OTGC
ORGANISMO TOSCANO GOVERNO CLINICO

SETTING DI SOMMINISTRAZIONE DELL'OZONOTERAPIA
AD INTEGRAZIONE DEL DOCUMENTO GIÀ APPROVATO CON DECISIONE N. 12 DEL 28/05/2020 "IL PERCORSO E LE INDICAZIONI D'USO DELL'OZONOTERAPIA NELLA PATOLOGIA DISCALE LOMBARE E NELLA LOMBOSCIATALGIA"
CORREDATI DALLA REVISIONE DEI DATI DELLA LETTERATURA

Allegato A

Decisione Comitato Tecnico Scientifico n. 25 del 12/07/2023

Regione Toscana

SST Servizio Sanitario della Toscana

Articolazione funzionale dell'Organismo Toscano per il Governo Clinico, ai sensi dell'art. 49 ter della l.r. 40/2005:

a) Coordinatore;
b) Ufficio di coordinamento;
c) Comitato tecnico scientifico

Coordinatore dell'OTGC
Prof. Stefano Grifoni

Supporto amministrativo:
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8. CODIFICHE DELLE PRESTAZIONI E SETTING ASSISTENZIALI

Le codifiche delle prestazioni sono quelle descritte nel Catalogo delle prestazioni specialistiche ambulatoriali
(http://dati.toscana.it/dataset/regione-toscana-catalogo-delle-prestazioni-ambulatoriali)
Via periferica

- 6E42 Nucleolisi percutanea intradiscale (guidata con immagini RX/TC/RM) con ozono
  setting assistenziale: erogabile in regime ambulatoriale (cod. ICD-9_CM 80.59) presso strutture sanitarie in possesso dei requisiti previsti per le attività di Radiologia diagnostica e interventistica e di Chirurgia ambulatoriale (DPGR 79/R 2016 e ss.mm.ii.);
- 6E45 Ossigeno – ozonoterapia intra-foraminale
  setting assistenziale: erogabile in regime ambulatoriale (cod. ICD-9_CM 80.59) presso strutture sanitarie in possesso dei requisiti previsti per le attività di Radiologia diagnostica e interventistica e di Chirurgia ambulatoriale (DPGR 79/R 2016 e ss.mm.ii.);
- 6E46 Ossigeno – ozonoterapia paravertebrale
  setting assistenziale: erogabile in regime ambulatoriale presso studi medici/strutture sanitarie in possesso dei requisiti previsti (DPGR 79/R 2016 e ss.mm.ii.);
- 1779 Iniezione di sostanze terapeutiche in articolazione – legamento
  setting assistenziale: erogabile in regime ambulatoriale presso studi medici/strutture sanitarie in possesso dei requisiti previsti (DPGR 79/R 2016 e ss.mm.ii.).

Sintesi delle evidenze disponibili sulla OZONOTERAPIA per ERNIA DISCALE nel periodo 2018-2023

Domanda: La ozonoterapia (OZO) intradiscale, intraforaminale o paravertebrale, rispetto ad altre opzioni terapeutiche, è utile per ridurre il dolore in soggetti con ernia discale se non recede con terapia medica entro alcune settimane?

Evidenze cercate: revisioni sistematiche di RCT pubblicate negli ultimi 5 anni; RCT pubblicati negli ultimi 5 anni e non inclusi nelle revisioni sistematiche.

Misura di esito primaria: pain relief come indicato dagli autori.

Metodi di valutazione della qualità delle evidenze: AMSTAR per le revisioni sistematiche; Cochrane Risk of Bias 2.0 (RoB2.0) per gli RCT

Revisioni sistematiche individuate:

Costa 2018 e Rimeika 2021 non sono considerate in quanto non valutano il rischio di bias (RoB) degli studi inclusi.

De Andrade 2019 include studi che confrontano intraforaminale o intramuscolare con diverse terapie (kenacort, depomedrol, radiofrequenza, placebo). La valutazione del RoB è effettuata con Cochrane RoB tool; su 6 studi inclusi, tutti hanno almeno un item 'unclear' e 5 almeno uno con 'high' RoB. La misura di esito primaria è il pain relief con varie metodiche di misura: a 3 mesi due studi danno risultati contrastanti (uno indifferente (RR 1.14 [0.99, 1.31]), l'altro favorevole (RR= 4.67 [1.16, 18.71])); a 6 mesi tre studi (717 pz) indicano un RR=2.2 (95%CI: 1.87-2.60) di pain relief (32% nei controlli). Conclusioni: "The systematic review has shown that ozone therapy used for six months for lum-bar pain relief is more effective than other therapies; however, this result is not definitive as data from studies with moderate to high risk of bias were used."

Huang 2019 conduce una network meta-analisi che confronta direttamente e indirettamente diversi trattamenti. L'ozonoterapia è inclusa, assieme alla chymopapain nella chemonucleolisi, ma alcuni degli studi inclusi usavano la chymopapain. Sono inclusi 56 studi: 48% con high risk of bias, 31% moderate risk. L'effetto a breve termine per la chemonucleolisi è ES= 0.28 (0.14,0.56) per pain relief (effetto da piccolo a moderato).

Sconza 2021 confronta OZO intradiscale, intraforaminale, epidurale, intramuscolare (eventualmente associato al trattamento convenzionale, anestetico locale, corticosteroidi, collagenasi, TENS, terapia psicosomatica e posturale o magnetoterapia) con diverse terapie (microdiscectomia, kenacort, TENS, radiofrequenza, placebo). Gli studi sono descritti singolarmente e non viene condotta una meta-analisi data la loro diversità. Sono inclusi 15 studi: nessuno di alta qualità, 3 di qualità discreta, 12 di qualità bassa. Estratti: "The analysis of literature revealed overall poor methodologic quality, withmost 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". "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". "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 papers26,32 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". Commento: la significatività statistica, ma non l'importanza clinica sono discusse, come non lo è l'eterogeneità degli effetti tra studi (tecniche, misure, confronti, ecc.).

RCT:

Clavo 2021 è uno studio a 3 bracci che confronta OZO intradiscale o ossigeno intraforaminale, associati a steroidi e anestetici vs (micro)discectomia. Lo studio intendeva includere 156 pazienti ma venne interrotto a 19 pazienti per arruolamento molto lento. Estratto "Five years after the treatment of the last recruited patient (median follow-up: 78 months), the requirement for further surgery was 20 % for patients in the ozone group and 60 % for patients in the oxygen group. 11 % of patients initially treated with surgery also required a second surgery". Lo studio è classificato ad alto RoB.

Kelesis 2022 confronta la chemonucleolisi con ossigeno-OZO intradiscale con la microdiscetomia in 49 pazienti con dolore (5-10 NRS) da almeno 6 settimane, con un disegno di non-inferiorità. Lo studio è di buona qualità metodologica. Estratto: "Intradiscal oxygen-ozone chemonucleolysis for single-level lumbar disc herniations unresponsive to medical management, met the non−inferiority criteria to microdiscectomy on 6-month mean leg pain improvement. Both treatment groups achieved similar rapid significant clinical improvements that persisted and overall, 71% undergoing intradiscal oxygen-ozone were able to avoid surgery." Per le piccole dimensioni dello studio, queste evidenze sono di qualità moderata, anche in presenza di buona qualità metodologica (limiti della Optimal Information Size).

Krahulik 2023 ha randomizzato 150 pazienti all'infiltrazione periradicolare di due steroidi (betametasone o methylprednisolone, con bupivacaina) o OZO paravertebrale. Outcome: pain relief (VAS) a 12 settimane. Estratto: "Clinical improvement occurred in all three groups but Diprophos showed the statistically best treatment effect compared to Depomedrone and ozone. Disc herniation resulting in radicular pain had a statistically significant better effect in comparison with spondylolisthesis in the Diprophos and ozone groups, but the ozone group showed heterogeneity depending on treatment effect and indication." Lo studio non adottava mascheramento del partecipante e outcome assessor ed è di bassa qualità metodologica.

Salehpour 2021 confrontava ossigeno-OZO intradiscale e terapia medica con sola terapia medica (Naproxene) in soggetti con ernia discale e dolore con radiculopatia da meno di 10 giorni. Estratto: "Mean pain intensities estimated by VAS and improvement of restless leg syndrome were not significantly different between the two groups during two weeks ($p=0.8$), three months ($p=0.5$) and six months ($p=0.9$) after the intervention. Pain intensity was found to be lower in both groups after the intervention compared with before treatment ($p=0.001$ for both). Moreover, significant differences were found between two groups in the Lasegue test during two weeks ($p=0.02$) and six months ($p=0.01$) after the intervention". Lo studio non adottava mascheramento del partecipante e outcome assessor ed è di bassa qualità metodologica.

Sucuoglu 2021 arruolava 46 pazienti con LBP acuto ed ernia discale randomizzandoli a "Intramuscular ozone injections (20 μg/ml for the first 4 sessions and 25 μg/ml for the next 4 sessions in the treatment group) vs 0.1 μg/ml for all the sessions in the placebo group". Pur essendo lo studio mascherato la randomizzazione era inadeguata e la qualità dello studio moderata. Estratto "As an additional treatment combined with conservative treatment, lumbar POI can lessen pain and disability in patients with acute LDH." A fronte di uno score di partenza di circa 7.5 nei due gruppi, lo score medio a 2 mesi era 2.6 per OZO e 4.8 per placebo ($p<0.05$). Un limite di questo studio è l'assenza di controllo attivo.

Bibliografia

Costa T et al. Ozone therapy for low back pain. A systematic review. ACTA REUMATOL PORT. 2018;43:172-181

Huang R et al. ***Nonsurgical medical treatment in the management of pain due to lumbar disc prolapse: A network meta-analysis.*** **Seminars in Arthritis and Rheumatism 49 (2019) 303-313**

de Andrade PR et al. ***Effectiveness of ozone therapy compared to other therapies for low back pain: a systematic review with meta-analysis of randomized clinical trials*****. Rev Bras Anestesiol. 2019;69(5):493-501**

Rimeika G et al. ***Metanalysis on the effectiveness of low back pain treatment with oxygen-ozone mixture: Comparison between image-guided and non-image-guided injection techniques*****. European Journal of Radiology Open 8 (2021) 100389**

Sconza C et al. ***Oxygen-ozone therapy for the treatment of low back pain: a systematic review of randomized controlled trials.*** **European Review for Medical and Pharmacological Sciences 2021**

Clavo et al, Ozone therapy versus surgery for lumbar disc herniation: A randomized double-blind controlled trial. Complementary Therapies in Medicine 59 (2021) 102724

Kelesis A et al, Intradiscal oxygen-ozone chemonucleolysis versus microdiscectomy for lumbar disc herniation radiculopathy: a non−inferiority randomized control trial.The Spine Journal 22 (2022) 895−909

Krahulik et al, Periradicular corticosteroid infiltration for radicular pain – comparison of Diprophos and Depomedrone and ozone effects, Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub. 2023 Mar; 167(1):80-84.

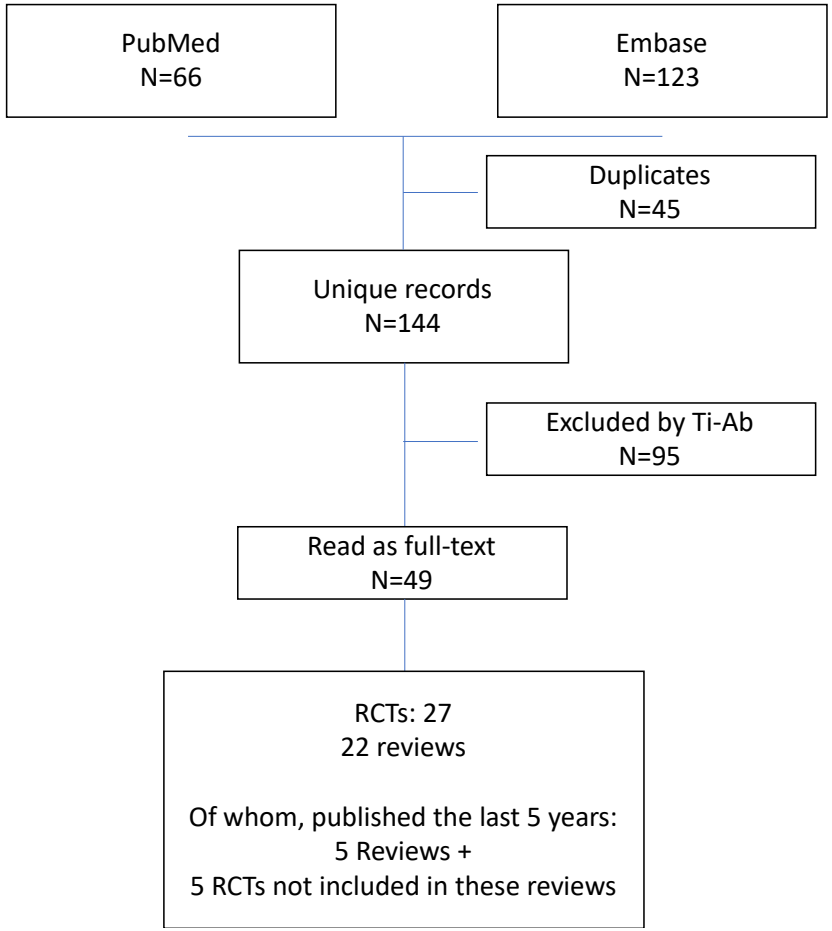
Salehpour et al, Ozone Therapy as a Minimally-invasive Alternative in patients with Acute Lumbar Disc Herniation: A Randomized Clinical Trial. Trauma Monthly 2021; 26(4): 206-212

Sucuog˘lu et al, Does paravertebral ozone injection have efficacy as an additional treatment for acute lumbar disc herniation? A randomized, double-blind, placebo-controlled study. Journal of Back and Musculoskeletal Rehabilitation 34 (2021) 725–733

PUBMED:
Stringa: (Oxygen-ozone OR ozone) AND (low back pain OR disc herniation)
Filters: **Clinical Trial, Meta-Analysis, Randomized Controlled Trial, Review, Systematic Review**

EMBASE:
('oxygen ozone' OR 'ozone'/exp OR ozone) AND ('low back pain'/exp OR 'low back pain' OR (low AND ('back'/exp OR back) AND ('pain'/exp OR pain)) OR 'disc herniation'/exp OR 'disc herniation' OR (disc AND ('herniation'/exp OR herniation))) AND ('clinical trial' OR guidelines OR [review]/lim)

Searched on 19 June 2023



Assessment of quality of systematic reviews published in the last 5 years (2018-2023) – AMSTAR tool

1. **Raul Ribeiro de Andrade et al. *Effectiveness of ozone therapy compared to other therapies for low back pain: a systematic review with meta-analysis of randomized clinical trials*. Rev Bras Anestesiol. 2019;69(5):493-501**	
N studies included	RS + MA; 6 RCTs selected (3 included in MA) Included RCTs: Bonetti 2005 (included in MA); Zambello 2006 (included in MA); Gallucci 2007; Canovas 2009; Paoloni 2009 (included in MA); Canovas 2015
Results	Of the 779 identified articles, six selected clinical trials show that ozone therapy is more effective for lumbar pain relief; however, they were mostly classified as having a high or uncertain risk of bias (Cochrane Handbook). The meta-analysis regarding the effectiveness of pain relief did not show a significant difference between groups in the three-month period (RR = 1.98, 95% CI: 0.46-8.42, p = 0.36; 366 participants). It also showed greater effectiveness of the ozone therapy at six months compared to other therapies (steroid and placebo) (RR = 2.2, 95% CI: 1.87-2.60, p < 0.00001; 717 participants).
AMSTAR checklist	
1.Did the research questions and inclusion criteria for the review include the components of PICO?	P: patients over 18 years old, male or female, diagnosed with low back pain in hospitals or clinics for pain management. I:percutaneous ozone therapy for low back pain C: another type of low back pain therapy, such as steroids and placebo O: Primary variable was pain relief, considering the effect and time of symptom follow-up in the studies. Relief was determined as total absence of pain reported by patients or by a score lower than 1 on the Visual Analogue Scale (VAS) Study: RCTs; excluded duplicate articles and those without full description of the data. Follow-up: 3-6 months
2.Did the report of the review contain an explicit statement that the review methods were established prior to the conduct of the review and did the report justify any significant deviations from the protocol?	Yes - A protocol was developed a priori and registered at the Prospero - International Prospective Register of Systematic Review (http://www.crd.york.ac.uk/PROSPERO/ displayrecord.php? ID = CRD42018090807) at York University on March 14, 2018, registration no. CRD42018090807. → link not accessible
3. Did the review authors explain their selection of the study designs for inclusion in the review?	No explanation for including only RCTs
4. Did the review authors use a comprehensive literature search strategy?	Consulted databases: Medline, Embase, Lilacs, ScopusPreview + the references of the included articles and previous systematic reviews on this subject. Timeframe: up to 2018. No search of gray literature; no info on the date in which search was conducted.
5. Did the review authors perform study selection in duplicate?	Yes (Two independent investigators (RRA and FBT) reviewed the titles and abstracts of the retrieved articles)
6. Did the review authors perform data extraction in	Not reported

duplicate?	
7. Did the review authors provide a list of excluded studies and justify the exclusions?	Yes (9 articles excluded by full text are cited in the reference list, with reasons for exclusion)
8. Did the review authors describe the included studies in adequate detail?	Yes (table 1)
9. Did the review authors use a satisfactory technique for assessing the risk of bias (RoB) in individual studies that were included in the review?	RoB assessed using the Cochrane Collaboration tool
10. Did the review authors report on the sources of funding for the studies included in the review?	Not reported
11. If meta-analysis was performed did the review authors use appropriate methods for statistical combination of results?	Authors did no justify combining the data in a meta-analysis and did not investigate the causes of any heterogeneity
12. If meta-analysis was performed, did the review authors assess the potential impact of RoB in individual studies on the results of the meta-analysis or other evidence synthesis?	No
13. Did the review authors account for RoB in individual studies when interpreting/ discussing the results of the review?	Yes, RoB was discussed when interpreting the results, but no sensitivity analysis including only low risk of bias
14. Did the review authors provide a satisfactory explanation for, and discussion of, any heterogeneity observed in the results of the review?	No (Authors state: During the 3-month follow-up, there was statistical heterogeneity in two studies15,16 (Tau2 = 0.89; chi2 = 4.52; df = 1, p = 0.03; I2 = 78%). However, withdrawing one of the studies in order to assess sensitivity precludes meta- analysis. Thus, sensitivity analysis was not necessary. During the six-month follow-up, there was no statistical heterogeneity between three studies15,16,19 (Tau2 = 0.00; chi2 =0.37; df=2, p= 0.83; I2 =0%).
15. If they performed quantitative synthesis did the review authors carry out an adequate investigation of publication bias (small study bias) and discuss its likely impact on the results of the review?	No, publication bias was not investigated nor discussed.
16. Did the review authors report any potential sources of conflict of interest, including any funding they received for conducting the review?	The authors reported no competing interests

2. Costa T. Ozone therapy for low back pain. A systematic review. ACTA REUMATOL PORT. 2018;43:172-181	
N studies included	7 studies (Zambello 2006, Bonetti 2005, Perri 2015 (T2 shine), Gallucci 2007, Paoloni 2009, Paradiso 2005, Apuzzo 2014)
Results	From 439 references retrieved after duplicates removal, inclusion and exclusion criteria were applied, and 7 studies were included in the final revision. One article compared treatment with ozone versus placebo, one ozone and global postural re-education versus global postural re-education alone, two the combina- tion of ozone with steroid versus steroid alone, two ozone versus steroid and one ozone versus micro-dis- cectomy.Allbutthestudycomparingozoneapplication with micro-discectomy, showed similar or better results in the experimental group. Only three studies evalua- ted the presence of side effects. In two papers no complication was reported, and in the other, a low per- centage of adverse effects was observed, not signifi- cantly different between the two study groups.
AMSTAR checklist	
1.Did the research questions and inclusion criteria for the review include the components of PICO?	P: patients with lumbar pain of degenerative causes I: ozone C: non-ozone intervention O: (according to studies) All performed at least a clinical evaluation such as Visual Analog Scale (VAS) for pain, Oswestry Disability Index (ODI) and McNabb method, and 4 also underwent a complementary assessment with Computed Tomography (CT) and/or Magnetic Resonance Imaging (MRI). Study: original articles. Follow-up: (according to studies) 2 weeks to 5 years
2.Did the report of the review contain an explicit statement that the review methods were established prior to the conduct of the review and did the report justify any significant deviations from the protocol?	No
3. Did the review authors explain their selection of the study designs for inclusion in the review?	Not applicable (no selection by study design)
4. Did the review authors use a comprehensive literature search strategy?	Searched Pubmed and Embase. No search of reference lists / bibliographies of included studies, grey literature etc.
5. Did the review authors perform study selection in duplicate?	Yes (two independent reviewers)
6. Did the review authors perform data extraction in duplicate?	Yes (The data was processed by two inde- pendent reviewers and the information was collected based on pre-defined variables)
7. Did the review authors provide a list of excluded studies and justify the exclusions?	No
8. Did the review authors describe the included studies in adequate detail?	Yes (table 1)
9. Did the review authors use a satisfactory technique for assessing the risk of bias (RoB) in individual studies that were included in the review?	No (RoB in individual studies was not assessed)
10. Did the review authors report on the sources of funding for the	Not reported

studies included in the review?	
11. If meta-analysis was performed did the review authors use appropriate methods for statistical combination of results?	NA
12. If meta-analysis was performed, did the review authors assess the potential impact of RoB in individual studies on the results of the meta-analysis or other evidence synthesis?	NA
13. Did the review authors account for RoB in individual studies when interpreting/ discussing the results of the review?	No (RoB not assessed)
14. Did the review authors provide a satisfactory explanation for, and discussion of, any heterogeneity observed in the results of the review?	No
15. If they performed quantitative synthesis did the review authors carry out an adequate investigation of publication bias (small study bias) and discuss its likely impact on the results of the review?	No
16. Did the review authors report any potential sources of conflict of interest, including any funding they received for conducting the review?	Not reported

3. Huang R et al. *Nonsurgical medical treatment in the management of pain due to lumbar disc prolapse: A network meta-analysis.* Seminars in Arthritis and Rheumatism 49 (2019) 303-313	
N studies included	Overall: 58 studies in global effects NMA and 74 studies in pain intensity NMA Ozone considered within the category chemonucleolysis agents (chymopapain, ozone) RCTs on ozone (from reference list): Melchionda 2012; Perri 2015 (t2 shine); Gallucci 2007; Paoloni 2009.
Results	Treatments with a statistically significant improvement compared with inactive control (A) at short-term follow-up included TESIs, caudal epidural steroid injections, interlaminar EIs, chemonucleolysis, and non-opioids. At the long-term follow-up, TESIs and chemonucleolysis continued to demonstrate significant improvement against placebo.
AMSTAR checklist	
1.Did the research questions and inclusion criteria for the review include the components of PICO?	P: patients 18 years or older, diagnosed with LDP clinically via a positive straight leg raised test or by CT/MRI imaging. If a trial included mixed popu- lations of LDP with other low back conditions (eg. spinal stenosis, degenerative disc disease) it was only included if data was presented separately, or if the data was presented together, only if more than 50% of the participants were diagnosed with LDP. I/C: placebo; standard/conventional care; chemonucleolysis agents (chymopapain, ozone); traction; auto- nomic drugs (clonidine); non-opioids (oral, intravenous, or

	intramus- cular including NSAIDs, acetaminophen, and local anesthetics); manipulation; steroids (oral/intramuscular/intravenous); interlami- nar/Intradiscal/nerve block epidural steroid injections; transforminal/ periradicular epidural steroid injections; caudal epidural steroid injections; cytokines/Immunomodulators (cytokine-modulating treatments targeting tumor necrosis factor alpha, adalimumab, inflix- imab, entanercept); and neuropathic pain modulators (tricyclic anti- depressants, amitryptiline, nortiptyline topiramate; gabapentin; pregabalin; 5HT receptor inhibitors) O: pain and/or global effect with sufficient data for estimating an odds ratio (OR) or weighted mean difference (WMD) with 95% credible interval (CI) Study: English language RCT (foreign language RCT were included if their data was reported in an English systematic review) Follow-up: 1 month, 3 months, 6 months, and 12 months
2.Did the report of the review contain an explicit statement that the review methods were established prior to the conduct of the review and did the report justify any significant deviations from the protocol?	No
3. Did the review authors explain their selection of the study designs for inclusion in the review?	No explanation for including only RCTs
4. Did the review authors use a comprehensive literature search strategy?	Consulted databases: Medline, Embase, Cochrane library + the references of the included articles and previous systematic reviews on this subject. From from inception to September 7th, 2017. No search of grey literature
5. Did the review authors perform study selection in duplicate?	Yes (two independent reviewers)
6. Did the review authors perform data extraction in duplicate?	Yes (two independent reviewers sing a standardized data extraction form in Excel)
7. Did the review authors provide a list of excluded studies and justify the exclusions?	No
8. Did the review authors describe the included studies in adequate detail?	Yes (Additional File 1)
9. Did the review authors use a satisfactory technique for assessing the risk of bias (RoB) in individual studies that were included in the review?	Yes (Cochrane Collaboration's ‘Risk of bias’ tool, performed by one reviewer and verified by a second reviewer)
10. Did the review authors report on the sources of funding for the studies included in the review?	Not reported
11. If meta-analysis was performed did the review authors use appropriate methods for statistical combination of results?	Bayesian random effects network meta-analysis
12. If meta-analysis was performed, did the review authors assess the potential impact of RoB in individual studies on the results of the meta-analysis or other evidence synthesis?	No
13. Did the review authors account for RoB in individual studies when interpreting/ discussing the results of the review?	Addressed only in the study limitations

14. Did the review authors provide a satisfactory explanation for, and discussion of, any heterogeneity observed in the results of the review?	Yes ("We evaluated the between study heterogeneity, by examining the findings of standard pairwise meta-analyses using visual inspection of the forest plots, as well as the I2 statistic. (P< 0.10 and I2> 50% indi- cated evidence of heterogeneity")
15. If they performed quantitative synthesis did the review authors carry out an adequate investigation of publication bias (small study bias) and discuss its likely impact on the results of the review?	eTable F1 for publication bias analysis + Fig. F1 for funnel plots.
16. Did the review authors report any potential sources of conflict of interest, including any funding they received for conducting the review?	None

4. Rimeika G et al. *Metanalysis on the effectiveness of low back pain treatment with oxygen-ozone mixture: Comparison between image-guided and non-image-guided injection techniques*. European Journal of Radiology Open 8 (2021) 100389	
N studies included	45 studies included for MA RCTs (retrieved from the list in the supplementary material): Andreula 2003 (Minimally Invasive Oxygen-Ozone Therapy for Lumbar Disk Herniation); Gallucci 2007; Wu 2009; Paoloni 2009; Gautam 2011; Li 2014; Morselli et al., J Pain Relief 2015; Perri 2016 (Indications); Perri 2015 (t2 shine); Elawamy 2018; Niu 2018; Rahimzadeh 2018; Ercalik 2020
Results	The overall treatment effect size shows a mean reduction in pain and pain perception of about − 4.48 in case of image-guided oxygen-ozone injection (95%CI: − 5.20 to − 3.75; p < 0.0001; variance:0.14), versus − 3.17 in case of non-image-guided oxygen-ozone injection (95% CI: − 4.3 to − 2.04; p < 0.0001; variance:0.33); mean difference in effect size and overall number of collected evidences between the two groups is statistically significant (p < 0.05).
AMSTAR checklist	
1.Did the research questions and inclusion criteria for the review include the components of PICO?	P: treatment of LBP and sciatica I: percutaneous image-guided and non-image-guided oxygen-ozone injections C: - O: Visual Analogue Scale for pain (VAS), Oswestry Disability Index (ODI), McNab and modified McNab clinical outcome score, Roland-Morris Disability Questionnaire (RMDQ), and Brief Pain Inventory (BPI) Study: Only case reports, congress posters/abstracts, animal model studies,

	reviews/meta-analyses, as well as methodological or ex-vivo researches were excluded. Follow-up: 1-6 months
2.Did the report of the review contain an explicit statement that the review methods were established prior to the conduct of the review and did the report justify any significant deviations from the protocol?	No
3. Did the review authors explain their selection of the study designs for inclusion in the review?	Both interventional and observational studies were included. Case reports, congress posters/abstracts, animal model studies, reviews/meta-analyses, as well as methodological or ex-vivo researches were not included.
4. Did the review authors use a comprehensive literature search strategy?	Only National Library of Medicine MEDLINE database; between January 1980 and December 2020
5. Did the review authors perform study selection in duplicate?	Not specified
6. Did the review authors perform data extraction in duplicate?	Not specified
7. Did the review authors provide a list of excluded studies and justify the exclusions?	No
8. Did the review authors describe the included studies in adequate detail?	No (only list of studies in supplementary table)
9. Did the review authors use a satisfactory technique for assessing the risk of bias (RoB) in individual studies that were included in the review?	No (RoB not assessed)
10. Did the review authors report on the sources of funding for the studies included in the review?	Not reported
11. If meta-analysis was performed did the review authors use appropriate methods for statistical combination of results?	Methods poorly described (logistic regression model with random-effects analysis)
12. If meta-analysis was performed, did the review authors assess the potential impact of RoB in individual studies on the results of the meta-analysis or other evidence synthesis?	No
13. Did the review authors account for RoB in individual studies when interpreting/ discussing the results of the review?	No
14. Did the review authors provide a satisfactory explanation for, and discussion of, any heterogeneity observed in the results of the review?	Only in the limitation section
15. If they performed quantitative synthesis did the review authors carry out an adequate investigation of publication bias (small study bias) and discuss its likely impact on the results of the review?	Publication bias assessed by funnel plot
16. Did the review authors report any potential sources of conflict of interest, including any funding they received for conducting the review?	The Authors declare that there is no conflict of interests.

5. Sconza. *Oxygen-ozone therapy for the treatment of low back pain: a systematic review of randomized controlled trials.* European Review for Medical and Pharmacological Sciences 2021	
N studies included	15 studies (Niu 2018, Bruno 2020; Rahimzadeh 2018; Perri 2015 (T2 shine); Li 2014; Zhang 2013; Melchionda 2012; Gautam 2011; Paoloni 2009; Arena 2008; Gallucci 2007; Zambello 2006; Bonetti 2005; Paradiso 2005; Buric 2005)
Results	Comparison of oxygen-ozone therapy (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.
AMSTAR checklist	
1.Did the research questions and inclusion criteria for the review include the components of PICO?	P: treatment of LBP (including: disc herniation with or without radicular irradiation and lumbar spine arthritis). I: ozone injections C: - O: patient's reported subjective scores and pain Study: RCT written in English, published on indexed journals in the period 2000-2021 Follow-up: 6 months
2.Did the report of the review contain an explicit statement that the review methods were established prior to the conduct of the review and did the report justify any significant deviations from the protocol?	Not reported
3. Did the review authors explain their selection of the study designs for inclusion in the review?	No explanation for including only RCTs
4. Did the review authors use a comprehensive literature search strategy?	Database searched: PubMed and Scopus (updated end of April 2021). Database searching was supplemented by screening reference lists and tracking citations included in trials to identify any additional studies. No grey literature.
5. Did the review authors perform study selection in duplicate?	The screening process and analysis were conducted separately by 2 independent observers
6. Did the review authors perform data extraction in duplicate?	The screening process and analysis were conducted separately by 2 independent observers. 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.
7. Did the review authors provide a list of excluded studies and justify the exclusions?	No
8. Did the review authors describe the included studies in adequate detail?	Yes (table 1)
9. Did the review authors use a satisfactory technique for assessing the risk of bias	Cochrane Risk of Bias tool for Randomized Controlled Trials

(RoB) in individual studies that were included in the review?	
10. Did the review authors report on the sources of funding for the studies included in the review?	Not reported
11. If meta-analysis was performed did the review authors use appropriate methods for statistical combination of results?	NA
12. If meta-analysis was performed, did the review authors assess the potential impact of RoB in individual studies on the results of the meta-analysis or other evidence synthesis?	NA
13. Did the review authors account for RoB in individual studies when interpreting/ discussing the results of the review?	Yes (comments on the overall modest quality of the available evidence concerning OOT in the treatment of LBP and radiculopathies.)
14. Did the review authors provide a satisfactory explanation for, and discussion of, any heterogeneity observed in the results of the review?	No
15. If they performed quantitative synthesis did the review authors carry out an adequate investigation of publication bias (small study bias) and discuss its likely impact on the results of the review?	NA
16. Did the review authors report any potential sources of conflict of interest, including any funding they received for conducting the review?	The authors declare no conflicts of interest.

RCTs mancanti non inclusi in queste RS:

- Clavo 2021
- Kelesis 2022
- Krahulik 2023
- ~~Perri MRI 2015 (più vecchio di 5 anni)~~
- Salehpour 2021
- Sucuoglu 2021

Assessment of quality of RCTs published in the last 5 years (2018-2023) and not included in the 5 reviews assessed above – ROB2 tool

Reference 1:	**Clavo et al, Ozone therapy versus surgery for lumbar disc herniation: A randomized double-blind controlled trial. Complementary Therapies in Medicine 59 (2021) 102724** - Short communication paper
Study design: ; N subjects randomized:	Randomized, double-blinded (patients and outcomes assessor) parallel group study; N=19 (premature closure for organizational issues)
Interventions/Comparators:	3 treatment groups: 1) surgery (standard treatment); discectomy or microdiscectomy; 2) ozone (experimental): intradiscal O2O3 infiltration (concentration: 27 μg/mL (μg O3/mL O2)) + foraminal infiltration of O2O3 + steroids + anesthetic; 3) O2 (sham control): intradiscal O2 infiltration + foraminal infiltration of "O2 + steroids + anesthetic"
Main outcome:	(the initial outcome was pain level assessment according to the VAS, but was not analyzed due to premature study closure) Evaluated outcomes: requirements of surgery, safety, and days and costs of hospitali- zation evaluated five years after treatment of the last recruited patient
Numerical result:	Three of the five patients initially treated with O2 infiltration (60 %) required surgery at the 4^{th}, 6^{th} and 14^{th} months, respectively. One out of the nine patients (11 %) initially treated with surgery required a second surgery eight months later, which was initially declined by the patient, but finally performed 33 months later. No adverse events related with infiltration procedure were observed. Compared to the surgery arm, patients treated with O2O3 in- filtrations required fewer inpatient days: 3 (3–3.5) vs. 0 (0–1.5), *P* = 0.012 and had lower costs: EUR 3702 (EUR 3283–7630) vs. 364 (364–2536), *P* = 0.029.
ROB2	
Domain 1: Risk of bias arising from the randomization process	
1.1 Was the allocation sequence random?	Randomized
1.2 Was the allocation sequence concealed until participants were enrolled and assigned to interventions?	Not specified
1.3 Did baseline differences between intervention groups suggest a problem with the randomization process?	Baseline features not reported
Risk-of-bias judgement	**Moderate**
Domain 2: Risk of bias due to deviations from the intended interventions	
2.1. Were participants aware of their assigned intervention during the trial?	No (blinded)
2.2. Were carers and people delivering the interventions aware of participants' assigned intervention during the trial?	No (The neurosurgeons and follow-up physicians were blinded with respect to the infiltration arm)
2.3. If Y/PY/NI to 2.1 or 2.2: Were there deviations from the intended intervention that arose because of the trial context?	NA
2.4 If Y/PY to 2.3: Were these deviations likely to have affected the outcome?	NA

2.5. If Y/PY/NI to 2.4: Were these deviations from intended intervention balanced between groups?	NA
2.6 Was an appropriate analysis used to estimate the effect of assignment to intervention?	No
2.7 If N/PN/NI to 2.6: Was there potential for a substantial impact (on the result) of the failure to analyse participants in the group to which they were randomized?	PN
Risk-of-bias judgement	**Low**
Domain 3: Missing outcome data	
3.1 Were data for this outcome available for all, or nearly all, participants randomized?	No (the initial outcome was pain level assessment according to the VAS, but was not analyzed due to premature study closure)
3.2 If N/PN/NI to 3.1: Is there evidence that the result was not biased by missing outcome data?	No
3.3 If N/PN to 3.2: Could missingness in the outcome depend on its true value?	Probably No
3.4 If Y/PY/NI to 3.3: Is it likely that missingness in the outcome depended on its true value?	Probably No
Risk-of-bias judgement	**High**
Domain 4: Risk of bias in measurement of the outcome	
4.1 Was the method of measuring the outcome inappropriate?	No
4.2 Could measurement or ascertainment of the outcome have differed between intervention groups?	No
4.3 If N/PN/NI to 4.1 and 4.2: Were outcome assessors aware of the intervention received by study participants?	NA
4.4 If Y/PY/NI to 4.3: Could assessment of the outcome have been influenced by knowledge of intervention received?	NA
4.5 If Y/PY/NI to 4.4: Is it likely that assessment of the outcome was influenced by knowledge of intervention received?	NA
Risk-of-bias judgement	**Low**
Domain 5: Risk of bias in selection of the reported result	
5.1 Were the data that produced this result analysed in accordance with a pre-specified analysis plan that was finalized before unblinded outcome data were available for analysis?	No (the study is registered in Clinicatrials.gov, but the SAP is not specified)
Is the numerical result being assessed likely to have been selected, on the basis of the results, from...	
5.2. ... multiple eligible outcome measurements (e.g. scales, definitions, time points) within the outcome domain?	PN
5.3 ... multiple eligible analyses of the data?	PN
Risk-of-bias judgement	**Moderate**

Reference 2:	**Kelesis A et al, Intradiscal oxygen-ozone chemonucleolysis versus microdiscectomy for lumbar disc herniation radiculopathy: a non−inferiority randomized control trial.The Spine Journal 22 (2022) 895−909**
Study design: ; N subjects randomized:	Multicenter pilot prospective non−inferiority blocked randomized control trial conducted in three European hospital spine centers. N=49
Interventions/Comparators:	intradiscal oxygen-ozone vs microdiscectomy
Main outcome:	Primary outcome was overall 6-month improvement over baseline in leg pain.
Numerical result:	Primary analyses with a non −inferiority margin of -1.94-point difference in 6-month cumulative weighted mean leg pain NRS scores were conducted using As-Treated (AT) and Intent-to-Treat (ITT) populations
ROB2	
Domain 1: Risk of bias arising from the randomization process	
1.1 Was the allocation sequence random?	Yes
1.2 Was the allocation sequence concealed until participants were enrolled and assigned to interventions?	No (Randomization procedures involved a block design stratified by center using a computerized random number generator allocation sequence. Randomization lists were prepared by the trial's statistician (DH) and the randomization schedule was unknown to treating physicians and allocation of assignments were only provided to them after their randomization request. The two procedures were performed by different specialties in different operative setting so it was not possible to blind the interventionalists or the patients to treatments)
1.3 Did baseline differences between intervention groups suggest a problem with the randomization process?	No
Risk-of-bias judgement	**Low**
Domain 2: Risk of bias due to deviations from the intended interventions	
2.1. Were participants aware of their assigned intervention during the trial?	It was not possible to blind the interventionalists or the patients to treatments.
2.2. Were carers and people delivering the interventions aware of participants' assigned intervention during the trial?	It was not possible to blind the interventionalists or the patients to treatments. Outcome assessors were blind to the treatment arm assignment of patients
2.3. If Y/PY/NI to 2.1 or 2.2: Were there deviations from the intended intervention that arose because of the trial context?	NA
2.4 If Y/PY to 2.3: Were these deviations likely to have affected the outcome?	NA
2.5. If Y/PY/NI to 2.4: Were these deviations from intended intervention balanced between groups?	NA
2.6 Was an appropriate analysis used to estimate the effect of assignment to intervention?	Post hoc analysis comparing differences between treatment groups in leg pain improvement over baseline were made at each follow-up point.
2.7 If N/PN/NI to 2.6: Was there potential for a substantial impact (on the result) of the failure to analyse participants in the group to which they were randomized?	Probably no
Risk-of-bias judgement	**Low**
Domain 3: Missing outcome data	

3.1 Were data for this outcome available for all, or nearly all, participants randomized?	Yes
3.2 If N/PN/NI to 3.1: Is there evidence that the result was not biased by missing outcome data?	NA
3.3 If N/PN to 3.2: Could missingness in the outcome depend on its true value?	NA
3.4 If Y/PY/NI to 3.3: Is it likely that missingness in the outcome depended on its true value?	NA
Risk-of-bias judgement	**Low**
Domain 4: Risk of bias in measurement of the outcome	
4.1 Was the method of measuring the outcome inappropriate?	No (The primary outcome of leg pain improvement assessed as 0−10-point Numerical Rating Scale (NRS), was based on all visits up to, and including 6 months. Other clinical outcome measures included separate NRS scores for back pain, Roland Morris Disability Index (RMDI), and the EQ-5D quality of life questionnaire)
4.2 Could measurement or ascertainment of the outcome have differed between intervention groups?	No
4.3 If N/PN/NI to 4.1 and 4.2: Were outcome assessors aware of the intervention received by study participants?	No
4.4 If Y/PY/NI to 4.3: Could assessment of the outcome have been influenced by knowledge of intervention received?	NA
4.5 If Y/PY/NI to 4.4: Is it likely that assessment of the outcome was influenced by knowledge of intervention received?	NA
Risk-of-bias judgement	**Low**
Domain 5: Risk of bias in selection of the reported result	
5.1 Were the data that produced this result analysed in accordance with a pre-specified analysis plan that was finalized before unblinded outcome data were available for analysis?	Specified in the Registered protocol (NCT02525120).
Is the numerical result being assessed likely to have been selected, on the basis of the results, from...	
5.2. ... multiple eligible outcome measurements (e.g. scales, definitions, time points) within the outcome domain?	No
5.3 ... multiple eligible analyses of the data?	No
Risk-of-bias judgement	**Low**

Reference 3:	**Krahulik et al, Periradicular corticosteroid infiltration for radicular pain – comparison of Diprophos and Depomedrone and ozone effects, Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub. 2023 Mar; 167(1):80-84.**
Study design: ; N subjects randomized:	Unclear study design N=150 patients
Interventions/Comparators:	3 groups: 1 mL of betamethasone (Diprophos) + 4 mL 0.25% bupivacaine, 1 mL of methylprednisolone (Depomedrone) + 4 mL 0.25% bupivacaine or 10 mL ozone generated by a TAO 80ozone generator.
Main outcome:	(unclear) change of leg pain VAS prior to and after therapy
Numerical result:	The statistically significant effect was higher in betamethasone (Diprophos) versus methylprednisolone (Depomedrone) (P=0.019) and Diprophos versus ozone (P<0.001). Diprophos also showed the highest decrease of VAS after therapy versus VAS prior to therapy (median decrease = 4) compared to Depomedrone and ozone (median decrease = 3 and 2, respectively). The statistically significant outcome was better with the indication of spondylolisthesis and disc herniation (P=0.019) indication for the Diprophos group and between spinal stenosis and spondylolisthesis (P=0.022) and spondylolisthesis and disc herniation (P=0.016) for the ozone group.
ROB2	
Domain 1: Risk of bias arising from the randomization process	
1.1 Was the allocation sequence random?	No (they state “randomized” only in the abstract)
1.2 Was the allocation sequence concealed until participants were enrolled and assigned to interventions?	No
1.3 Did baseline differences between intervention groups suggest a problem with the randomization process?	No
Risk-of-bias judgement	**High**
Domain 2: Risk of bias due to deviations from the intended interventions	
2.1. Were participants aware of their assigned intervention during the trial?	Yes
2.2. Were carers and people delivering the interventions aware of participants' assigned intervention during the trial?	Yes
2.3. If Y/PY/NI to 2.1 or 2.2: Were there deviations from the intended intervention that arose because of the trial context?	Not reported
2.4 If Y/PY to 2.3: Were these deviations likely to have affected the outcome?	NA
2.5. If Y/PY/NI to 2.4: Were these deviations from intended intervention balanced between groups?	NA
2.6 Was an appropriate analysis used to estimate the effect of assignment to intervention?	No
2.7 If N/PN/NI to 2.6: Was there potential for a substantial impact (on the result) of the failure to analyse participants in the group to which they were randomized?	Not assessable
Risk-of-bias judgement	**High**

Domain 3: Missing outcome data	
3.1 Were data for this outcome available for all, or nearly all, participants randomized?	Yes
3.2 If N/PN/NI to 3.1: Is there evidence that the result was not biased by missing outcome data?	
3.3 If N/PN to 3.2: Could missingness in the outcome depend on its true value?	
3.4 If Y/PY/NI to 3.3: Is it likely that missingness in the outcome depended on its true value?	
Risk-of-bias judgement	**Low**
Domain 4: Risk of bias in measurement of the outcome	
4.1 Was the method of measuring the outcome inappropriate?	No
4.2 Could measurement or ascertainment of the outcome have differed between intervention groups?	No
4.3 If N/PN/NI to 4.1 and 4.2: Were outcome assessors aware of the intervention received by study participants?	Yes
4.4 If Y/PY/NI to 4.3: Could assessment of the outcome have been influenced by knowledge of intervention received?	Probably yes
4.5 If Y/PY/NI to 4.4: Is it likely that assessment of the outcome was influenced by knowledge of intervention received?	Probably yes
Risk-of-bias judgement	**Moderate**
Domain 5: Risk of bias in selection of the reported result	
5.1 Were the data that produced this result analysed in accordance with a pre-specified analysis plan that was finalized before unblinded outcome data were available for analysis?	No
Is the numerical result being assessed likely to have been selected, on the basis of the results, from...	
5.2. ... multiple eligible outcome measurements (e.g. scales, definitions, time points) within the outcome domain?	Probably no
5.3 ... multiple eligible analyses of the data?	Probably no
Risk-of-bias judgement	**Low**

Reference 4:	**Salehpour et al, Ozone Therapy as a Minimally-invasive Alternative in patients with Acute Lumbar Disc Herniation: A Randomized Clinical Trial. Trauma Monthly 2021; 26(4): 206-212**
Study design: ; N subjects randomized:	Randomized phase 3 trial; 100 patients
Interventions/Comparators:	ozone therapy (25 mcg/mL in 5 cc volume) plus medical therapy (naproxen 500 mg and baclofen 10 mg, both two times a day) vs medical treatment alone
Main outcome:	Changes in pain intensity (VAS) and basal test
Numerical result:	Mean pain intensities estimated by VAS and improvement of restless leg syndrome were not significantly different between the two groups during two weeks (p=0.8), three months (p=0.5) and six months (p=0.9) after the intervention. Pain intensity was found to be lower in both groups after the intervention compared with before treatment (p=0.001 for both). Moreover, significant differences were found between two groups in the Lasegue test during two weeks (p=0.02) and six months (p=0.01) after the intervention.
ROB2	
Domain 1: Risk of bias arising from the randomization process	
1.1 Was the allocation sequence random?	Yes
1.2 Was the allocation sequence concealed until participants were enrolled and assigned to interventions?	envelopes
1.3 Did baseline differences between intervention groups suggest a problem with the randomization process?	Probably Yes (sex)
Risk-of-bias judgement	**Moderate**
Domain 2: Risk of bias due to deviations from the intended interventions	
2.1. Were participants aware of their assigned intervention during the trial?	Yes (blinding not feasible)
2.2. Were carers and people delivering the interventions aware of participants' assigned intervention during the trial?	Yes (also the evaluator)
2.3. If Y/PY/NI to 2.1 or 2.2: Were there deviations from the intended intervention that arose because of the trial context?	Not reported
2.4 If Y/PY to 2.3: Were these deviations likely to have affected the outcome?	NA
2.5. If Y/PY/NI to 2.4: Were these deviations from intended intervention balanced between groups?	NA
2.6 Was an appropriate analysis used to estimate the effect of assignment to intervention?	No
2.7 If N/PN/NI to 2.6: Was there potential for a substantial impact (on the result) of the failure to analyse participants in the group to which they were randomized?	NA
Risk-of-bias judgement	**Moderate**
Domain 3: Missing outcome data	
3.1 Were data for this outcome available for all, or nearly all, participants randomized?	Yes
3.2 If N/PN/NI to 3.1: Is there evidence that the result was not biased by missing outcome data?	NA
3.3 If N/PN to 3.2: Could missingness in the outcome depend on its true value?	NA
3.4 If Y/PY/NI to 3.3: Is it likely that missingness in the outcome depended on its true value?	NA
Risk-of-bias judgement	**Low**
Domain 4: Risk of bias in measurement of the outcome	
4.1 Was the method of measuring the outcome inappropriate?	No

4.2 Could measurement or ascertainment of the outcome have differed between intervention groups?	Probably no
4.3 If N/PN/NI to 4.1 and 4.2: Were outcome assessors aware of the intervention received by study participants?	Yes
4.4 If Y/PY/NI to 4.3: Could assessment of the outcome have been influenced by knowledge of intervention received?	Probably yes
4.5 If Y/PY/NI to 4.4: Is it likely that assessment of the outcome was influenced by knowledge of intervention received?	Probably yes
Risk-of-bias judgement	
Domain 5: Risk of bias in selection of the reported result	
5.1 Were the data that produced this result analysed in accordance with a pre-specified analysis plan that was finalized before unblinded outcome data were available for analysis?	No previous protocol
Is the numerical result being assessed likely to have been selected, on the basis of the results, from...	
5.2. ... multiple eligible outcome measurements (e.g. scales, definitions, time points) within the outcome domain?	Probably no
5.3 ... multiple eligible analyses of the data?	Probably no
Risk-of-bias judgement	**Moderate**

Reference 5:	**Sucuog˙lu et al, Does paravertebral ozone injection have efficacy as an additional treatment for acute lumbar disc herniation? A randomized, double-blind, placebo-controlled study. Journal of Back and Musculoskeletal Rehabilitation 34 (2021) 725–733**
Study design: ; N subjects randomized:	randomized, double-blind, placebo-controlled study; 46 patients randomized (38 completed)
Interventions/Comparators:	Intramuscular ozone injections (20 µg/ml for the first 4 sessions and 25 µg/ml for the next 4 sessions in the treatment group) vs 0.1 µg/ml for all the sessions in the placebo group
Main outcome:	pain (VAS) and disability related to the LBP (ODI) at baseline (V1), during the treatment period (15 (V2) and 30 (V3) days after the treat- ment started), and after the treatment ended (one month (V4)).
Numerical result:	A significant improvement was seen in the VAS and ODI scores in the final follow-up (V4) as compared with the baselines scores (V1) in both groups ($P < 0.05$). The patients in the OT group had lower mean VAS and ODI scores in V2, V3, and V4 follow-ups compared with the patients in the PC group. This significant difference reached its peak in the final follow-up (V4) ($P < 0.05$)
ROB2	
Domain 1: Risk of bias arising from the randomization process	
1.1 Was the allocation sequence random?	Yes
1.2 Was the allocation sequence concealed until participants were enrolled and assigned to interventions?	No (The participants were asked to choose a number between 1 and 10. Those who selected odd numbers were included in the PC group and those who selected an even number were included in the OT group)
1.3 Did baseline differences between intervention groups suggest a problem with the randomization process?	No
Risk-of-bias judgement	**Moderate**
Domain 2: Risk of bias due to deviations from the intended interventions	
2.1. Were participants aware of their assigned intervention during the trial?	No
2.2. Were carers and people delivering the interventions aware of participants' assigned intervention during the trial?	No (The physician and patient who performed the assess- ment, administered the POI, planned the PT program and medication, were blinded to the OT doses. A dif- ferent physician adjusted the ozone concentration from the generator and drew it into the syringe. Additionally, only this physician knew the ozone dose to be applied to the patient.)
2.3. If Y/PY/NI to 2.1 or 2.2: Were there deviations from the intended intervention that arose because of the trial context?	
2.4 If Y/PY to 2.3: Were these deviations likely to have affected the outcome?	
2.5. If Y/PY/NI to 2.4: Were these deviations from intended intervention balanced between groups?	
2.6 Was an appropriate analysis used to estimate the effect of assignment to intervention?	No
2.7 If N/PN/NI to 2.6: Was there potential for a substantial impact (on the result) of the failure to analyse participants in the group to which they were randomized?	
Risk-of-bias judgement	**Low**
Domain 3: Missing outcome data	
3.1 Were data for this outcome available for all, or nearly all, participants randomized?	38 out of 46 (but ITT analysis)

3.2 If N/PN/NI to 3.1: Is there evidence that the result was not biased by missing outcome data?	No
3.3 If N/PN to 3.2: Could missingness in the outcome depend on its true value?	No
3.4 If Y/PY/NI to 3.3: Is it likely that missingness in the outcome depended on its true value?	No
Risk-of-bias judgement	**Moderate**
Domain 4: Risk of bias in measurement of the outcome	
4.1 Was the method of measuring the outcome inappropriate?	No
4.2 Could measurement or ascertainment of the outcome have differed between intervention groups?	No
4.3 If N/PN/NI to 4.1 and 4.2: Were outcome assessors aware of the intervention received by study participants?	No
4.4 If Y/PY/NI to 4.3: Could assessment of the outcome have been influenced by knowledge of intervention received?	
4.5 If Y/PY/NI to 4.4: Is it likely that assessment of the outcome was influenced by knowledge of intervention received?	
Risk-of-bias judgement	**Low**
Domain 5: Risk of bias in selection of the reported result	
5.1 Were the data that produced this result analysed in accordance with a pre-specified analysis plan that was finalized before unblinded outcome data were available for analysis?	No previous protocol
Is the numerical result being assessed likely to have been selected, on the basis of the results, from...	
5.2. ... multiple eligible outcome measurements (e.g. scales, definitions, time points) within the outcome domain?	Probably no
5.3 ... multiple eligible analyses of the data?	Probably no
Risk-of-bias judgement	**Moderate**