxygen and ozone and then reinfused into the donor).[12,13]

At present, the therapeutic mechanism of ozone is possibly associated with the regulation of endogenous growth factors, antioxidant capacity, hemorheology modulations, pathogen inactivation, but with no precision mechanism yet.[14-19] Alarms have sounded for ozone's toxicity to the respiratory passage, skin irritation including dermatitis, and burning sensation during treatment.[20] However, the effectiveness and safety of ozone are equivocal now because the convergence of findings from randomised controlled trials (RCTs) of various chronic wounds is deficient. Some defects exist in only two systematic evaluations now.[13,21] In this research, we found ozone treatment significantly improves the wound area and lowers the amputation rate for diabetic foot ulcers. As to other wound types, no meta-analysis can be conducted because of the lack of studies for other wound types. All quality of evidence is not high. In addition, there is insufficient evidence to judge the net efficacy and safety of ozone. This study will provide an up-to-date summary of evidence and guidance for clinical applications and research studies.

2 | MATERIALS AND METHODS

2.1 | Database and search strategies

This systematic review and meta-analysis has been registered in INPLASY (https://inplasy.com/) – registration

number is INPLASY202040148) – and was conducted according to a previously published protocol.[22] And the results were reported following the PRISMA statement. Five common databases – the Cochrane Library, PubMed, Ovid Embase, Web of Science, Chinese Biomedical Literature Database – were searched from its inception to May 2020. Google Scholar and Baidu Scholar were also searched to find missing research. The Chinese Clinical Registry and references of review and meta-analysis articles were searched to find out more research. In addition, we contacted the study authors for more information. A search strategy that combined MeSH terms and free text words was used to capture as many trials as possible. The search MeSH terms were as follows: 'Chronic Disease & Wound Healing', 'Skin Ulcer', 'Diabetic Foot', 'Leg Ulcer', 'Foot Ulcer', 'Pressure Ulcer', 'Burns', 'Wound Infection', 'Wounds, Penetrating', 'ozone'. For detailed retrieval strategies, see Supplementary Information S1. Two authors (DL and XW) searched and screened all the citations independently.

2.2 | Inclusion criteria

Study design: Only randomised controlled trials were included in our research, irrespective of publication status or language.

Participants: Included human participants were of any age with refractory wounds, including war wounds, burns, non-healing diabetic foot ulcers, venous, or arterial ulcers and cutaneous ulcers of any aetiology whether clinically infected or uninfected in any care setting.

Interventions: The primary intervention will be any formulation of ozone topically or systematically applied by any means, alone or in combination with other dressings or components. There was no restriction on the use of the controls in this study.

2.3 | Exclusion criteria

Study design: In addition to RCTs, other types of studies were excluded, such as cluster-randomised clinical trials, quasi-randomised studies, cohort studies, case–control studies, animal research or studies in the atmosphere, and meteorology.

Participants: People with ulcers or wounds in dentistry, palatal epithelial fields, jaw, lung, and disc are excluded.

Interventions: Studies in which multiple interventions were used at the same time, or ozone was not the main intervention were excluded. In addition to the use of ozone treatment, people with chronic wounds often combine with other basic treatments, such as conventional debridement and dressing or hypoglycaemic agents for diabetic foot ulcers. If this addressing was used in both the experimental and control groups, the study could be included. If the use of ozone is not balanced between the experiment and control groups, the study would be excluded.

Other reasons: Conference articles with no data to extract were excluded. For articles published repeatedly, the one with more data was chosen.

2.4 | Study selection and data extraction

Two researchers read the title, abstract, and full text, and selected the eligible literature based on the inclusion and exclusion criteria independently. A data extraction Excel form template was created to extract the following information: first authors and publication time, country, wound type, wound site, enrolment date, end date, random sequence, funding, interventions in experimental and control groups, time of treatment, baseline data including age, sex, number of patients and ulcers, duration of wound and diabetes, baseline wound area of both groups, outcomes including follow-up time, participants and ulcers with complete healing of both arms, participants and ulcers with complete not-healing, treatment efficient ratio of both groups, post-intervention wound area and time to achieve complete ulcer healing in both groups, reduction ratio in the wound area, side effects, recurrence, amputation, length of hospital stay, quality of life, cost, and number of participants discontinued the study. If articles lacked enough data, we contacted the corresponding authors for more details by email. We transformed all standard error of mean (SEM) into standard deviation (SD). The data extraction was carried out independently by two researchers, and a final decision was made through discussion when discrepancies exist.

2.5 | Risk of bias assessment

Two review authors used the Cochrane Collaboration tool for assessing the risk of bias on the following specific domains: random sequence generation (selection bias), allocation concealment (selection bias), blinding of participants and personnel (performance bias), blinding of outcome assessors (detection bias), incomplete outcome data (attrition bias), selective outcome reporting (reporting bias), and other biases. The risk of the bias table of each item was classified as 'low risk', 'unclear risk', and 'high risk'. Any disagreements were solved by the discussion of all reviewers.

2.6 | Data analysis

Review Manager version 5.4 was used to create a forest plot and conduct subgroup analysis. Sensitivity analysis was administrated by Stata 14.0 software. The dichotomous variable was represented with RR and 95% CI, and continuous outcomes were represented with SMD, a statistic that can standardise the results to a uniform scale, and 95% CI. We applied a random-effects model provided $I^2 > 50\%$, $P < .05$ considered being indicative of substantial heterogeneity.[23] If $I^2 < 50\%$, $P > .05$, which represents negligible heterogeneity with a fixed-effects model. No meta-regression was conducted for further analysis. Sensitivity analysis was conducted by excluding the study one by one and compared with the total effects. Publication bias did not conduct because of insufficient studies.

TABLE 1 Subgroup analysis for outcomes

	Number of comparisons	Results	*P* value for overall effect	I^2	*P* value for subgroup difference
The proportion of participants with complete healing		RR[95%CI]			
All comparisons	3	1.13[0.91,1.41]	.28	0%	
Different ozone applications					.58
Only local administration	2	1.32[0.74,2.35]	.35	0%	
combination of local and systematic administration	1	1.10[0.87,1.40]	.43	NA	
Different control treatments					.58
Topical and systemic antibiotics	1	1.10[0.87,1.40]	.43	NA	
Conventional standard treatment	2	1.32[0.74,2.35]	.35	0%	
Course of treatment					.90
≤20 d	2	1.13[0.89,1.42]	.33	0%	
>20 d	1	1.18[0.61,2.26]	.62	NA	
Duration of diabetes					.37
≥15 y	2	1.11[0.89,1.39]	.36	0%	
<15 y	1	2.00[0.56,7.12]	.28	NA	
Change in wound size(%)		SMD95%CI			
All comparisons	3	66.54[46.18.86.90]	.00001	91%	
Different ozone application					.91
Only local administration	1	65.07[55.86,74.28]	<.00001	NA	
Combination of local and systematic administration	2	67.43[29.22105.64]	.0005	95%	
Different follow-up time					.03
No follow-up time	2	75.65 [54.16, 97.15]	<.00001	87%	
14 wk follow-up time	1	48.03 [36.93, 59.13]	<.00001	NA	
Different baseline wound size					.91
≥50 cm2(mean wound area)	2	67.43[29.22105.64]	.0005	95%	
<50 cm2(mean wound area)	1	65.07 [55.86, 74.28]	<.00001	NA	
Different duration of diabetes					.91
≤20 y (mean duration of diabetes)	2	67.43[29.22105.64]	.0005	95%	
>20 y (mean duration of diabetes)	1	65.07 [55.86, 74.28]	<.00001	NA	

Abbreviation: NA: not available.

TABLE 2 Summary of findings' tables

Wound type	Certainty assessment								
	No. of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other consideration	Certainty	Importance
Ozonated oil and α-bisabolol for chronic venous leg ulcers	Proportion of participants with completely healed wounds								
	1	Randomised trials	Serious [a]	Not serious	Serious [b]	Serious [c]	None	⊕◯◯◯ VERY LOW	IMPORTANT
	The reduction in the mean surface of the wound								
	1	Randomised trials	Serious [a]	Not serious	Serious [b]	Serious [c]	None	⊕◯◯◯ VERY LOW	IMPORTANT
Endovenous laser therapy +local ozone gas bath for lower limb venous ulcers	Proportion of participants with completely healed wounds								
	1	Randomised trials	Serious [d]	Not serious	Not serious	Serious [e]	None	⊕⊕◯◯ LOW	IMPORTANT
	Adverse events								
	1	Randomised trials	Serious [d]	Not serious	Not serious	Serious [f]	None	⊕⊕◯◯ LOW	IMPORTANT
Oxygen-ozone gas bath for DUs in systemic sclerosis (SSc)	Proportion of participants with completely healed wounds								
	1	Randomised trials	Serious [a]	Not serious	Not serious	Serious [g]	None	⊕⊕◯◯ LOW	IMPORTANT
	Change in wound size								
	1	Randomised trials	Serious [a]	Not serious	Not serious	Serious [g]	None	⊕⊕◯◯ LOW	IMPORTANT
Ozone-based autologous blood immunomodulation therapy (IMT) for ischemic ulcer of critical limb ischemia (CLI)	Adverse event								
	1	Randomised trials	Serious [a]	Not serious	Serious [h]	Not serious	None	⊕⊕◯◯ LOW	IMPORTANT
	Amputations								
	1	Randomised trials	Serious [a]	Not serious	Not serious	Very serious [c]	None	⊕◯◯◯ VERY LOW	IMPORTANT
	Change in wound size								
	1	Randomised trials	Serious [a]	Not serious	Not serious	Very serious [c]	None	⊕◯◯◯ VERY LOW	IMPORTANT
Ozone therapy compared with control therapy for diabetic foot ulcers	Proportion of participants with completely healed wounds								
	3		Serious [a]	Not serious	Not serious	Not serious	None	⊕⊕⊕◯	IMPORTANT

(Continues)

TABLE 2 (Continued)

Wound type	Certainty assessment								
	No. of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other consideration	Certainty	Importance
		Randomised trials						MODERATE	
	Time to achieve complete ulcer healing								
	1	Randomised trials	Serious [i]	Not serious	Not serious	Serious [j]	None	⊕⊕◯◯ LOW	IMPORTANT
	Change in wound size								
	3	Randomised trials	Serious [a]	Serious [k]	Not serious	Serious [l]	None	⊕◯◯◯ VERY LOW	IMPORTANT
	Amputations								
	4	Randomised trials	Serious [a]	Not serious	Not serious	Not serious	None	⊕⊕⊕◯ MODERATE	IMPORTANT
	Length of hospital stay								
	2	Randomised trials	Serious [a]	Serious [k]	Not serious	Not serious	None	⊕⊕◯◯ LOW	IMPORTANT

[a]The risk of bias assessment is mostly 'unclear risk' because there are no enough details in articles.
[b]Because this study used the combination of ozonated oil and α-bisabolol rather than ozone oil only compared with control cream.
[c]No confidence interval available and no statistic method because there were no specific data and statistic test.
[d]Blinding of personnel and performance bias exists.
[e]The sample size is too small and only one study.
[f]No specific incidence rate of adverse events in two groups and no statistic test.
[g]The 95% confidence interval is too wide.
[h]There are no enough details about adverse events directly related to IMT therapy.
[i]The risk of bias assessment is mostly 'high risk' because there are no enough details in this study.
[j]No specific time data of the control group can be compared.
[k]There is significant heterogeneity between studies.
[l]The standard deviation is borrowed from other studies in the same pooled group.

2.7 | Subgroup analysis

Subgroup analysis was conducted according to these predefined variables: duration of follow-up and different control treatments; In addition, the following post-hoc subgroup analysis was conducted: duration of diabetes, course of treatment, different ozone applications, and baseline wound size (Table 1).

2.8 | Certainty assessment of the evidence

GRADEpro, produced by the GRADE Working Group, is a widely used online evidence evaluation system. It classified the quality of evidence as four grades: 'high', 'moderate', 'low', and 'very low' with seven sub-domains in certainty assessment: no. of studies, study designs, risk of bias, inconsistency, indirectness, imprecision, and other considerations. We can evaluate the quality of the evidence for this outcome and showed the judgement results in Table 2.

3 | RESULTS

3.1 | Search results

A total of 138 related citations were initially retrieved. Twelve RCTs contributed to this systematic review.[11,15,24-33] A diagram of the selection of studies is shown in Figure 1. A list of records excluded by reading the full text is shown in Table 3.

3.2 | Study characteristic

Among all the included 12 articles with 1055 participants, the publishing dates ranged from 2005 to 2020. Involved wound types include second- or third-degree actinic ulcers following a radiotherapy cycle,[25] chronic venous leg ulcers,[28,33] digital ulcers (DUs) in systemic sclerosis (SSc),[32] critical limb ischemia (CLI),[24] and diabetic foot ulcers.[11,15,26,27,29-31] The proportion of participants with completely healed wounds were reported in seven trials.[11,15,26,28,29,32,33] Nine trials[11,15,24,27,29-33] reported a change in the wound size during treatment, but three of which only figures are available and no numerical variation.[24,31,33] One trial[29] directly reported the total area closed after treatment. In three studies,[25,26,31] the intervention period was until the complete healing of wounds, 20 days in four studies,[11,15,27,32] 30 days in one study,[33] 3 weeks in one study,[30] 12 weeks in one study,[29] and at least 22 weeks or until study completion in one trial.[24] One study for lower-limb venous ulcers was conducted until wounds suitable for skin puncture.[28] The follow-up time varied between trials. Eight trials reported outcomes immediately following the course of therapy.[11,15,24-26,30,32,33] Two followed patients for 1 year after therapy.[28,31] One study followed 14 weeks or until they met the treatment outcome,[27] and one gave results at week 24.[29]

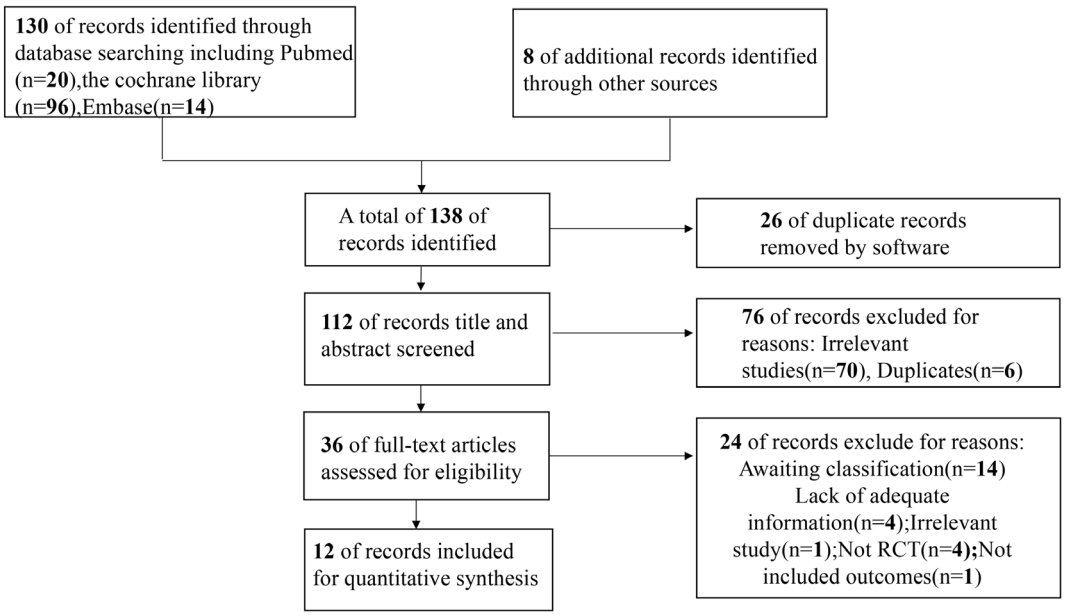

FIGURE 1 Diagram of the selection of studies

TABLE 3 A list of excluded studies by reading the full text

Awaiting classification (no full text)	Rokitansky O et al, Clinic and biochemistry of ozone therapy [in German].Hospitals 1982;52:643–647. Romero Valdes A et al, Ozone therapy in the advanced stages of arteriosclerosis obliterans. Angiologia 1993;45:146–148. Rovira Duplaa G et al, Ozone therapy in the treatment of chronic ulcers of the lower extremities. Angiologia 1991;2:47–50. Verrazzo G et al, Hyperbaric oxygen, oxygen-ozone therapy, and rheologic parameters of blood in patients with peripheral occlusive arterial disease, Undersea Hyperbar Med 1995;22:17–22. Bocci V. Oxygen-Ozone Therapy: A Critical Evaluation. Dordrecht, Netherlands: Kluwer Academic Publisher, 2002. Bocci V. Ozone: A New Medical Drug. Dordrecht, Netherlands:Springer, 2005 Matassi R,et al. Ozonetherapy in chronic limb ischemia. Il Giornale di Chiruga 1987;8:108–111. Wolff HH. Method for ozonated autohemotherapy in peripheral vascularities [in German]. Erfahr Hk 1974;23:181–184. NO experimental outcomes are reported in Cochrane library: NCT03742466:Local Injection of Ozone Vs Methylprednisolone Acetate in Carpal Tunnel Syndrome of Scleroderma Patients. IRCT20181105041563N3:Comparative study on the effect of ozone therapy, honey dressing and combination of ozone and honey dressing on healing of diabetic foot ulcer. IRCT20130317012830N32:Comparison of ozone therapy and honey therapy on diabetic foot ulcers. NCT02448511: Local Application of Ozone Gas for Infected Ulcers. IRCT2014012511898N: Therapeutic effect of ozone therapy on open fracture. NCT01643967: Clinical Trial to Evaluate the Efficacy and Safety of the Use of Ozone Vs Sunflower Oil in Treating Diabetic Foot.
Lack of adequate information	Di Paolo N, Bocci V, Salvo DP, et al. Extracorporeal blood oxygenation and ozonation (EBOO): a controlled trial in patients with peripheral artery disease. *The International journal of artificial organs* 2005; 28(10): 1039–50. Filippi A. The effects of ozonized water on epithelial wound healing. *Deutsche zahnarztliche zeitschrift* 2001; 56(2): 104–8. Quelard B, Cordier ME, Regent MC, Tenette M. Comparative study to determine the relative efficiency of two types of treatment of decubitus ulcers of sacro and ischial tuberosities: topical ozone treatment vs the traditional methods. *Annales medicales de nancy ET de l'est* 1985; 24(OCT.): 329–34. Zagirov UZ, Isaev UM, Salikhov MA. [Clinicopathologic basis of ozonomagnetophoresis in treatment of festering wounds]. *Khirurgiia (Mosk)* 2008; (12): 24–6.
Irrelevant study	Falanga V, Saap LJ, Ozonoff A. Wound bed score and its correlation with healing of chronic wounds. *Dermatol Ther* 2006; 19(6): 383–90.
NOT RCT	Kadir K, Syam Y, Yusuf S, Zainuddin M. Ozone Therapy on Reduction of Bacterial Colonies and Acceleration of Diabetic Foot Ulcer Healing. *Home healthcare now* 2020; 38(4): 215–20. Liu J, Zhang P, Tian J, et al. Ozone therapy for treating foot ulcers in people with diabetes. *The Cochrane database of systematic reviews* 2015; (10): Cd008474. Campanati A, De Blasio S, Giuliano A, et al. Topical ozonated oil vs hyaluronic gel for the treatment of partial- to full-thickness second-degree burns: A prospective, comparative, single-blind, non-randomised, controlled clinical trial. *Burns* 2013; 39(6): 1178–83. Turcić J, Hancević J, Antoljak T, Zic R, Alfirević I. Effects of ozone on how well split-thickness skin grafts according to Thiersch take in war wounds. Results of prospective study. *Langenbecks Arch Chir* 1995; 380(3): 144–8.
Not included outcomes	Karatieieva S, Plesh I, Yurkiv O, Semenenko S, Kozlovskaya I. NEW METHOD OF TREATMENT OF PYOINFLAMMATORY SOFT TISSUE COMPLICATIONS IN PATIENTS WITH DIABETES MELLITUS. *Georgian medical news* 2017; (264): 58–60.

The local ozone gas bath (either with or without other ozone administration) was applied in eight trials[11,15,26-30,32]: four of which used ozone only in the bag with a concentration between 35 and 60 mg/L for 30 ~ 60 minutes per day[11,27,28,30] and one of which the concentration is not found although wounds remain in special bag consisting of only ozone gas for 30 minutes,[26] while the rest used locally oxygen-ozone combination with 40 ~ 80ug/ml for between 26 and 30 minutes and gave once a day or four times each week, then gradually decreasing to twice a week.[15,29,32] The additional four trials were applied locally (bagging and ozonised oil

TABLE 4 Characteristics of included studies

First author and year	Country	Wound type	Wound site	Enrolment date	End date	Random sequence	Measurement tool	Funding		
Francesco Inchingolo, 2015	Italy	Second- or third-degree actinic ulcers following a radiotherapy cycle	NR	NR	NR	NR	NR	NR		
Laura Gheuca˘ Solova˘stru, 2015	Romania	Chronic venous Leg ulcers	Leg	NR	NR	Randomly divided	A centimetre ruler	NR		
Yi-Ting Zhou, 2016	China	Lower limb venous ulcers	Lower limb	2006.04	2012.07	Computer-generated random numbers and numbered envelopes	computerised planimetry	NR		
Hassanien M, 2018	Egypt	Digital ulcers (DUs) in systemic sclerosis (SSc)	Digital	NR	NR	Assigned by computer-based selection as ratio of 1:1	Modified Rodnan skin score (MRSS)	NR		
Raffaele Marfella, 2009	Italy	Critical limb ischemia (CLI)	Foot	NR	NR	Computer-generated code lists	EZ graph	NR		
Enas Mohamed Ali, 2013	Egypt	DFU	Foot	2012.01	2012.04	Randomised	Polythene sheet placed with a marker	NR		
Qin Xinyuan, 2020	China	DFU	Foot	2018.09	2019.09	Random number table	Medical area measuring camera	The Capital Clinical Characteristic Application Research and Achievement Promotion. NO. Z171100001017070		
Xiaoxiao Hu, 2019	China	DFU	Foot	2016.04	2017.08	Randomised	A digital camera and analysed by ImageJ software	The project of Lnc-MALAT1 which regulates the homing and biological function mechanism of endothelial progenitor cells in diabetic vascular disease (No.81671793) and the Fundamental Research Funds for the Central Universities (No.22120170092.		
Jing Zhang, 2014	China	DFU	Foot	2012.03	2013.01	Randomised	film transparency tracings using grid paper	the 2010 special technological development of Guangdong industries, no. 2060403.		
Julio Wainstein, 2011	Israel	DFU	Foot	NR	NR	Randomised	A transparent grid onto the wound	NR		
Morteza Izadi, 2019	Iran	DFU	Foot	NR	NR	Randomised	A ruler	NR		
Gregorio Martínez-Sánchez,2005	Cuba	DFU	Lower extremities	NR	NR	Randomised	A computer program (DIGIPAT).	NR		
Duration of diabetes (treatment group/ control group)	*Duration of wound (treated group/ control group)*	*Ages (treated group/control group) years*	*Intervention of treatment*	*Intervention of control*	*Man of treatment group N (%)*	*Woman of treatment group N (%)*	*Man of control group N (%)*	*Woman of control group N (%)*	*Number of patients (treatment/ control)*	
/	5/7 (days)	62–65/62–65	A mixture called ozolipoile.	Hyaluronic acid gel	NR	NR	NR	NR	NR (both groups are 13 participants)	
/	13/16(months)	58/59	Ozonated oil +α-Bisabolol spray	Standard epithelialisation cream	9 (60%)	6 (40%)	10 (71%)	4 (29%)	15/14	
/	At least 2 mo	61.1 ± 11.2/60.2 ± 9.7	Endovenous laser therapy (EVLT) + local ozone gas bath	Endovenous laser therapy (EVLT) + sham	29(58%)	21(42%)	20(47%)	22(53%)	50/42	
/	NR	38.83 ± 12.32/ 44.08 ± 10.42	Calcium channel blockers +oxygen-ozone gas bath	Calcium channel blockers	0	25(100%)	0	25(100%)	25/25	

(Continues)

TABLE 4 (Continued)

First author and year	Country	Wound type	Wound site	Enrolment date	End date	Random sequence	Measurement tool	Funding	
/	NR	70 ± 5/69 ± 7	Ozone-based autologous blood immunomodulation therapy (IMT)	Sham therapy	54(70%)	23(30%)	51(70%)	23(31%)	77/74
mean duration 11.3 y (range 2–20 y.)	NR	48–69(all participants)	Rectal insufflation+ ozone gas bath+ ozonised olive oil	Antifungal group: fluconazole; control group: standard care only	NR	NR	NR	NR	20/20/20
23.8 y (range 9–40 y.)/22.9 y (range 11–39 y)	0.95 y (1 mo–3 y) ; 0.98 y (1 mo–2 y)	67.1(39–78)/65.5(40–75)	Cleaning the wound with local normal saline+ozone gas bath	Cleaning the wound with local normal saline+sham therapy	27(54%)	23(46%)	25(50%)	25(50%)	50/50
13.3 ± 6.9 y/13.5 ± 6.1 y	91.5 ± 25.3 d/ 94.5 ± 21.5 d	53.5 ± 8.6/56.4 ± 10.4	Ozone water flush +VAC	0.9% saline flush +VAC	37(54%)	31(45%)	35(51%)	33(48%)	68/68
8.64 ± 5.35 y/10.24 ± 5.47 y	45.04 ± 8.6 d/46.6 ± 10.79 d	61.12 ± 10.90/59.72 ± 12.20	Standard treatment + ozone gas bath	standard treatment	12(48%)	13(52%)	14(56%)	11(44%)	25/25
15.2 ± 9.7 y (1-41 y)/16.4 ± 11.0 y (2-45 y)	At least 8 wk	62.6 ± 10.2 /62.6 ± 9.5	Ozone gas bath + usual diabetic foot ulcer care	Sham treatments + usual diabetic foot ulcer care	19 (59%)	13(41%)	19 (66%)	10(34%)	32/29
NR	NR	59.03 ± 12.593/53.5 ± 10.212	Appropriate medical and surgical treatments + local ozone gas bath+ozonised olive oil +ozonated gel (ozolive) + injection of ozone-oxygen + systemic ozone through rectal or intravenous administration	Appropriate medical and surgical treatments	50(50%)	50(50%)	50(50%)	50(50%)	100/100
17 ± 11/18 ± 8 y	NR	20–40(5);40–60(17); ≥60 (30)/20–40(7);40–60(20); ≥60(22)	Rectal insufflation+ ozone bag +ozonised sunflower oil	Topical and systemic antibiotics	26(50%)	26(50%)	30(61%)	19(38%)	51/49
Number of ulcers(treatment/ control)	*Time of treatment*	*Followed up time*	*Baseline wound area of treatment/control (X ± SD)*	*Participants with complete healing of treated /control group and ratio*	*Participants with complete not-healing and ratio of treated/ control group*	*Ulcer number with complete healing of treated/control group*	*Ulcer number and ratio with complete not-healing of treated /control group*	*Treatment efficient ratio of treated /control group*	*Wound area after treatment in the treated/control group (X ± SDcm^2)*
NR	NR (until complete healing)	NR	3.8 ± −/4.1 ± −cm2 (standard deviations NR)	NR	NR	NR	NR	NR	NR
20/17	30 d	NR	4.36 ± 5.61/4.59 ± 3.46 cm^2	5(25%)/none (0)	NR	5/none	NR	NR	1.85 cm2 ± −/ NR
50/42	NR (until the ulcer area was improved and suitable for skin puncture.)	12 mo	5.3 ± 1.25/5.2 ± 1.34 cm2	46(92%) /32(76.19%)	4(8%)/10 (23.81%)	46/32	4/10	92%/76.19%	NR
25/25	20 d	NR	3.61 ± 0.8/4.18 ± 0.38 mm	7(28%)/3(12%)	1(4%)/14(56%)	7/3	1(4%)/14(56%)	96%/44%	0.75 ± 0.3/2.44 ± 0.80 mm
53/49	At least 22 wk or until study completion.	NR	4.6 ± 2.4/4.4 ± 2.1 cm2	32(41%)/NR	NR	NR	NR	NR	NR
20/20/20	20 d	14 wk	140 ± 2.8/142 ± 3(antifungal group)/133.5 ± 2.5(control group) cm^2	NR	NR	NR	NR	NR	9.5 ± 0.1/88.5 ± 1.5 (antifungal group)/98.7 ± 1.0 cm2(control group)

TABLE 4 (Continued)

First author and year	Country	Wound type	Wound site	Enrolment date	End date	Random sequence	Measurement tool	Funding	
50/50	3 wk	NR	25.85 ± 8.77/23.29 ± 7.91 cm^2	NR	NR	NR	NR	NR	4.65 ± 1.93/21.47 ± 8.14 cm^2
68/68	NR (until ulcer closure.)	1 y	37.5 ± 21.6/39.3 ± 22.8 cm^2	NR	NR	NR	NR	NR	NR
25/25	20 d	NR	11.74 ± 0.72/10.82 ± 0.93 cm^2	6(24%)/3(12%)	2(8%)/9(36%)	6/3	2(8%)/9(36%)	23(92%)/16(64%)	NR
32/29	12 wk	12 wk	4.9 ± 4.4/3.5 ± 3.8 cm^2	13(41%)/10(33%)	NR	13/10	NR	NR	NR
100/100	Until wound closure and epithelialisation (about 180 d)	NR	13.41 ± 14.092/ 12.72 ± 0.911 cm^2	NR/75(75%)	NR/25(25%)	NR/75	NR/25(25%)	NR	NR
51/49	20 d	NR	57.97 ± 0.52/54.84 ± 0.39 cm^2	39(78%)/34(69%)	12(24%)/15 (30%)	39/34	12(24%)/15(30%)	39(78%)/34(69%)	23.31 ± 0.36/40.72 ± 0.35 cm^2
Total wound closure area of the two groups (treatment/ control)	*Percentage reduction of wound area in treatment /control group*	*Time to achieve complete ulcer healing (treated/ control group) (day)*	*Adverse events*	*Recurrence participants and ratio of treatment/control group*	*Amputation participants and ratio of treatment /control group*	*Length of hospital stay of treatment/control group*	*Life quality*	*Cost expenditure*	*Suspending the research*
NR	NR	NR	No side effect	NR	NR	NR	NR	NR	NR
NR	73%/13%	NR	No side effects	NR	NR	NR	NR	NR	NR
NR	NR	NR	pain, laser-induced burn, paresthesia	3(6.52%)/ 8(25.00%)	NR	NR	NR	NR	NR
NR	NR	NR	No side effect	NR	NR	NR	NR	NR	NR
NR	NR	NR	53.2%/52.7% (treatment /control)	NR	NR	NR	NR	NR	NR
NR	93.27%/37.6% (antifungal group)/26.06% (control group)	NR	3 (control group) and 7 (antifungal group) patients anincrease in wound surface area	NR	NR	NR	NR	NR	NR
NR	93.22 ± 1.86%/3.28 ± 0.55%	NR	No side effects	NR	NR	NR	NR	NR	NR
NR	NR	12.6 ± 4.2 /25.8 ± 4.3	NR	6(8.8%)/8(11.8%)	3(4.4%)/4(5.9%)	12.6 ± 4.2 d/25.8 ± 4.3 d	NR	NR	NR
6.84 ± 0.62/3.19 ± 0.65 cm2	NR	NR	NR	NR	NR	NR	NR	NR	NR
/−2.0 ± 3.9/−1.6 ± 1.7 cm2	NR	NR	Amputation and infection (control group) vs osteomyelitis, fever, wound infection, and pulmonary congestion (treatment group.)	NR	0/1 (3%)	NR	NR	NR	Treatment: 16 Control: 11
NR	NR	69.44 ± 36.055/NR	No side effect	NR	19.1%/57%	NR	NR	NR	0
NR	74.58 ± 0.35/50.30 ± 0.17(%)	NR	An increase in the area and perimeter of the lesion four patients (antibiotic group)	NR	3(5%)/7(16%)	26 ± 13/34 ± 18 d	NR	NR	NR

Abbreviations: NR, not reported; VAC, vacuum-assisted closure.

and solution) in combination with systemic ozone (rectal insufflation or intravenous administration of autologous blood immunomodulation therapy[IMT]).[11,24,26,27] The characteristics of included studies are shown in Table 4.

3.3 | Risk of bias in included studies

Overall, the quality of reporting was poor because of insufficient formation obtainable in the literature. The risks of bias of the articles were mostly 'unclear risk'. All risk of bias assessment data is summarised in Figures 2 and 3.

3.4 | Effects of interventions

Given the clinical diversity and methodological heterogeneity of the evidence, it was not appropriate, therefore, to combine the trials in a single meta-analysis to produce a summary statistic for ozone overall. This review is organised by subgroup summary statistics based on the wound type. Within the subgroups (actinic ulcer following radiotherapy, venous leg ulcers, DUs in SSc, critical limb ischemia, diabetic foot ulcer), trials have been combined in meta-analysis where appropriate. Otherwise, the trials have been summarised narratively.

3.4.1 | Actinic ulcers following radiotherapy

One trial (13 participants) randomly allocated participants with actinic ulcers following a radiotherapy cycle because of oncological pathology to ozolipoile and hyaluronic acid gel.[25] Although the ulcers appeared less deep and reduced in size from the figure, no concrete change in the wound size was obtained. And other outcomes we focused on were not reported.

3.4.2 | Chronic venous leg ulcers

One trial recruited 29 participants (37 ulcers) compared ozonated oil and α-bisabolol spray formulation with the daily application of an epithelialisation cream.[33] The proportion of ulcers healed are not significantly different between groups after treatment (25% vs 0%, $P = .16$). The differences between the two arms were confirmed by the significant difference ($P < .05$) observed in the reduction in the mean surface of the wound after therapy (73% vs 13%), in favour of the group of ozonated oil/α-bisabolol spray. Neither group of patients reported adverse events. This trial does not report other residual outcomes.

One study (92 participants) compared ozone gas bath and endovenous laser therapy (OEVLT), preconditioning wounds once a day before endovenous laser therapy (EVLT) until the necrosis and infection in the ulcer area were improved and suitable for skin puncture, with only EVLT.[28] The proportion of ulcer healing of the OEVLT group (92.00%) was no significantly higher than EVLT alone (76.19%) at 12-month follow-up ($P = .05$). Uncertainty exists about whether there are more or fewer side effects without a specific incidence value between groups. Other remaining outcomes are unknown.

3.4.3 | Digital ulcers (DUs) in systemic sclerosis (SSc)

One trial enrolled 50 patients with 25 participants randomised to the treatment group (oxygen-ozone gas bath and calcium channel blockers [Epilat retard ®40 mg

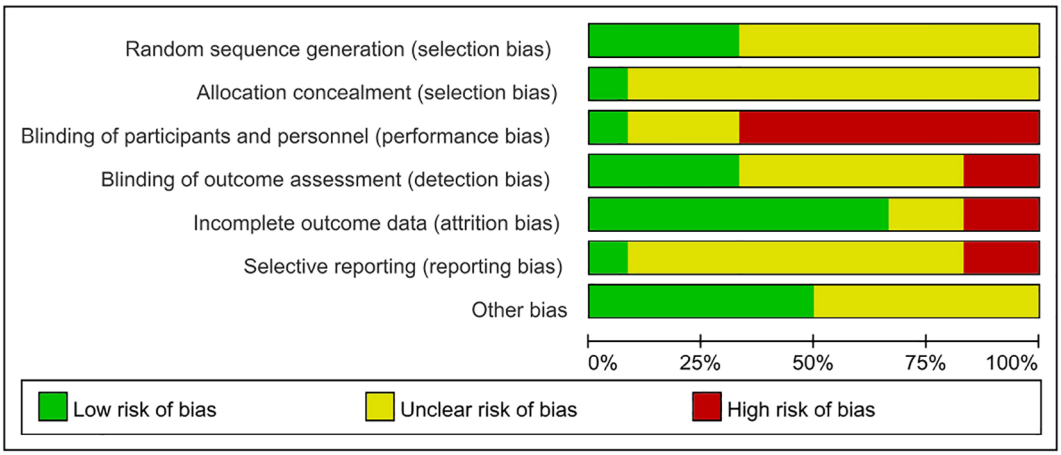

FIGURE 2 Risk of bias of included studies

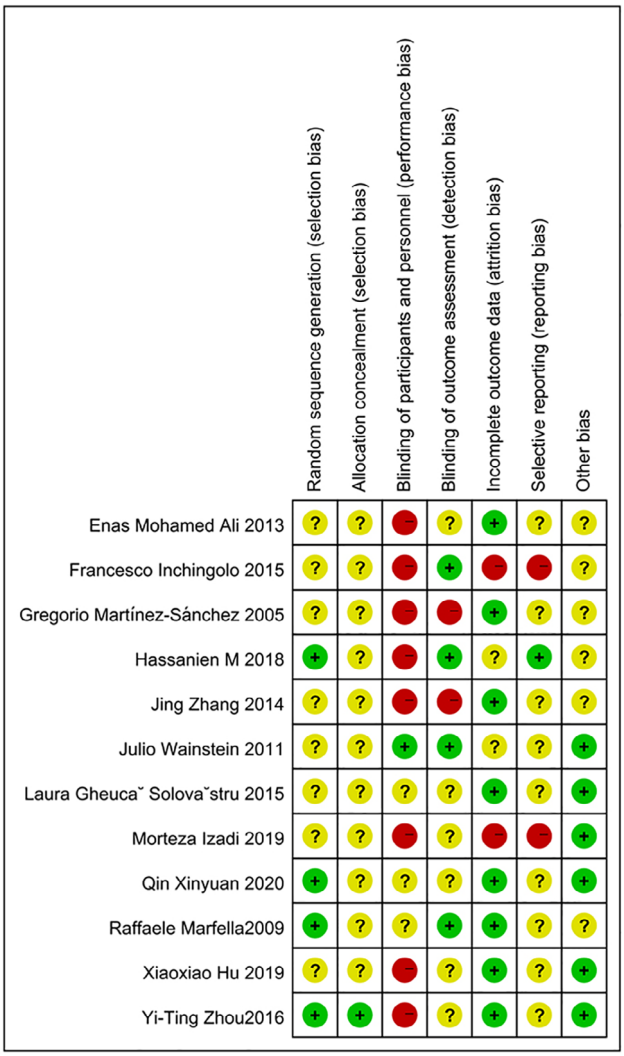

FIGURE 3 Summary of risk of bias

/day]) and 25 to the control (calcium channel blockers alone).[32] There was no significant difference in the proportion of people with ulcers completely healed between groups (28% vs 12%, $P = .18$), which are probably related to a very short observation time and no follow-up. There was a significantly greater reduction in the wound area in the oxygen-ozone group of 2.44 ± 0.80 mm compared with 0.75 ± 0.30 mm($P < .00001$). No data were available for other outcomes.

3.4.4 | Ischemic ulcer of CLI from peripheral arterial disease

One trial (156 participants) randomly allocated participants with CLI to ozone-based IMT, autologous blood exposed to the oxygen/ozone gas mixture by intragluteal injection, and sham treatment of applications of sterile saline in the placebo group.[24] Not only ischemic ulcers (120 participants, about 77%) but also rest pain of CLI is appropriate in this trial. There were no significant differences in the incidence of at least one serious adverse event mainly due to CLI or to the comorbid conditions between groups (52.7% vs 53.2%, $P = .95$). However, the incidence of adverse events directly related to IMT therapy is unclear as there are no enough details. There were no differences in the incidence of major amputation between comparisons and more reduction in wound ulcers in the IMT group at 22 weeks from the figure while lacking specific data. This article does not report other outcomes.

3.4.5 | Diabetic foot ulcers

Seven studies recruited participants with diabetic foot ulcers, and more than one trials contributed results to partial outcomes of our review. Thus, we classified pooled data for meta-analysis. However, publication biases were not conducted due to insufficient studies.

The proportion of participants with completely healed wounds

Three trials were reported this outcome,[11,15,29] involving 211 participants with 103 participants randomised to antibiotics or standard treatment or sham therapy and 108 to ozone. The trial by Martínez-Sánchez et al contributes 85.4% of the weight to this analysis.[11] There was no statistically significant increase in the proportion of ulcers completely healed following combination with the ozone application compared with control therapy or topical and systemic antibiotics alone ($P = .28$) (RR = 1.13, 95%CI [0.91,1.41], $I^2 = 0\%$) using a random-effects model (Figure 4). Sensitivity analysis indicated that the result was robust (Supplementary Information S1). Subgroup analysis by different ozone applications, different control treatments, course of treatment, and duration of diabetes did not significantly affect this outcome (Table 1).

Time to achieve complete ulcer healing

Only one study reported average healing time was 69.44 ± 36.005 days with local and systemic ozone therapy, which is significantly lower than the median healing time with conventional treatments ($P = .012$) but a specific time is not published in the control group.[26]

Change in wound size

Five trials reported this outcome.[11,15,27,29,30] We find too significant heterogeneity ($I^2 = 99\%$, $P < .00001$) when pooling a single meta-analysis. Consequently, a separate analysis was performed.

Three of them mentioned this outcome by the change percentage compared with the baseline wound size,[11,27,30] among which significant heterogeneity exists ($I^2 = 91\%$, $P < .0001$). According to the random-effects model, ozone gas bath locally alone or in combination with rectal insufflation and ozonised olive oil applied by monotherapy or combined control therapy could significantly promote the improvement of the wound area compared with the control group (standard care only or antibiotics) (pooled SMD = 66.54%, 95%CI [46.18,86.90], $P < .00001$) (Figure 5). Sensitive analysis showed the results were robust. (Supplementary Information S1). No significant difference between the subgroup of different ozone applications ($P = .91$), different baseline wound sizes ($P = .91$), different durations of diabetes ($P = .91$), but different follow-up times were probably responsible for obvious heterogeneity ($P = .03$).

The remaining two studies showed the wound size reduction by a concrete transformed value.[15,29] The course of treatment and ulcer size at baseline and the calculation method of the change area are totally different so that we use descriptive analysis. Wound area reduction after 20 days was significantly greater in the ozone gas bath group than the standard treatment of control limbs in this study, which recruited a mean baseline wound size of about $10cm^2$ ($6.84 \pm 0.62cm^2$ vs $3.19 \pm 0.65\ cm^2$, $P < .001$),[15] whereas there was no difference in ulcer area reduction between the two groups that included participants with a much smaller mean baseline wound size of about $4cm^2$ ($-\ 2.0 \pm 3.9\ cm^2$ vs $-1.6 \pm 1.7\ cm^2$, $P = .78$).[29]

Incidence of adverse events

Among seven studies about diabetic foot ulcers, no adverse events were believed to be related to the ozone treatment.

Amputations

The amputation rate data across the four trials[11,26,29,31] were pooled (random effects); Overall, there was a significantly lower amputation rate with combined ozone therapy than control treatment (RR = 0.36, 95% CI = [0.24,0.54], $P < .00001$, $I^2 = 0\%$) (Figure 6). Sensitive analysis indicated that the result was robust (Supplementary Information S1). The size of amputation (major or minor) is not reported.

Length of hospital stay

Two studies mentioned the length of hospital stay, and a random-effects model was used because of the significant heterogeneity ($I^2 = 98\%$). Meta-analysis suggested the length of hospital stay was not significantly different following ozone monotherapy or combined control treatment compared with the control group (SMD = −1.79, 95%CI = [−4.32,0.74], $P = .16$)[11,31] (Figure 7). The high heterogeneity may be due to different baseline wound sizes and ozone application modes so that we must approach this outcome with caution. Sensitive analyses were not conducted due to the lack of studies.

Quality of life and Cost

No data were available for these two outcomes in all seven studies.

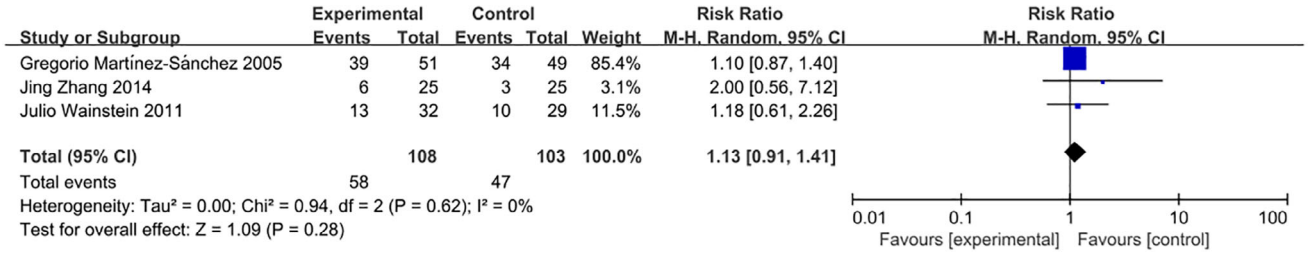

FIGURE 4 Forest plot for the proportion of participants with completely healed wounds

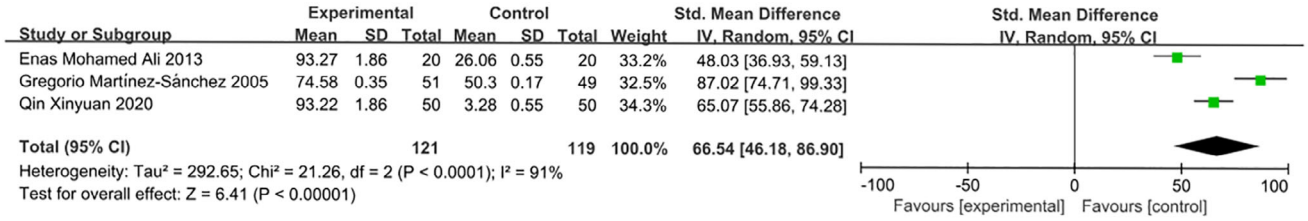

FIGURE 5 Forest plot for the change in the wound size

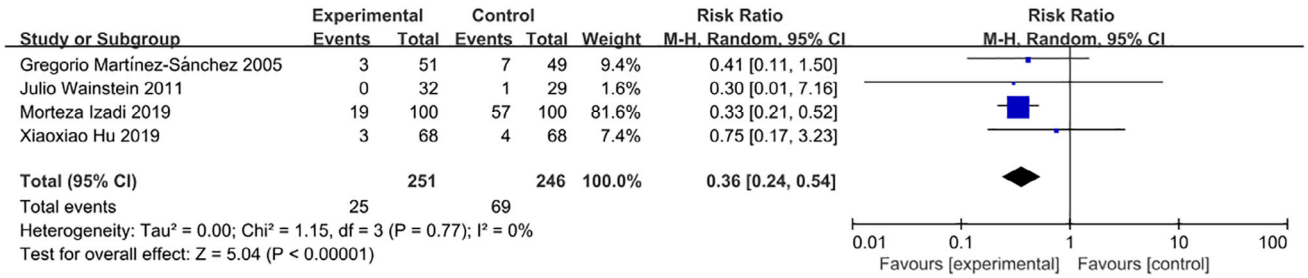

FIGURE 6 Forest plot for amputations

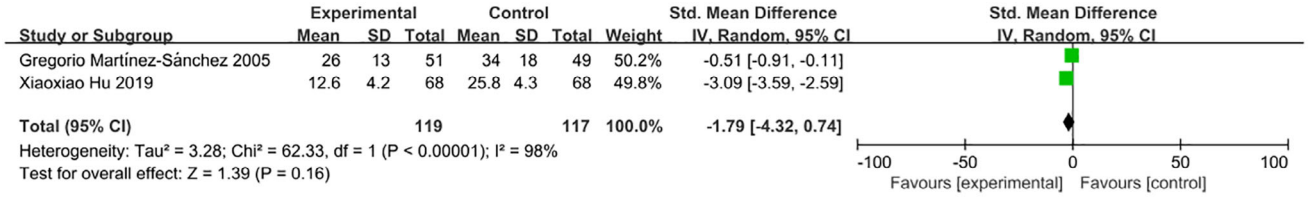

FIGURE 7 Forest plot for the length of hospital stay

4 | DISCUSSION

4.1 | Summary of main results

We found some results by combining existing literature. Firstly, the methodological quality of the included studies was poor. Secondly, as to diabetic foot ulcers, ozone regardless of monotherapy or combined control treatment markedly accelerated the improvement of the wound area and reduced amputation rate compared with standard control therapy. But there is no superiority of the proportion of participants with completely healed wounds and length of hospital stay with ozone intervention by meta-analysis. The results were robust by sensitivity analysis. In terms of other wound types, the narrative analysis suggested ozone therapy significantly improved the wound area for chronic venous leg ulcers and DUs in SSc, whereas the proportion of participants with completely healed wounds is no significantly high. But we are not confident of the accuracy of the results because we used only one study for each wound type. Other pre-specified outcomes are unclear on account of limited information and studies. Thirdly, the general quality of the above evidence was not high.

4.2 | Overall completeness and applicability of evidence

Overall, there are significant weaknesses. Involved seven studies about diabetic foot ulcers evaluated a wide range of treatment application options and dosage of ozone, leading to inevitable heterogeneity and lacking specific comparisons of interest. Furthermore,implementation in these studies maybe are different from varied real practice. This variation is shown in our research often viewing systematic and the local application of ozone as a 'class', despite the apparent variations within these treatments. And studies have not yet directly compared the effects of these different ozone applications.[21] A study reported a diabetic patient with a non-healing wound who developed severe foot necrosis and infection following intralesional ozone injections, so ozone local subcutaneous injection is not recommended for deep, heavily infected, or necrotic wounds.[20,34] Thus, readers should bear this in mind when interpreting findings even if there is no pooling heterogeneity. The toxicity of ozone relies upon the dosage and applying ways so that controlling dosage well and administration method's option are very cautious.[12,35] It is irrational to judge the best application method, dosage and treatment course, and safety and tolerability for ozone because of no specific comparison about these events. The results of ozone's advantage over antibiotics are unknown based on only one small study. We, therefore, urge that future research will find out specific wound indications of ozone as clearly as possible. This very weak evidence base makes it impossible to draw firm conclusions with confidence for other chronic wound types.

4.3 | Quality of evidence

We evaluated the quality of evidence by GRADEpro (Table 2). The reporting information of included studies

was limited so the quality of outcomes' evidence is not high. The downgrade of evidence quality was mainly due to low methodological quality, obvious heterogeneity, and small numbers of participants and documented outcome events. Admittedly, these methodological flaws may exaggerate the treatment effect mainly due to imprecision and risk of bias.[36] Over 75% of included studies were at unclear risk for selection bias and reporting bias. Poor reporting is a major issue, and the majority of studies were unclear for one or more important bias domains in the risk of bias assessment.[4] More than 50% were at high risk for performance, whereas the risk of performance bias is not yet clear in wound care studies.[37] Partial subgroup analyses are post-hoc analyses, making the outcomes less reliable.[38]

4.4 | Potential biases in the review

There are several biases in this research, driven largely by the nature of included studies. Firstly, there is still unpublished literature suitable for inclusion that was not retrieved and the risk of publication bias is increased. Secondly, time to healing should be treated as a type of time-to-event outcome rather than a continuous measure and this may enable all participants to contribute data to the analysis irrespective of whether they experienced the outcome or remained in the study.[4] However, we were limited to using a common means of measurement wherever possible. Thirdly, some subgroup analyses based on different wound baseline sizes and duration of diabetes were post-hoc analyses. Finally, it was difficult to estimate the overall possibility of publication bias.

4.5 | Agreements and disagreements with other studies and reviews

Two relevant systematic reviews have been published before our research. In 2018, one review found evidence in favour of ozone treatment for chronic wounds showed a significant improvement in healing when compared with the control.[21] There are key methodological differences compared with our review. Firstly, literature searches are not rigorous, and no RCTs are involved actually although RCTs only were considered for inclusion. Meanwhile, we add some recent research studies about other systematic ozone applications to more comprehensive evaluation instead of only topical application. Secondly, the effect size of all comparisons was not chosen correctly based on the data type. Thirdly, GRADE was not used to evaluate the quality of the evidence. In addition, the authors do not further explore heterogeneity and find its reasons. In terms of another similar systematic review,[13] it is now 6 years since its publication, so it is necessary to update the evaluation results. Compared with these articles, the methods used and designs reported in our research were standard and valid, which ensured the accuracy of the results.

4.6 | Strength and limitations

The aim of systematic review is to supply a comprehensive assessment and presentation of the issue to provide health decision-makers.[39] Thus, ensuring reliability is very important. This research was implemented strictly following the methodological requirement of the systematic review. In addition, a research protocol was conducted and published in advance, which minimised post-hoc decisions and lower bias. We interpreted the results prudently and avoided misleading as much as possible. Despite these advantages, we also should pay attention to some limitations. Firstly, high-quality multi-centre, randomised, double-blind, placebo-controlled clinical trials of ozone therapy for chronic wounds are not enough, which leads to insufficient convincing results. Due to incomplete information provided by the articles and the flaws of the study design, the overall methodological quality of the included studies was poor. Secondly, as a new therapy, it needs to be taken with exact dosage and application manner for a long time, ensuring safety. However, we are not able to judge the safety of ozone as inadequate studies. Thirdly, the publication bias needs to be considered and may affect the reliability and accuracy of the results.

5 | CONCLUSIONS

5.1 | Implication for practice

There is no related evidence of zone from only one trial of actinic ulcers following radiotherapy. As to chronic venous leg ulcers, ozonated oil combined with α-bisabolol spray heals more reduction in the wound area with very low-quality evidence; the addition of ozone gas bath to standard EVLT therapy does not bring a higher proportion of ulcer healing with low-quality evidence. There is low-quality evidence that showed more reduction in the wound area after ozone gas bath associated with calcium channel blockers for DUs of SSc, but this benefit is not evident in the proportion of people with ulcers completely healed. IMT may be more effective in reducing the wound area with low-quality evidence and major amputation rate with very low-quality evidence for ischemic ulcer of CLI.

As to diabetic foot ulcers on this analysis, ozone therapy lowers the amputation rate but does not appear to

significantly increase the proportion of participants with ulcers completely healed with moderate-quality evidence, whereas there is a significant improvement of the wound area with low quality. And the incidence of adverse events is unclear.

It behoves physicians to cautiously interpret these findings limited by reporting and methodological flaws of involved trials and small numbers of participants.

5.2 | Implications for research

This systematic review found some common problems in current research, and improving these problems will be helpful to define the true extent of benefit from the administration of ozone. Firstly, it is essential to conduct multicentre clinical trials to incorporate people from different regions for appropriate sample sizes with the power to detect expected differences. Secondly, more information is required on the careful definition and selection of chronic wounds and the subset of disease severity or classification most likely to benefit from this therapy. In the next stage of clinical research, research should compare appropriate ozone dose and application time per treatment session with specific comparator therapy even including suitable outcome measures for sufficient follow-up time. And last but not least, the safety of ozone has become an increasing concern. The occurrence of clinical adverse events is related to the lack of sufficient attention given to the safety of ozone administration; thus, the safety of ozone usage should be paid more attention.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

AUTHORS CONTRIBUTIONS

Qing Wen, Yanli Zhang, and Qiu Chen proposed the idea and designed the whole research; literature research, study selection, and data extraction were conducted by Dongying Liu and Xian Wang; and Qing Wen and Xianliang Qiu performed the data analysis. The article was written by Qing Wen and revised by Dongying Liu and Song Fang. All authors approved the final manuscript before submission.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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