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Oxygen-ozone therapy for the treatment of low back pain: a systematic review of randomized controlled trials

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Abstract. – OBJECTIVE: The aim of the study was to review the available literature on the application of oxygen-ozone therapy (OOT) in the treatment of low back pain (LBP), to understand its therapeutic potential and compare it with other available treatment options.

MATERIALS AND METHODS: A systematic review was performed on the PubMed and Scopus databases, with the following inclusion criteria: (1) randomized controlled trials (RCTs), (2) published in the last 20 years, (3) dealing with OOT in patients with LBP and herniated disc, (4) comparing the results of OOT with those of other treatments. The risk of bias was assessed by the Cochrane Risk of Bias tool.

RESULTS: Fifteen studies involving 2597 patients in total were included. Patients in the control groups received different treatments, from oral drugs to other injections, instrumental therapy and even surgery: corticosteroids were used in 5 studies, analgesic therapy in 2 studies; placebo, microdiscectomy, laser-therapy, TENS and postural rehabilitation, percutaneous radiofrequency intradiscal thermocoagulation and psoas compartmental block were tested in the other trials. Looking at the quality of the literature, none of the studies included reached "good quality" standard, 3 were ranked as "fair" and the rest were considered "poor". Comparison of OOT results with other approaches showed that, in the majority of studies, OOT was superior to the control treatment, and also when compared to microdiscectomy, ozone showed non inferiority in terms of clinical outcomes.

CONCLUSIONS: The analysis of literature revealed overall poor methodologic quality, with most studies flawed by relevant bias. However, OOT has proven to be a safe treatment with beneficial effects in pain control and functional recovery at short to medium term follow-up.

Key Words:
Ozone therapy, Ozone injection, Ozone, Back pain, Spine, Hernia, Herniated disc, Disc.

Introduction

Low back pain (LBP) is a major health problem around the world, that accounts for considerable socio-economic and health care burden in terms of loss of working days and public health. The prevalence is estimated at 22-65% per year: it is higher between the fifth and sixth decade of life, and up to 80% of the population present a mild to severe LBP at some point in life[1,2]. In approximately 60-80% of cases, no specific cause is diagnosed, and the pain is attributed to muscle or ligament tension. Although the pathogenesis of LBP remains unclear, it is often induced by lumbar disc herniation (LDH) and spine degeneration[2]. Specifically, LDH can create mechanical, biochemical, and inflammatory stimuli on the lumbar region and nerve roots, also inducing neurological symptoms and radicular pain[1,2]. In most cases, it is a self-limiting condition, but relapses are common, and significant disability and chronic pain may develop[3]. A wide number of therapeutic interventions have been studied and

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performed for the treatment of LBP. A conservative approach is generally considered as the first line treatment. Also, minimally invasive strategies, such as percutaneous injections, have been shown to be well-tolerated and to yield good clinical results[4]. In particular, the use of oxygen-ozone (O_2-O_3) injections is gaining more and more attention as one of the minimally invasive treatments for LBP due to lumbar disc herniation, either as part of a conservative therapeutic program before surgery or when surgery is contra-indicated[3,5,6]. Ozone (O_3), or trioxygen, is an inorganic gas, an allotrope of oxygen with lower stability than the diatomic dioxygen (O_2)[7]. The therapeutic mechanism of action can be identified in its high reactivity: once injected, ozone is able to produce a short and self-limiting oxidant action with a consequent increase in the biological antioxidant cell response. In this light, ozone acts as a prodrug, activating endogenous mediators that cause a change in cellular metabolism[8]. Its benefits range from the inhibition of inflammation and correction of ischemia and venous stasis, to the reflex induction of endorphin release, as well as the promotion of antinociceptive-analgesic effects[9]. Oxygen-ozone therapy (OOT) might exert its action in reducing LBP with a coupled mechanical and anti-inflammatory effect: the oxidizing action might break glycosaminoglycan chains in the nucleus pulposus, reducing its ability to retain water, thus decreasing the size of the herniated portion, thus helping to reduce hernia conflict[10,11]. This is supposed to be the main mechanism of action of intradiscal administration of OOT. O2-O3 could also activate an anti-inflammatory cascade by altering the breakdown of arachidonic acid into inflammatory prostaglandins[12,13]. This is instead the rationale behind O2-O3 injections in the paravertebral muscles corresponding to the metamer of the herniated disc[14]. Although these procedures are considered well tolerated and unexpensive, data regarding their safety and efficacy have not been systematically analyzed yet. The aim of the present systematic review is to investigate the available evidence on the application of OOT for the treatment of LBP to understand how OOT compares to other conservative treatment options in terms of pain relief and functional improvement.

Materials and Methods

A systematic review of the literature was performed on the use of injective treatment with oxygen-ozone for low back pain. A search was conducted for English articles published up to the end of April 2021. The electronic databases PubMed and Scopus were investigated, using the following formula: ("ozone therapy" OR "ozone injection" OR "ozone") AND ("back pain" OR "spine" OR "hernia" OR "herniated" OR "disc").

Database searching was supplemented by screening reference lists and tracking citations included in trials to identify any additional studies. The screening process and analysis were conducted separately by 2 independent observers (CS and GL). First, the articles were screened by title and abstract. The following inclusion criteria for relevant articles were used during the screening: (1) randomized controlled trials (RCT), (2) written in English, (3) published on indexed journals in the last 20 years (2000-2021) and (4) dealing with the use of ozone injections for the treatment of LBP (including: disc herniation with or without radicular irradiation and lumbar spine arthritis). Exclusion criteria were: articles written in other languages; reviews; non-randomized studies; trials analyzing other applications of ozone not directly related to low back pain. In the second step, the full texts of the selected articles were screened, with further exclusions according to the previously described criteria. A flowchart of the systematic review is provided in Figure 1. Relevant data were then extracted and collected in a unique database, with the consensus of the two observers, to be analyzed for the purposes of the present manuscript. In particular, the following data were retrieved: (1) treatment groups, (2) sample size and patients' features (3) ozone preparation method, (4) therapeutic protocols, (5) outcome measures, (6) timepoints of follow-up evaluations, (7) summary of clinical results. Any discrepancy was discussed with and resolved by the senior investigator (BDM), who made the final judgement. The primary outcome of the present review was the analysis of patient's reported subjective scores and pain at 6 months' follow-up. The risk of bias was assessed using the Cochrane Risk of Bias tool for Randomized Controlled Trials, which evaluates seven different types of bias. Each of them, based on specific criteria, was classified as "Low risk", "High risk" or "Unclear risk". Subsequently, the results of this assessment were converted to AHRQ (Agency for Healthcare Research and Quality) Standards, which ultimately rank the RCTs in "Good quality", "Fair quality" and "Poor quality".

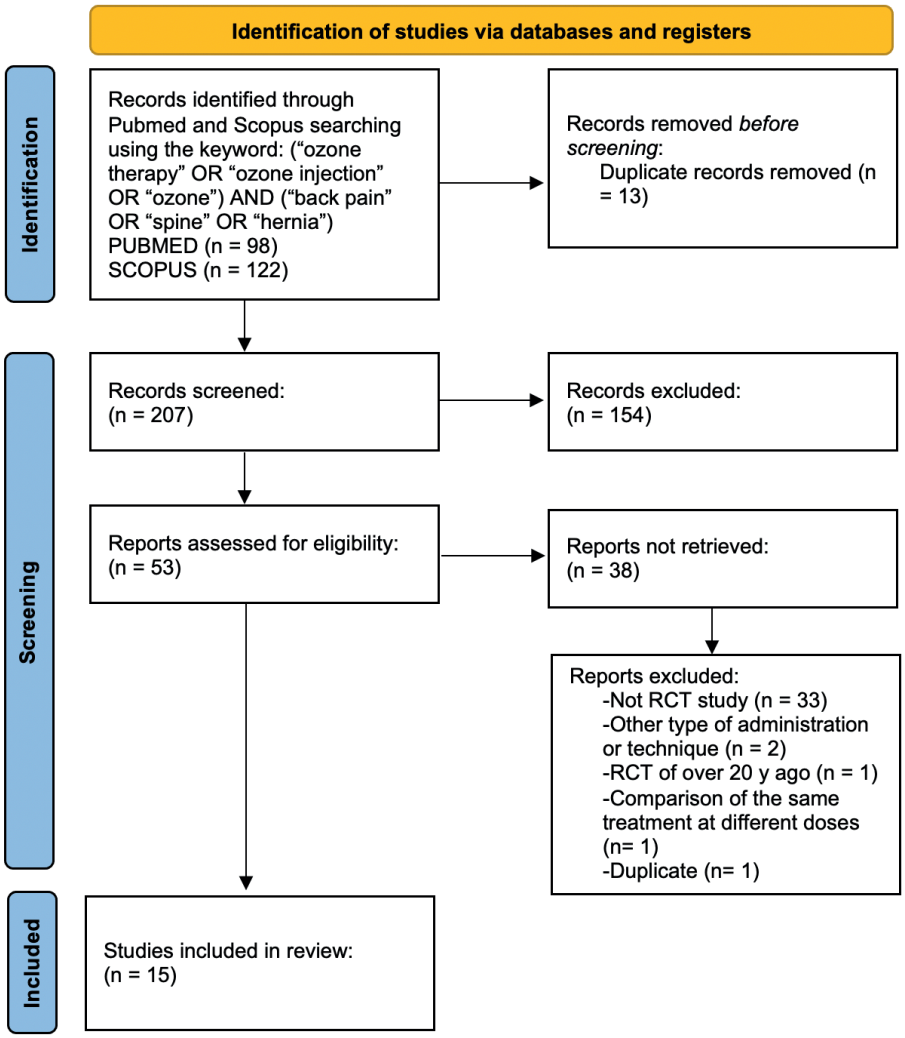

Figure 1. Preferred Reporting Items for Systematic Reviews and Meta-analyses flowchart resuming the paper's selection process.

Results

A total of 15 studies published from 2005 to January 2020 dealing with O2-O3 injection outcomes in the treatment of LBP were ultimately included in this review (Figure 1). A detailed description of each study has been provided in Table I.

Study Design and Quality

All studies were, as per the inclusion criteria, RCTs. The studies' features were highly variable. Patients in the control groups received different injections or treatments: corticosteroids (CS) in 5 studies; analgesics drugs in 2 studies; simulated injections, microdiscectomy, OOT + Laser, OOT + TENS and postural rehabilitation, OOT + CS, OOT + percutaneous intradiscal radiofrequency thermocoagulation (PIRFT), OOT + psoas compartment block (PBC) in the other 10 studies. Looking at the quality of the available literature by AHRQ standard, we found that none of the studies included reached a "good quality" standard, whereas 3 were ranked as "fair quality", and the rest were considered as "poor quality". The results of the analysis performed with the Cochrane Risk of Bias tool for RCT are detailed in Table II. In regard to the random sequence generation process, it was not specified in 2 papers[15,16]. Except for three papers, the method of allocation concealment was described in nearly none of the studies included[2,17,18]. Five papers reported outcomes incompletely[5,6,16,19,20]. Regarding simple size calculation, only in two trials[2,17] the power analysis methods were fully clarified. Seven trials were

Table I. Synopsis of all the RCTs included in the systematic review.

Publication	Study Design	Score	Patients Features	Ozone preparation Method	Therapeutic Protocol and follow-up	Results	Overall performance of OOT
Niu et al[6] 2018	RCT (Intradiscal low, medium and high medical ozone *vs.* Conventional drug treatment)	VAS and SOD activity, IgM and IgG levels by ELISA	80 (20 *vs.* 20 *vs.* 20 *vs.* 20) **Age:** 48 y **Sex:** 49 M:31 F	**Machine:** NA **V & Conc:** 20 μg/ml 40 μg/ml 60 μg/ml	Single injection F-up at admission and at 6-and 12-mo after injection	VAS score gradually decreased over time among all groups (p<0.05), with increasing treatment duration and the most over time at an ozone dose of 40 ug/ml	+
Bruno et al[5] 2020	Single-blind RCT (Intradiscal ozone *vs.* Periradicular LA+CS)	VAS and ODI	60 (30 *vs.* 30) **Age**: 47.52 *vs.* 48.49 **Sex**: 17 M:13 F *vs.* 18 M:12 F	**Machine:** NA **V & Conc:** 10 mL of 27 μg/ml	Single injection F-up at 1-7 days before the injection and 1 mo after	Greater success rate in the O2-O3 group than in those who received corticosteroid injection, with improvement of VAS and ODI scores (p<0.001) at 1 mo	+
Rahimzadeh et al[17] 2018	Double-blind RCT (Intradiscal ozone + LA + CS *vs.* Laser disc decompression + LA + CS)	VAS and ODI	40 (20 *vs.* 20) **Age:** 20-70y *vs.* 20-70y **Sex:** NA	**Machine:** NA **V & Conc**: 6 mL of 30 μg/mL	Single injection F-up before the injection and at 1,3,6 and 12 mo after	No statistically significant differences in VAS score between OOT and CG (p=0.1). ODI score was significantly different (p=0.02) at 3,6 and 12 mo when OOT showed better results than the CG (60% ODI reduction)	+
Perri et al[23] 2015	Double-blind RCT (Percutaneous LA+CS + Intradiscal ozone *vs.* Percutaneous LA+CS)	VAS questionnaire ranging from 0 to 10 classifying as successful if was no greater than 2, and unsuccessful otherwise.	154 (77 *vs.* 77) **Age:** 44.4 *vs.* 43.8 **Sex:** 42 M:35 F vs 47 M:30 F	**Machine:** Ozone Generator OZO2 Futura, Alnitec S.r.l. **V & Conc:** 10 mL of 28 μg/mL	Single injection F-up at admission and at 2,4 and 6 mo after the treatment	The difference in VAS score was statistically significant at 6 mo, when success rate was 75.3% in OOT and 38.9% in CG (p<0.001)	+
Li et al[24] 2014	RCT (Intradiscal ozone+collagenease *vs.* Intradiscal ozone+collagenase +LA+CS+PBC)	VAS and ODI	192 (95 vs 97) **Age:** 45.2 *vs.* 46.2 **Sex:** 54 M:41 F vs 57 M:40 F	**Machine:** Ozone Generator HERRMANN **V & Conc:** 5 mL of 40 μg/mL	Single injection F-up before the injection and at 1 wk,1 mo, 3 mo and 6 mo after	VAS and ODI scores were significantly decreased in both groups, at all timepoints. The addition of LA+CS+PBC produced a greater reduction in the VAS scores, and ODI at 1 wk,1,3 and 6 mo (P=0.000)	(both SG and CG included OOT)

Table continued

Table I. ***(Continued).*** Synopsis of all the RCTs included in the systematic review.

Publication	Study Design	Score	Patients Features	Ozone preparation Method	Therapeutic Protocol and follow-up	Results	Overall performance of OOT
Zhang et al[25] 2013	Single-blind RCT (Intradiscal and Intraforaminal ozone *vs.* Intradiscal ozone and Intraforaminal ozone + CS)	VAS and JOA	172 (90 vs 82) **Age:** 41.5 *vs.* 43.6 **Sex:** 49 M:41 F 43 M:49F	**Machine:** Ozone Generator Ozoneline E80 **V & Conc:** 10 mL of 25-30 µg/ml	Single injection F-up before the injection, at 3 wk, at 6 and 12 mo after	Statistically significant reduction of VAS score at all point of F-up both in OOT and CG (P<0.05). Improvements of mean JOA score at every F-up time in both groups	= (both SG and CG included OOT)
Melchionda et al[3] 2012	Double-blind RCT (Paravertebral ozone *vs.* antinflammatory-analgesic drugs)	VAS and ODI	38 (20 vs 18) **Age:** 53.2 *vs.* 52.7 **Sex:** 12 M:8 F 10 M:8 F	**Machine:** Multiossigen PM95 Generator **V & Conc:** 20 mL of 40 µg/mL	12 injections F-up at baseline, at 1, 2, 4 wk and at 3 and 6 mo after the treatment	Strong improvement of VAS and ODI scores after 2 wks in SG for up to 6 mo (p<0.5), when 80% of OOT turned out pain free compared with half of CG	+
Gautam et al[18] 2011ì	Double-blind RCT (Intradiscal ozone *vs.* Intradiscal ozone + PIRFT)	VAS and ODI	91 (41 *vs.* 43) **Age:** 43.5 *vs.* 45.1 **Sex:** 27 M:14 F 25 M:18 F	**Machine:** NA **V & Conc:** 4 to 7 mL of 30 µg/mL	Single injection F-up at 2 wk, at 1, 3, 6 mo and at 1 y after the treatment	VAS and ODI scores were significantly decreased in both groups at all points of F-up; ozone-PIRFT produced a greater reduction in the VAS scores and ODI at 2 wk,1,3,6 mo and 1 y	- (both SG and CG included OOT)
Paoloni et al[2] 2009	Double-blind RCT (Paravertebral ozone *vs.* simulated treatment)	VAS, BACKILL, KELLNER and SF-36	60 (36 *vs.* 24) **Age:** 48.8 *vs.* 47.2 **Sex:** 18 M:18 F 10 M:14 F	**Machine:** Multiossigen PM95 Generator **V & Conc:** 20 mL of 20 µg/mL	15 infiltrations F-up at 30 days, at 2 wk, at 3 and 6 mo after the treatment	Significant difference in VAS score at 6 mo (SG 61% vs CG 33%, p: 0.05) and in Backill score in the SG alone at 3, 4, 5 and 6 mo. No statistically significant differences in Kellner and SF-36 scores	+
Arena et al[15] 2008	RCT (TENS+ psychosomatic postural rehabilitation *vs* bioresonance magnetotherapy-+TENS+ psychosomatic postural rehabilitation vs Paravertebral ozone+TENS+ psychosomatic postural rehabilitation *vs.* Paravertebral ozone+TENS + psychosomatic postural rehabilitation+bioresonance magnetotherapy)	VAS and BARTHEL	549 (135 *vs.* 139 vs 137 *vs.* 139) **Age:** 50-75 y **Sex:** NA	**Machine:** NA **V & Conc:** 10 ml of 20 µg/ml	15 sessions with a biweekly schedule for the first 8 wks and weekly from the 9°application on, F-up after 11 wks and at, 1, 6 and 12 mo	The improvements from the basal value were significant and similar among groups (p<0.05) at 11 wks and up to 12 mo.	role of OOT not clearly assessable
Gallucci et al[19] 2007	Double-blind RCT (Intradiscal and Intraforaminal LA + CS *vs.* Intradiscal and Intraforaminal LA + CS + ozone)	ODI classified as successful if was no greater than 20% at F-up, and unsuccessful otherwise	159 (77 *vs.* 82) **Age:** 41 *vs.* 40 **Sex:** 43 M:34 F 45 M:37 F	**Machine:** Ozone Generator OZO2 Futura, Alnitec S.r.l. **V & Conc:** 5-7 ml of 28 µg/mL	Single injection, F-up at the day of the procedure and at 2 wk, at 3 and 6 mo	Satisfactory outcomes at 6 mo in 47% of LA+CS and in 74% of LA+CS+OOT. The difference was significant (p<.01)	+

Table continued

Table I. (*Continued*). Synopsis of all the RCTs included in the systematic review.

Publication	Study Design	Score	Patients Features	Ozone preparation Method	Therapeutic Protocol and follow-up	Results	Overall performance of OOT
Zambello et al[16] 2006	Double-blind RCT (Epidural CS *vs.* Paravertebral ozone)	McNab's method and "Excellent" "Good" "Satisfactory" or "Poor" OUTCOME	351 (171 vs 180) **Age:** 48 *vs.* 51 **Sex:** 91 M:80 F 100 M:80 F	**Machine:** CE class 1B equipment, Alnitec S.r.l. **V & Conc:** 5 ml of 10-20 µg/ml	Max 3 injections at weekly intervals F-up at 3 wk and at 6 mo	In the short-term 59% of CS and 88.2% of OOT ($p<0.05$) had a total or near total remission of pain. Long-term outcome remained excellent or good in 47.3% of CS and 77.1% of OOT ($p<0.05$)	+
Bonetti et al[20] 2005	Single-blind RCT (Intraforaminal ozone *vs.* Periradicular CS)	McNab's method and "Excellent" "Good" or "Poor" OUTCOME	306 (166 *vs.* 140) **Age:** 48 y **Sex:** 178 M:128 F	**Machine:** CE mark class 1B, Alnitec S.r.l. **V & Conc:**3 ml of 25 µg/mL	Single injection F-up at 1-wk, at 3 and 6 mo after treatment	At 6 mo, significant differences in favour of O2-O3 treatment in patients with disk disease (p=.0021). Clinical outcomes were poor in 15.1% of SG and in 22.5% of CG (p= .2226)	+
Paradiso et al[21] 2005	RCT (Microdiscectomy *vs.* Intradiscal ozone)	VAS, ODI and JOA	300 (150 *vs.* 150) **Age:** 51 *vs.* 50 **Sex:** 78 M:71 F 76 M:74 F	**Machine:** NA **V & Conc:** NA	Single injection F-up before surgery and at 4, 6 mo and at 1 and 3 y after	Microdiscectomy had a greater improvement in ODI score at short term. Regression of pain 3y after surgery was similar in the two groups	=
Buric et al[22] 2005	Single-blind RCT (Intradiscal ozone vs microdiscectomy)	VAS, RMDQ and OPSR	45 (30 *vs.* 15) **Age:** 45 *vs.* 45 **Sex:** 14 M:16 F 9 M:6 F	**Machine:** Ozone Generator Ozonline E 80, Medica S.r.l. **V & Conc:**10-15 ml of 30 µg	Single injection F-up at 1 day before the treatment, at 6,12 and 18 mo after	Both groups achieved a statistically significant improvement in pain and disability at 18 mo F-up and there was no statistically significant difference in results	=

NOTE: OOT for the treatment of low back pain: data extracted from the 15 RCTs included in the review (+, =, and - signs reflect the overall performance of OOT compared with the control group(s) of any study analyzed). BACKILL, Backill questionnaire; BARTHEL, Barthel index; CG, control group; Conc., concentration; CS, corticosteroids; ELISA, Enzyme-Linked Immunosorbent Assay; F, female; FU, follow-up; IgG, Immunoglobulin G; IgM, Immunoglobulin M; JOA, Japanese Orthopedic Association; KELLNER, Kellner rating scale; LA, local anesthetic; M, male; MO, month; NA, not available; ODI, Oswestry Disability Index; OOT, oxygeneozone therapy; OPRS, Overall Patient Rating Scale; PBC, Psoas compartment block; PIRFT, Percutaneous Intradiscal RadioFrequency Thermocoagulation; RCT, randomized controlled trial; RMDQ, Roland–Morris Disability Questionnaire; SF-36, the 36-Item Short Form Health Survey; SG, study group; SOD activity, Superoxide Dismutase Activity assay; V, volume; VAS, visual analog scale; WK, week.

Table II. Quality Assessment of the Included Studies by using the Cochrane Risk of Bias tool for Randomized Controlled Trials and the AHRQ (Agency for Healthcare Research and Quality) Standards.

Publication	Random Sequence Generation	Allocation Concealment	Selective Reporting	Other Bias	Blinding of Partecipants and Personnel	Blinding of Outcome Assessment	Incomplete Outcome Data	AHRQ Standard
Bruno et al[5] 2020	High	Unclear	High	Unclear	High	Unclear	High	Poor
Niu et al[6] 2018	High	Unclear	High	Unclear	Unclear	Unclear	High	Poor
Rahimzadeh et al[17] 2018	Low	Low	Low	Unclear	Low	Low	Low	Fair
Perri et al[23] 2015	High	Unclear	High	High	Low	Low	Unclear	Poor
Li et al[24] 2014	Low	Unclear	High	High	Unclear	Unclear	Unclear	Poor
Zhang et al[25] 2013	High	Unclear	High	Unclear	High	Unclear	Unclear	Poor
Melchionda et al[3] 2012	High	Unclear	High	Unclear	Low	Low	Low	Poor
Gautam et al[18] 2011	Low	Low	Low	Unclear	Low	Low	Low	Fair
Paoloni et al[2] 2009	Low	Low	Low	Unclear	Low	Low	Low	Fair
Arena et al[15] 2008	Unclear	Unclear	High	High	Unclear	Unclear	Unclear	Poor
Gallucci et al[19] 2007	High	Unclear	High	High	Low	Low	High	Poor
Zambello et al[16] 2006	Unclear	Unclear	High	High	Low	Low	High	Poor
Bonetti et al[20] 2005	High	High	High	Unclear	High	Low	High	Poor
Paradiso et al[21] 2005	High	High	High	High	Unclear	Unclear	Low	Poor
Buric et al[22] 2005	High+	High	High	Unclear	High	Unclear	Low	Poor

"Good quality": All criteria met (i.e., low for each domain); "Fair quality": One criterion not met (i.e., high risk of bias for one domain) or 2 criteria, and the assessment that this was unlikely to have biased the outcome, and there is no known important limitation that could invalidate the results; "Poor quality": One criterion not met (i.e., high risk of bias for one domain) or 2 criteria unclear, and the assessment that this was likely to have biased the outcome, and there are important limitations that could invalidate the results; Poor quality: two or more criteria listed as high or unclear risk of bias.

double-blinded, 5 were single-blinded, and the others were unblinded. Moreover, the risk of attrition bias was unclear for the majority of the studies: in most cases, it was not specified how many patients were screened, how many were excluded from randomization or why, how many were lost to follow-up, and for which specific reason. Flow diagrams depicting the patients' selection process were reported only in 6 papers[2,3,17,18,21,22]. Finally, we found that only 1 out of 15 trials was registered in a public clinical trial registry[17], which should be mandatory according to the Consolidated Standards of Reporting Trials 2010 guidelines.

Patients and Evaluation Methods

Fifteen studies involving a total of 2597 patients with LBP were included in this review. The mean age was 50 years. Fourteen papers included patients affected by LDH assessed by computer tomography (CT) and/or magnetic resonance (MR). Only one study[15] considered patients with degenerative lumbar disease. Baseline and follow-up assessments were based on clinical scores in all studies. Visual Analogue Scale (VAS), Oswestry Disability Index (ODI) and Japanese Orthopedic Association (JOA) were the most used evaluation scores. In only 2 cases[16,20] they were replaced by a modified version of the MacNab score and clinical results were expressed as "excellent" "good" "satisfactory" or "poor" outcomes.

Beyond clinical questionnaires, also other outcomes were considered: six trials reported MRI scores[2,5,22,23], other two studies included lumbosacral X-rays, CT, and electromyography (EMG) examinations[3,21]. Lastly, Niu et al[6] measured also the serum levels of IL-6, IgM, IgG, and SOD activity by enzyme-linked immunosorbent assay (ELISA).

Treatment

With regard to the method of preparation of the injected gas, various machines were tested: 9 papers specifically reported the device adopted for the production of the O2-O3 mixture[2,3,16,19,20,22-25] (Table I), while the rest of the Authors did not specify it. Furthermore, the injected O3 volume and concentration were widely different, ranging from 3 to 20 mL and from 10 to 60 μg/mL of concentration (Table I). Regarding the methods of O2-O3 administration, it was injected by an intradiscal and / or intra-foraminal approach in 11 studies and by a paravertebral approach in 4 studies[2,3,15,16]. The most commonly used intradiscal dosage of O2-O3 was 10 mL at 30 μg/mL concentration, whereas, for paravertebral injections, it was 20 mL at 20 μg/mL. The treatment protocols were very different in terms of the number of injections and frequency (Table I).

Complications

No major complications or serious adverse events were reported in any of the trials included. Only one study[3] described a case of a patient experiencing a fainting phenomenon immediately after gas injection, two drop-outs related to gastrointestinal symptoms and other two related to hypertension.

Reported Clinical Outcome

Paoloni et al[2] conducted the only controlled study against sham therapy demonstrating that paravertebral injections of O2-O3 are safe and effective in relieving pain, as well as reducing both disability and analgesic drug use compared to the control group. Niu et al[6] showed similar results using an intradiscal O2-O3 approach compared to conventional drug treatment. They demonstrated that lower concentrations of medical ozone (20 μg/ml and 40 μg/ml) reduced the expression of IL-6, IgG and IgM in serum, thus presenting analgesic and anti-inflammatory effects, while higher concentrations (60 μg/ml) can increase their expression, presenting therefore pro-inflammatory effects correlated with increased pain perceived by patients. Bruno et al[5] and Bonetti et al[20] found a higher success rate in the O2-O3 group compared to peri-radicular corticosteroid injections at 1 and 6 months of follow-up, respectively. In another study[16], paravertebral OOT results more effective than the use of peridural steroid injections in short- and long-term evaluation. Perri et al[23], Gallucci et al[19], and Zhang et al[25] demonstrated that combined treatment with O2-O3 and CS was superior to CS alone at both 6 and 12 months of follow-up, although the differences between two groups were not always significant. One study[3] focusing on the treatment of radiculopathy demonstrated that OOT is effective and appears to have persistent action in reducing pain for up to 6 months after injection. Two studies compared the use of intradiscal OOT with microdiscectomy, demonstrating similar results between the two techniques in pain control and disability reduction at long-term follow-up[21,22]. Microdiscectomy appeared to be superior in the short term in patients with severe pain due to large migrated fragments, whereas OOT in patients with con-

tained disc herniation; anyway, at mid-term follow-up, no significant difference between the two techniques were documented, thus supporting the beneficial role of OOT with respect to surgery. The other studies have shown that OOT provides better results than intradiscal laser irradiation at the ODI score[17], and that OOT in combination with PIRFT[18] or PCB[24] may provide superior clinical outcomes at short- and medium-term follow-up compared to ozone alone. Finally, Arena et al[15] documented that the integration of OOT with TENS, bio-resonance magnetotherapy, and postural rehabilitation could guarantee a longer maintenance of improvement in patients affected by lumbar degenerative disease.

Discussion

The present systematic review has confirmed the strong interest of the scientific community in the use of ozone for the treatment of muscolo-skeletal diseases, as evidenced by the increasing number of papers published in the last years, which investigated OOT use in different clinical scenarios, from degenerative conditions like OA to more complex systemic diseases[9,26-30]. Upregulation of endogenous antioxidant systems and the activation of pathways suppressing inflammatory processes are the main mechanisms of action of OOT, which is also a versatile approach: it can be used both as a "local" treatment, such as in the case of intra-articular[8] or peri-lesional application, or as a systemic therapy, as in the case of fibromyalgia, where auto-hemotransfusion or rectal insufflations with ozone can provide significant pain reduction and symptomatic improvement[29,30], associated to systemic effects, such as the increase of serum serotonin and decreased concentration of some markers of oxidative stress[30].

Despite this increasing interest, the main finding of our review is the overall modest quality of the available evidence concerning OOT in the treatment of LBP and radiculopathies. Criticisms have already been published on the quality of studies on the use of OOT for the treatment of other conditions, such as knee osteoarthritis[8,31]. Despite only including RCTs, the critical assessment revealed relevant bias in all the 15 studies considered, thus limiting our possibility to clearly understand how ozone therapy compares with "standard" approaches currently used. Furthermore, the differences in clinical scores, in the samples tested, and other methodological biases did not allow to perform a meta-analysis of the results. All the analyzed papers were characterized by weak power analysis and the lack of a clear statement concerning the primary outcomes and the sample size calculation, with the exception of two trials[2,17], where these details were fully clarified. Consequently, a high risk of underpowered sample size is present, with obvious consequences on the significance and reliability of results. Most of the articles did not report on losses to follow-up and their management, on blinding and randomization and/or allocation methods, when applicable. There was an overall weak adherence to the Consolidated Standards of Reporting Trials guidelines for reporting methods and results in RCTs, and therefore none of the studies included could be evaluated as a "good-quality" RCT according to the AHRQ standard.

The second issue emerged concerns the remarkable differences in ozone therapeutic protocols in terms of concentration and volumes, injection techniques, duration and timing of the treatment: all these variables make study comparison very hard and shed shadows on the standardization of the procedure, which would be anyway essential to avoid potential drawbacks, especially considering that reports already exist suggesting that certain concentration of O2-O3 might be even detrimental[6]. Despite these flaws, some useful clinical considerations can be drawn from the analysis of the literature. The results from the included studies are overall positive and support the efficacy of OOT in reducing pain and improving the functional status of LBP patients, including those affected also by radicular irradiation (sciatica in the majority of cases). In fact, as suggested by some studies[19,20,25], intraforaminal or peri-radicular application of OOT contributes to normalize nerve function and its micro-environment[38], thanks to an eutrophic effect played by ozone, which favors the normalization of cytokines and prostaglandins concentrations, promotes the increase of superoxide dismutase levels, and improves perineural microcirculation by reducing local hypoxia due to both arterial compression and venous stasis.

Furthermore, OOT proved to be a safe therapy, with a very low adverse event rate, as revealed by all the RCTs included. Beyond the findings of the present review, some recent papers[26,32] focused on the potential risks of OOT in the development of major complications, reporting four cases of severe spine infections in patients treated with ozone therapy. However, these cases were bare-

ly described, missing most of the crucial details of the treatment: therapy protocol, asepsis technique, and who performed the therapy. Another study[33] reported the case of a 57-year-old patient who developed fulminant septicemia after 6 cycles of paravertebral injections. The most likely physio-pathological mechanism was direct inoculation of bacteria during the procedure. This is the only case of fatal complications secondary to paravertebral ozone therapy reported in the literature, although it seems more likely related to the procedure rather than to the substance. Finally, Vanni et al[34] reported the possible formation of hard adhesions between the nerve root and the dural sac/herniated discs in patients in whom ozone was administered intra-foraminally. Despite these rare cases, O2O3 injections remain a practice considered safe by most of the literature with few and infrequent adverse events[35]. However, it is fundamental to emphasize the crucial role of using all necessary precautions to ensure sterility and uncontaminated gas mixture.

In the available literature, mean follow-up was performed in the short to medium term, with the exception of 2 studies[21,22] that reported data beyond one year follow-up. In light of this, our primary outcome was to understand the potential of OOT in terms of pain relief at mid-term evaluation, the median follow-up of all included studies being 6 months (range 1- 12), which was therefore considered the reference time frame for the present review.

Among the 9 studies in which OOT was statistically more effective than control group, 6 papers[2,3,16,19-21] showed the most significant improvements in clinical and functional outcomes up to 6-month follow-up. This performance was maintained at 1-year follow-up in 3 other studies[6,17,25], and persisted up to 18 months in one[22]. These data suggest that OOT shows its greatest efficacy in patients with LBP during midterm evaluation. This finding, if confirmed by further research, could be an accurate estimation of the expected duration of beneficial effects following OOT in LBP, and it would help clinicians in their patients' counselling. Studies with longer follow-up are anyway needed to allow us to reach more definitive conclusions.

Currently, in the "real world" setting, the most widely used O2-O3 technique to treat LBP patients consists of paravertebral intramuscular injection and, less frequently, of intra-discal and intra-foraminal approaches[14]. In spite of this, there is an abundancy of RCTs analyzing the results of intra-discal/intra-foraminal techniques (11 *vs.* 4), reflecting therefore the lack of high evidence studies on the use of paravertebral ozone injections. The intradiscal approach consists of X-rays guided injections of high concentrations of O2-O3 aimed at reducing intradiscal pressure through glycosaminoglycan lysis, proteoglycan reduction, and disc dehydration[36]. The need for fluoroscopy or tomographic guidance has limited the uptake of this technique in the common clinical rehabilitation setting[14], where radiologic equipment may not be easily available. Comparing the efficacy of intradiscal ozone with other treatments, we found that four studies demonstrated overall superiority over corticosteroids[16,19,20,23] in reducing pain and disability, especially in the middle term follow-up (up to 6 months). In two of these trials, ozone was used alone or even in combination with CS. According to some authors[19,23], it would seem that the combination of the two can provide better results in achieving simultaneous short- and medium-term pain control. These main effects could be related to the action on different and independent metabolic pathways. The indirect anti-inflammatory action determined by O3 through the activation of antioxidant systems should work synergistically with the direct anti-inflammatory and immunosuppressive actions exerted by steroid therapy[8].

The results of OOT were more uncertain when compared to more invasive procedures. Intradiscal ozone injections were superior in terms of pain reduction and functional improvement compared to intradiscal laser irradiation[17], whereas no significant superiority over microdiscectomy was documented[21,22]. The combined treatment of PIRFT[18] or PCB[24] and ozone resulted in a better outcome than the use of ozone alone. While intradiscal injections appear to use the direct, mechanical action of ozone, intramuscular paravertebral injections might indirectly affect the inflammatory cascade by altering the degradation of arachidonic acid into inflammatory prostaglandins and stimulating fibroblastic activity, increasing collagen deposition and the initiation of the repair process[37]. The advantage of intramuscular OOT is that it can be performed in an outpatient setting, without exposing patients to X-rays, and could also produce a therapeutic effect on trigger points in the paraspinal musculature[38]. On the other hand, a potential flaw could be related to the inaccuracy of the landmark-guided technique, especially in obese patients or in cases of lumbosacral junction abnormalities, such as sacralization of the

L5 vertebra or lumbarization of S1[39]. New studies are exploring even more accurate methods of administration, particularly the use of ultrasound guidance[37]. Comparing the efficacy of paravertebral ozone with other treatments, we found that all studies considered demonstrated overall superiority over placebo, CS, and analgesic medications. Interesting results emerged from an article[15] that analyzed the use of an integrated approach combining OOT with TENS, bio-resonance magnetotherapy, and postural rehabilitation: this multidisciplinary conservative management achieved better and longer lasting results in terms of pain reduction and functional improvement compared to the use of ozone alone.

Limitations

The present manuscript suffers some major limitations. First of all, the lack of a meta-analysis of data, which was not possible due to the low number of trials comparing the same treatment groups and, above all, the poor homogeneity of data, with unmatched follow-up evaluations and different clinical scores adopted. Another limitation is the fact that, in some studies, OOT was combined to other therapeutic strategies, thus negatively affecting the possibility of evaluating the sole ozone contribution to the clinical outcome. Lastly, despite being a systematic review of RCTs, the poor methodological quality of the trials prevents from defining clear indications for OOT use and drawing reliable conclusion on the efficacy of OOT compared to other approaches.

Conclusions

OOT is a promising approach for LBP, with a good safety profile and therapeutic potential, and it could be included among the armamentarium of the conservative management of this common condition. Based upon the evidence gained from the present review, OOT provides better outcomes compared to local administration of CS and systemic drugs. Less clear is its efficacy against surgical procedures such as microdiscectomy. Nonetheless, the current paucity of high-quality trials warrants further studies to elucidate some fundamental issues regarding the optimal therapeutic protocols, i.e., the number of injections, dosages, and site of administration (paravertebral *vs.* peri-radicular *vs.* intra-discal).

Conflicts of interest

The authors declare no conflicts of interest.

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