ACCSCIENCE PUBLISHING

INNOSC Theranostics and Pharmacological Sciences

ORIGINAL RESEARCH ARTICLE

Observational study on the effectiveness of oxygen-ozone treatment vs. pharmacological treatment in patients with chronic low back pain after previous spinal stabilization surgery

Matteo Bonetti[1]*, Michele Frigerio[1], Mario Muto[2], Giannantonio Pellicanò[3], Alessio Zambello[4], Cosma Andreula[5], Irene Volonghi[6], Serena Miglio[7], and Federico Maffezzoni[7]

[1]Department of Neuroradiology, Istituto Clinico Città di Brescia, Brescia, Brescia, Italy

[2]Department of Neuroradiology, Ospedale Cardarelli, Naples, Naples, Italy

[3]Department of Neuroradiology, Azienda Ospedaliera–Universitaria Careggi, Florence, Florence, Italy

[4]Department of Anesthesia and Pain Therapy, Casa di Cura Borghi, Brebbia, Varese, Italy

[5]Coordinator of Radiology and Neuroradiology, Anthea Hospital, Bari, Bari, Italy

[6]Department of Neurology, ASST Spedali Civili di Brescia, Brescia, Brescia, Italy

[7]Oberdan Specialist Outpatient Clinic, Brescia, Brescia, Italy

***Corresponding author:**
Matteo Bonetti
(dottorbonetti@gmail.com)

Citation: Bonetti M, Frigeriio M, Muto M, *et al.* Observational study on the effectiveness of oxygen-ozone treatment vs. pharmacological treatment in patients with chronic low back pain after previous spinal stabilization surgery. *INNOSC Theranostics and Pharmacological Sciences.* doi: 10.36922/ITPS026110009

Received: March 13, 2026

Revised: May 9, 2026

Accepted: May 9, 2026

Published online: May 28, 2026

Copyright: © 2026 Author(s). This is an Open-Access article distributed under the terms of the Creative Commons Attribution License, permitting distribution, and reproduction in any medium, provided the original work is properly cited.

Publisher's Note: AccScience Publishing remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Abstract

Persistent low back pain after spinal stabilization with instrumentation remains a frequent and clinically demanding condition. In many cases, pain persists despite pharmacological therapy and rehabilitation, limiting quality of life. Among emerging non-surgical options, oxygen–ozone (O_2–O_3) therapy has been proposed for its analgesic, anti-inflammatory, and neuromodulatory properties. This study aims to evaluate the clinical effectiveness of computed tomography (CT)-guided infiltrative O_2–O_3 treatment in patients with chronic low back pain following spinal fusion and to compare outcomes with those of standard pharmacological therapy within structured rehabilitative care. This observational retrospective study included 168 adult patients with low back pain persisting for more than six months after posterior spinal fusion with screws and plates. Group A ($n = 72$) received CT-guided paravertebral infiltrations of an O_2–O_3 gas mixture at 20 µg/mL, followed by individualized physiotherapy. Group B ($n = 96$) received a combination of pharmacological agents (oxycodone/paracetamol and pregabalin) alongside the same rehabilitative program. Clinical evaluation was performed at baseline (T0), 1 month (T1), and 6 months (T2) using the Visual Analog Scale (VAS) and the modified McNab scale. Group A showed a significant mean VAS reduction (>3 points) already at T1, with benefits maintained at T2. Functional recovery was rated as "good" or "excellent" in approximately 60% of cases. In contrast, Group B exhibited a transient response at T1, with VAS scores returning close to baseline at T2. Intergroup differences in both pain reduction and functional improvement were statistically significant ($p < 0.001$). CT-guided O_2–O_3 infiltrative therapy, integrated with physiotherapy, was associated with better clinical outcomes compared to pharmacological treatment alone in patients with persistent low back pain after spinal fusion. These findings suggest a potential role for O_2–

O_3 therapy within a multimodal treatment approach for selected post-surgical patients.

Keywords: Low back pain; Spondylolisthesis; O2–O3 therapy; Oxygen–ozone therapy; Computed tomography-guided injections; Interventional neuroradiology; Chronic post-surgical pain

1. Introduction

Low back pain, with or without radicular involvement (lumbosciatica), is one of the most common musculoskeletal conditions globally, with a lifetime prevalence of up to 80% in the general population. It is the leading cause of work absenteeism in industrialized countries, having a significant impact in terms of disability and overall healthcare costs.[1,2]

Among the various causes of chronic low back pain, spondylolysis plays a significant role. It is a discontinuity of the vertebral neural arch—particularly in the region between the superior and inferior articular processes—which can be associated with spondylolisthesis, the anterior slippage of one vertebral body over the one below. The term "spondylolisthesis" was first introduced by Kilian in 1854, and today the most commonly used clinical classification is that of Meyerding, which quantifies the degree of slippage as a percentage relative to the underlying vertebral body (Figure 1).[3,4] The Meyerding classification is the most widely used standard for assessing the extent of anterior vertebral body slippage in spondylolisthesis. It is based on the percentage of translation of the superior vertebral body over the base of the vertebra below, measured on lateral radiographic projections.[5]

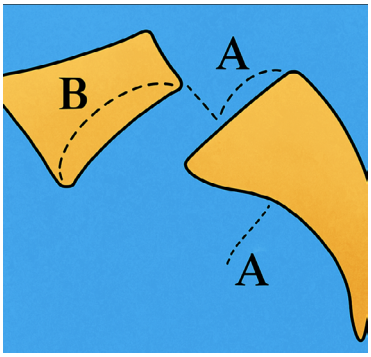


Figure 1. Schematic representation of the Meyerding classification. Grade I is characterized by minimal anterior vertebral displacement, not exceeding 33% of the anteroposterior diameter of the inferior vertebral body. Grade II denotes moderate slippage, ranging from 34% to 66%. Grade III corresponds to severe displacement, between 67% and 99%, often associated with marked disruption of vertebral alignment. Grade IV indicates complete anterior displacement, with 100% loss of contact between adjacent vertebral bodies. When the superior vertebra displaces entirely beyond the anterior border of the subjacent vertebra, the condition is termed spondyloptosis, representing a highly unstable scenario with complete vertebral body dislocation.

Grade I spondylolisthesis is often asymptomatic and incidentally identified. However, in a significant proportion of patients, it may manifest with chronic low back pain, sometimes accompanied by radicular symptoms. The clinical presentation may include low back pain of variable intensity, accentuation of lumbar lordosis, and, in more severe cases, functional limitations in daily activities. Diagnosis is based on a detailed medical history and thorough physical examination, supported by diagnostic imaging: lateral and oblique X-rays complemented by morphodynamic tests in flexion-extension and right-left bending, computed tomography (CT) and/or magnetic resonance imaging (MRI), which are essential tools to identify the degree of slippage, spinal stability, and any associated structural alterations.[5–10] Initial treatment of low-grade spondylolisthesis and symptomatic spondylolysis is usually conservative, involving physiokinesitherapy programs, sometimes in association with analgesic therapy.

In cases resistant to conservative treatment, surgical spinal stabilization may become necessary. Although surgery has long been considered the standard treatment, long-term clinical effectiveness remains variable, leading in recent years to growing interest in minimally invasive and conservative approaches.[10–12]

The treatment of post-stabilization spinal pain represents a complex clinical challenge, requiring a multimodal and individualized assessment. Pain persistence may result from multiple factors: post-surgical scar fibrosis with perineural adhesions, pseudoarthrosis, articular overload above or below the stabilization, or possibly associated discopathies.[13–17]

Severe epidural fibrosis is present in over 80% of patients with persistent pain following spinal surgery[13], while symptomatic pseudoarthrosis can occur in up to 2.7% of cases within 10 years of the procedure.[14] Adjacent segment disease affects up to 36% of patients in the long term[17], contributing to the clinical complexity of these conditions.

The management of post-stabilization spinal pain requires a multimodal and individualized approach.

Conservative options include rehabilitation protocols aimed at restoring segmental functionality and stability, along with individualized pharmacological treatments, comprising analgesics, anti-inflammatory drugs, and medications for neuropathic pain.[18] In addition to these strategies, minimally invasive procedures are playing an increasingly prominent role, including epidural or selective nerve root injections[18], pulsed radiofrequency of the dorsal root ganglia[18,19], epiduroscopy for adhesion lysis[19], and, in more complex cases, spinal cord stimulation, which has demonstrated efficacy in improving pain and quality of life.[20,21]

Beyond these options, physiotherapeutic rehabilitation holds a crucial role, constituting one of the cornerstones of the post-surgical therapeutic pathway. Multiple randomized controlled trials and systematic reviews have shown that early, supervised exercise programs—ideally initiated within the first 4–12 weeks post-surgery—can significantly reduce pain, improve functional disability, and promote global recovery of dynamic spinal stability.[22–24] Contemporary rehabilitation protocols incorporate muscle-strengthening exercises, motor control retraining, proprioceptive work, and cognitive-behavioral interventions aimed at reducing fear of movement (kinesiophobia) and improving psychological adaptation to residual chronic pain.[24–26]

Thus, the integration of personalized physiotherapy not only improves primary clinical outcomes but also helps prevent recurrence and reduces the risk of chronic pain, ultimately optimizing patients' long-term quality of life. These techniques, well-tolerated and repeatable, allow targeted action on pain generators, providing significant benefits to patients.

Among various therapeutic strategies, oxygen–ozone (O_2–O_3) therapy has gained increasing attention and adoption. Introduced in the 1980s for the treatment of discogenic pain, paravertebral or intraforaminal infiltration of an oxygen–ozone mixture has shown encouraging results in the management of low back pain and lumbosciatica not only of discal origin, but also extradiscal (facet joint syndromes, inter- and intra-articular synovitis, spondylolysis, and spondylolisthesis). The mechanism of action identifies controlled modulation of oxidative stress as the primary biological trigger: through the transient generation of reactive oxygen species (ROS) and lipid oxidation products (LOPs), ozone induces activation of the Nuclear factor erythroid 2–related factor 2/Kelch-like ECH-associated protein 1/antioxidant response element (Nrf2/Keap1/ARE) pathway, resulting in increased expression of cytoprotective, antioxidant, and anti-inflammatory genes.[27–38]

At the same time, inflammatory pathways mediated by nuclear factor-kappa B (NF-κB) are modulated, and vasomodulatory and microcirculatory effects are promoted through increased expression of inducible nitric oxide synthase (iNOS) and improved tissue oxygen bioavailability. These synergistic effects lead to reduced periradicular edema, enhanced tissue trophism, and significant pain relief.[39–48]

Numerous studies have reported therapeutic success rates ranging from 70% to 85% in patients with chronic low back pain, with or without radicular involvement of discogenic origin.[1–12,48–64] However, the application of O_2–O_3 therapy in patients who have already undergone spinal stabilization surgery remains scarcely documented, highlighting the need for further evaluation in this specific clinical subgroup. Recent evidence indicates that the combination of physiotherapy and O_2–O_3 injections can significantly reduce pain and disability, with marked and long-lasting effects, even in subacute cases, potentially due to the synergy between the biochemical action of ozone and the neuromuscular activation promoted by rehabilitation.[65-67] These findings provide a strong rationale for investigating whether similar benefits may be observed in patients with persistent pain after spinal stabilization.

The objective of the present study is therefore to evaluate the clinical effectiveness of infiltrative O_2–O_3 treatment, compared with standard pharmacological therapy, in a selected sample of 72 patients with chronic low back pain persisting for more than 6 months following vertebral arthrodesis with screws and metal plates. Specifically, 58 patients presented with Grade I spondylolisthesis according to the Meyerding classification, while the remaining 14 underwent surgery based on clinical decision despite the absence of spondylolisthesis, due to possible isthmic lysis and documented vertebral instability. In all cases included in the study, the surgical procedure did not involve the placement of interbody cages.

The research hypothesis underlying the present study is that CT-guided paravertebral O_2–O_3 infiltration, combined with a structured physiotherapy program, results in a significantly greater clinical improvement—both in pain reduction and functional recovery—compared to pharmacological treatment combined with the same physiotherapy program, in patients with persistent chronic low back pain following spinal stabilization surgery.

2. Materials and methods

2.1. Study design and population

The study used a retrospective, observational, and comparative design, covering the period from January

2022 to April 2025. The aim of the study is to evaluate the effectiveness of O_2–O_3 therapy, in combination with a targeted rehabilitation physiotherapy program, in the treatment of chronic low back pain persisting for more than six months in patients who had previously undergone spinal arthrodesis using screw and plate stabilization (Figures 2 and 3). The clinical outcomes of this treatment (Group A: O_2–O_3 therapy + physiotherapy) were compared with those of a group (Group B) treated with analgesic pharmacological therapy combined with rehabilitation physiotherapy, in order to investigate the potential added value of the infiltrative O_2–O_3 mixture approach in the management of chronic post-surgical low back pain. Clinical evaluations were conducted at three time points: baseline (T0), 1 month (T1), and 6 months (T2).

The study was conducted and reported in accordance with the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines, where applicable to the study design.

2.2. Patient allocation and eligibility criteria

A total of 168 patients were included in the final analyzed cohort. Among them, 72 patients (Group A; mean age: 58.4 ± 13.6 years; 42 females, 30 males) underwent CT-guided O_2–O_3 therapy, while 96 patients (Group B; mean age: 56.1 ± 16.2 years; 54 females, 42 males) received pharmacological treatment. Initially, 104 patients had been allocated to pharmacological treatment. However, 8 patients who experienced treatment-related adverse effects (constipation, headache, nausea and vomiting) discontinued therapy before the final eligibility assessment and were therefore excluded from the analyzed cohort.

All patients had previously undergone spinal stabilization surgery with screw and plate implantation and continued to experience chronic low back pain (Figures 2 and 3).

Patients were allocated to two groups (Group A: O_2–O_3 therapy plus physiotherapy; Group B: pharmacological therapy plus physiotherapy) without randomization. Given the retrospective design, pre-existing cohorts were analyzed, reflecting treatments administered according to individualized clinical indications established by a multidisciplinary team. Treatment allocation was based on clinical judgment, previous therapeutic response, and patient preference, in agreement with the treating physician. This approach was chosen to reflect real-world clinical practice.

To improve comparability between groups, the following criteria were applied:

(i) Inclusion criteria:

- Age > 18 years
- Chronic low back pain lasting > 6 months after spinal stabilization
- Availability of at least 6 months of follow-up
- Signed informed consent for the use of anonymized clinical data

(ii) Exclusion criteria:

- Documented malposition of pedicle screws
- Evidence of active osteolysis
- Incomplete follow-up data

2.3. Treatment protocols and rehabilitation program

Group A patients were treated with CT-guided (Supria 16, Hitachi Medical Systems, Japan) paravertebral infiltrations of an O_2–O_3 gas mixture, without concomitant pharmacological therapy during the infiltration phase. Following completion of the infiltrative treatment, all patients were referred to a structured physiotherapy program consisting of individualized functional rehabilitation over a period of six months. Etiological factors responsible for lumboradicular symptoms in patients from Group A included: pseudarthrosis of the stabilized segment (n = 10; 13.9%), periradicular scar fibrosis (n = 34; 47.2%), functional overload on non-stabilized facet joints (n = 20; 27.8%), and in 8 patients (11.1%) the presence of protrusions or herniated discs was identified as the cause of residual pain symptoms.

Group B patients received pharmacological therapy combined with the same rehabilitative program from the outset. The therapeutic protocol included the combined administration of the following:

- Oxycodone/paracetamol: A fixed-dose combination of oxycodone hydrochloride 5 mg + paracetamol 325 mg per tablet, administered four times daily (every 6 h), with individual dose titration based on pain control.
- Pregabalin: 75 mg oral suspension, administered twice daily.

This combination was selected to reflect a real-world multimodal pharmacological strategy for chronic post-surgical low back pain, where nociceptive and neuropathic components may coexist. Oxycodone/paracetamol was used to address the nociceptive component, while pregabalin was included to target neuropathic or lumboradicular pain features, consistent with commonly adopted clinical pain-management protocols.[14,18,20] Treatment could be adjusted or discontinued in case of adverse effects.

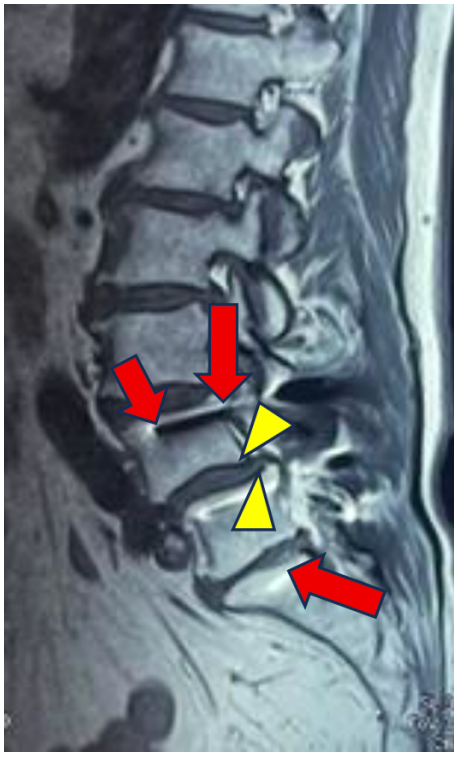


Figure 2. Sagittal magnetic resonance imaging: vertebral stabilization from L4 to S1 with metal screw-plate system (red arrows) in Grade I Meyerding spondylolisthesis of L4 over L5 (yellow arrowheads)

Pharmacological therapy and physiotherapy were administered concurrently for a minimum of 30 days, with subsequent continuation or adjustment based on individual clinical evolution. The physiotherapy protocol was identical in both groups and consisted of three weekly sessions (45–60 min) for at least four weeks. The program included postural re-education, core stabilization, proprioceptive neuromuscular facilitation, passive and active mobilization, and targeted muscle stretching. Adjunctive therapies (e.g., low-intensity laser or infrared thermotherapy) were applied when clinically indicated.

Pharmacological treatment and the physiotherapy program were carried out concurrently for a minimum duration of 30 days. In the following months, patients continued with personalized physiotherapy protocols, with potential continuation or adjustment of pharmacological therapy depending on individual clinical evolution.

In all patients (Groups A and B), the initial physiotherapy program consisted of three weekly sessions lasting 45–60 min each, for a minimum period of four weeks. The treatment was administered by licensed physiotherapists, following a structured protocol with common components, and allowing for personalization based on individual functional assessment. The protocol included:

- Postural re-education and core stabilization exercises aimed at improving spinal alignment and muscle control;
- Proprioceptive neuromuscular facilitation techniques to enhance coordination and neuromuscular response;
- Passive and active mobilization to promote lumbar segmental mobility;
- Targeted stretching of the lumbar paravertebral, gluteal, and hamstring muscles; and
- Adjunctive therapies, such as low-intensity laser or infrared thermotherapy, were used when clinically indicated (Figure 4).

2.4. Clinical assessment and follow-up

All patients underwent clinical evaluation at baseline and during follow-up using standardized outcome measures. Two standardized tools were used to assess treatment effectiveness[61]:

- Visual Analog Scale (VAS): A validated psychometric tool widely used for measuring perceived pain intensity. It consists of a 10 cm straight line with no intermediate numeric indications, where the endpoints are labeled "0 = no pain" and "10 = worst imaginable pain." The patient was asked to mark a point on the line that best represents their current level of pain. The VAS was administered at three time points (T0, T1, and T2) to monitor the course of pain symptoms.
- Modified McNab scale: Used for the subjective assessment of functional outcomes, this scale provides a simple yet clinically meaningful classification of perceived improvement, divided into three categories:
  (i) Excellent: Complete resolution of pain and full return to pre-symptom daily activities;
  (ii) Good or satisfactory: More than 50% reduction in pain compared to baseline;
  (iii) Fair or poor: Partial reduction in pain of less than 50%, with persistent functional limitations.

2.5. O_2–O_3 infiltration procedure

The O_2–O_3 treatment was administered at the vertebral level previously subjected to stabilization surgery, following local skin anesthesia with ethyl chloride spray. CT guidance was used to confirm the correct positioning of the spinal needle in the periganglionic region (Figure 5).

A total of 3–4 cm^3 of an O_2–O_3 gas mixture at 20 µg/mL

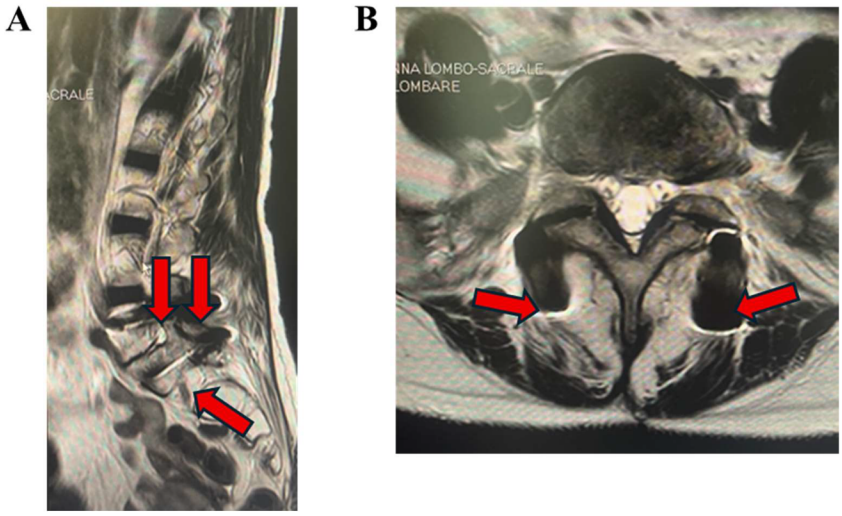


Figure 3. Magnetic resonance imaging (MRI) in multiple planes**.** (A) Sagittal MRI: Vertebral stabilization at L5–S1 (arrows). (B) Axial MRI: Scan at the L5–S1 disc level showing a mild bilateral median–paramedian protrusion of the annulus, with greater focality in the left intraforaminal region (arrows).

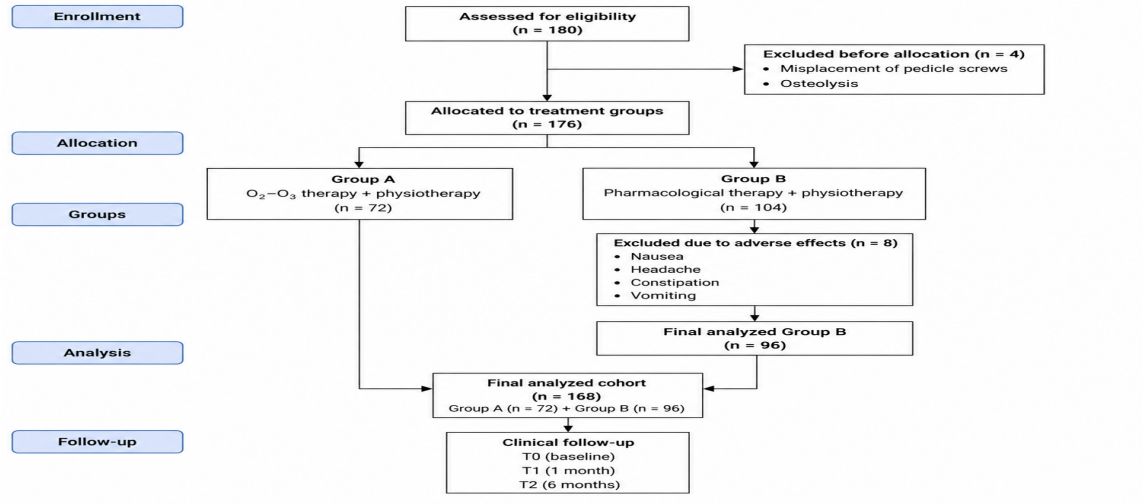


Figure 4. Flowchart of the study design, illustrating the process of patient selection, allocation to treatment groups, and clinical follow-up

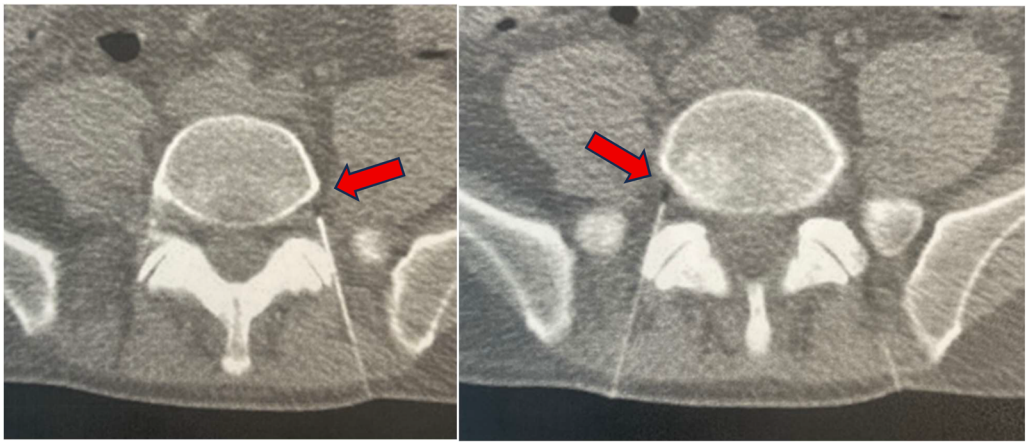


Figure 5. Computed tomography verification of correct needle placement in the periganglionic region (arrows)

was injected as a single shot in the periganglionic region. The needle was then withdrawn by a few centimeters, and an additional 3–4 cm^3 of the gas mixture was injected into the paravertebral musculature and over the articular mass. CT guidance was used throughout the procedure to confirm the correct distribution of the gas mixture (Figures 6 and 7).

The O_2–O_3 gas mixture was produced with the Alnitec OZO 2 Futura PLUS generator (Alnitec Srl, Italy), a Class II medical device with Conformité Européenne (CE; European Conformity) marking, equipped with a high-precision Alnitec photometer featuring fully automated controls (Alnitec Srl) for automatic calibration and precise concentration control. The system, with no manual adjustments, uses medical-grade oxygen and generates ozone from 1 to 100 μg/mL, with 10 programmable sequences. It features an integrated catalyst for neutralizing residual ozone and operates at a supply voltage of 230 V at 50 Hz. All treatments were carried out at the standard concentration of 20 μg/mL, in accordance with the guidelines of the Italian Federation of Oxygen–Ozone Therapy.[68]

No adverse events related to O_2–O_3 were observed in our sample. This finding is consistent with the literature, as complications associated with O_2–O_3 therapy are extremely rare and, in the vast majority of cases, attributable to procedural errors (malpractice).[48-50] The use of CT guidance and the application of standardized concentrations and volumes are key factors in ensuring treatment safety.

Patients in whom the pedicle screws were deemed to be improperly positioned (Figure 8) were excluded from the study and referred for specialist reassessment. Additionally, one patient presenting with evident bone resorption around the pedicle screws (Figure 9) was also excluded.

Assessments were performed at baseline (T0), 1 month (T1), and 6 months (T2) after treatment initiation to monitor clinical progression and compare the medium-term effectiveness of the two treatment strategies (Figure 4). In patients who did not achieve satisfactory clinical improvement at one month or who experienced significant adverse effects, adjunctive therapy with serotonin–norepinephrine reuptake inhibitors was introduced. These patients also underwent neurosurgical reassessment to evaluate potential indications for further interventions.

2.6. Statistical analysis

Statistical analyses were performed using SPSS Statistics, version 29.0 (IBM Corp., United States of America). Continuous variables were summarized as means with 95% confidence intervals (CIs), while categorical variables were reported as frequencies and percentages. The normality of VAS score distributions was assessed using the Shapiro–Wilk test.

As VAS scores were not normally distributed, within-group changes over time were analyzed using non-parametric tests. The Friedman test was used to assess overall differences across the three time points (T0, T1, and T2), followed by Wilcoxon signed-rank tests for pairwise comparisons. Between-group comparisons of VAS scores were performed using the Mann–Whitney *U* test.

Categorical outcomes based on the modified McNab scale were compared using the Chi-square test or Fisher's exact test, as appropriate. Effect sizes were calculated using the *r* coefficient for non-parametric comparisons and partial eta squared (η^2) for repeated-measures analyses. A repeated-measures ANOVA with the Greenhouse–Geisser correction was also performed as a robustness analysis to evaluate temporal trends in pain scores. Statistical significance was set at $p < 0.05$, two-tailed. A post hoc power analysis was conducted using G*Power 3.1.9.7 (Heinrich Heine University Düsseldorf, Germany), assuming $\alpha = 0.05$ and a moderate effect size (Cohen's $d = 0.5$). The available sample size provided statistical power greater than 80%, supporting the adequacy of the study to detect clinically meaningful between-group differences.

3. Results

According to the modified McNab scale, in Group A (O_2–O_3 therapy + physiotherapy), 43 out of 72 patients (59.7%; 95% CI: 47.4–71.1%) reported a "good" or "excellent" outcome at 1 month (T1), while 37 out of 72 patients (51.4%; 95% CI: 39.0–63.6%) maintained this level of improvement at 6 months (T2). The change over time was statistically significant ($\chi^2 = 12.35$, $p = 0.015$).

In Group B (pharmacological therapy + physiotherapy), 24 out of 96 patients (25.0%; 95% CI: 17.0–34.3%) reported "good" or "excellent" outcomes at T1, and 25 out of 96 (26.0%; 95% CI: 18.0–35.1%) at T2 (Table 1).

Pain intensity analysis (VAS) in Group A showed a reduction from a mean of 7.5 (95% CI: 7.3–7.7) at baseline (T0) to 4.2 (95% CI: 4.0–4.4) at T1, with partial maintenance at T2 (mean 4.6; 95% CI: 4.4–4.8) (Table 1). The Friedman test confirmed a statistically significant variation over time ($\chi^2 = 128.4$, $p < 0.001$). Pairwise comparisons (Wilcoxon signed-rank test) showed significant differences between T0 and T1 ($p < 0.001$), T1 and T2 ($p = 0.004$), and T0 and T2 ($p < 0.001$).

In Group B, VAS scores decreased from a mean of 7.4 (95% CI: 7.2–7.6) at T0 to 5.3 (95% CI: 5.1–5.5) at

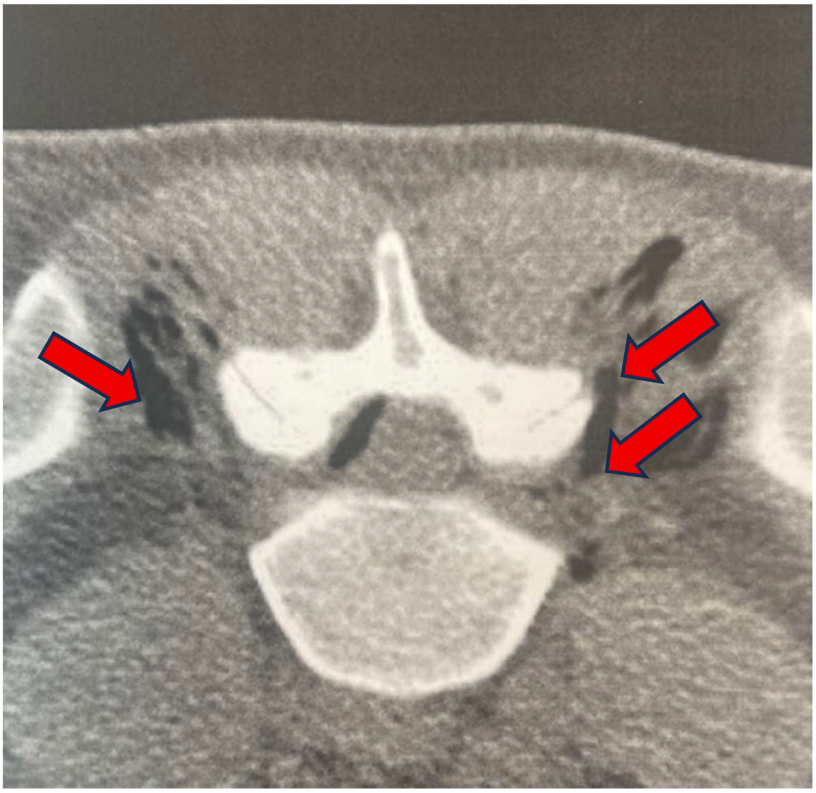


Figure 6. Axial computed tomography scan confirming the distribution of the O_2–O_3 gas mixture (arrows)

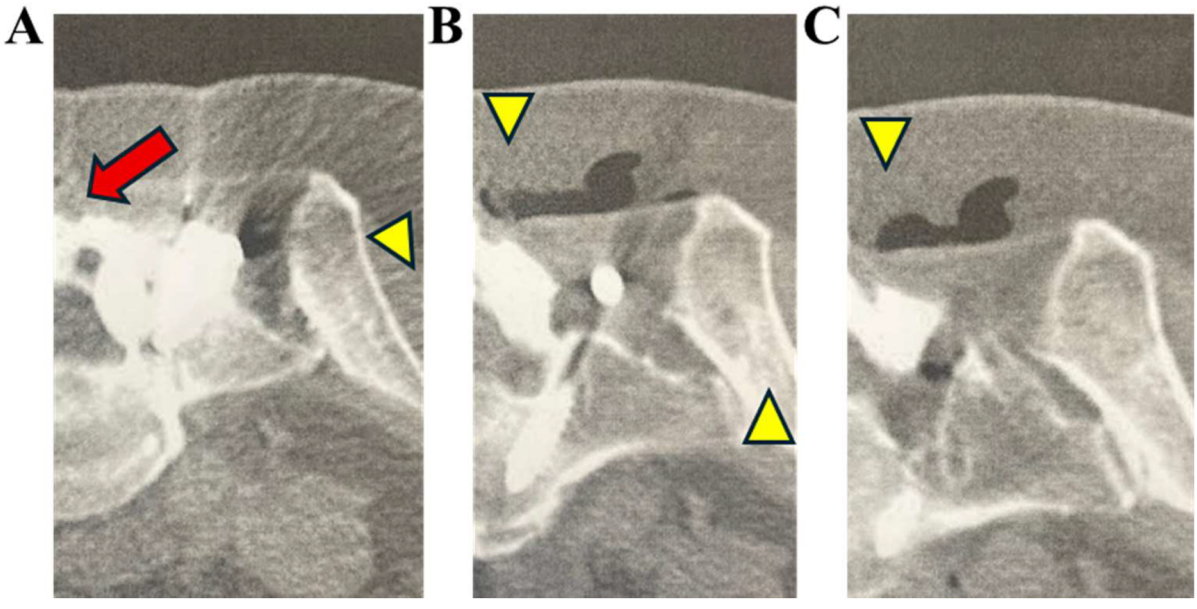


Figure 7. Patient with chronic low back pain following spinal stabilization, complicated by left-sided sciatica. Electromyographic examination showed chronic neurogenic impairment in the S1 left distribution territory. (A) Low-dose parenchymal algorithm computed tomography (CT) scan confirming needle placement (arrow). (B–C) CT control scans documenting the distribution of the gas mixture (arrowheads).

T1, followed by an increase to 6.2 (95% CI: 6.0–6.4) at T2 (Table 1; Figure 10). Pairwise comparisons showed differences between T0 and T1 (p = 0.003) and between T1 and T2 (p = 0.021), whereas no clear difference was observed between T0 and T2 (p = 0.146). Repeated-measures ANOVA indicated an overall temporal effect (F = 55.12, p < 0.001).

At 6 months, VAS values in Group B approached baseline levels, whereas Group A maintained a lower mean pain intensity (Figures 10 and 11). The mean VAS reduction in Group A (−3.3 points at T1 and −2.9 at T2 compared to baseline) exceeded the minimal clinically important difference for chronic low back pain, typically estimated between 1.5 and 2.0 points on a 0–10 scale.[69–71]

4. Discussion

The findings of the present study clearly underscore the clinical efficacy of O_2–O_3 therapy in managing chronic low back pain persisting after spinal stabilization surgery. The comparison with the control group (Group B), which received only pharmacological treatment, revealed significant differences in both pain intensity reduction (as measured by the VAS) and functional recovery (as assessed by the modified McNab scale). A mean reduction of more than 3 points on the VAS, already observed one month after treatment and sustained at six months, along with 59.7% of patients reporting "good" or "excellent" outcomes, indicates a strong and lasting therapeutic impact.

The physiological rationale underlying this efficacy is supported by recent advancements in molecular biochemistry. Ozone, a highly reactive triatomic molecule, does not act directly on cells but induces an adaptive response in the biological microenvironment via controlled oxidative stress. This phenomenon, referred to as oxidative hormesis, leads to the generation of ROS and LOPs, which serve as intracellular second messengers.[27-38]

The key mechanism behind O_2–O_3 therapy's effectiveness lies in the activation of the Nrf2/Keap1/ARE pathway, which is recognized as the central regulator of the cytoprotective antioxidant response. Under baseline conditions, the transcription factor Nrf2 is sequestered by Keap1, a cytosolic protein that facilitates its proteasomal degradation. ROS and LOPs generated by ozone modify thiol residues on Keap1 cysteines, triggering conformational changes that release Nrf2 and allow its nuclear translocation. Once in the nucleus, Nrf2 binds to ARE and promotes the transcription of key antioxidant and anti-inflammatory genes, including glutathione peroxidase, superoxide dismutase, catalase, heme oxygenase-1 (HO-1), and NAD(P)H quinone dehydrogenase 1 (NQO1). The result is a robust endogenous enhancement of cellular defenses, a reduction in systemic oxidative stress, and suppression of pro-inflammatory pathways mediated by NF-κB, leading to decreased levels of cytokines such as interleukin (IL)-1β, IL-6, and tumor necrosis factor-alpha (TNF-α). Moreover, Nrf2 pathway activation is known to interact with the hypoxia-inducible factor 1-alpha (HIF-1α) pathway, which is responsible for cellular adaptation to hypoxia by promoting angiogenesis, erythropoiesis, and improved tissue perfusion—effects of particular relevance in the post-surgical vertebral context. The hormetic response elicited by O_2–O_3, when properly dosed (10–40 μg/mL), does not induce oxidative damage. Instead, it supports the regulated production of 4-hydroxynonenal (4-HNE), a bioactive aldehyde that functions as a physiological signaling

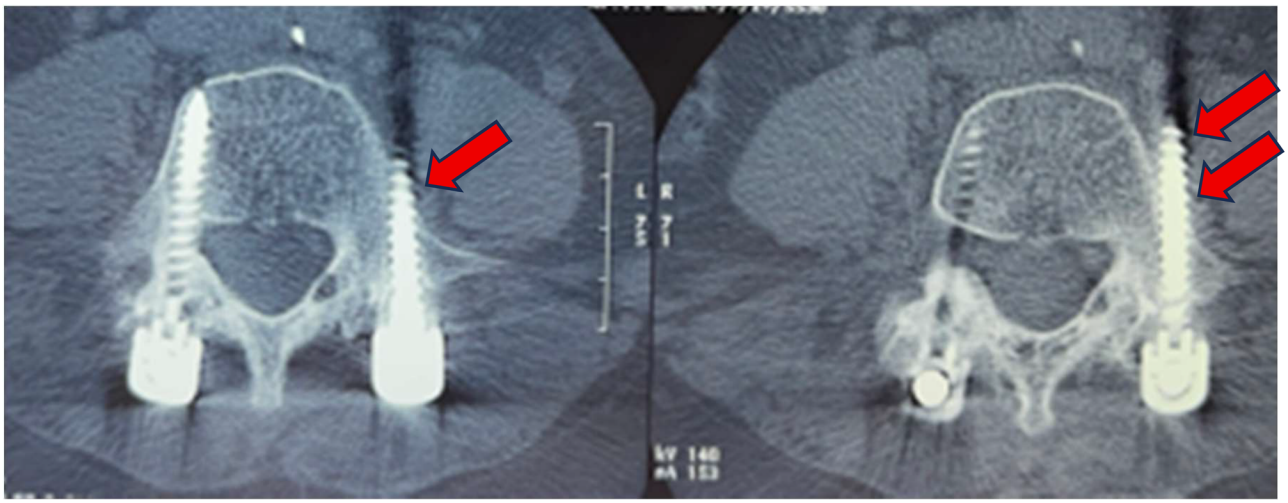


Figure 8. Computed tomography imaging demonstrates lateral misplacement of the left pedicle screw (arrows), leading to the exclusion of the patient from the study and prompting referral for further specialist evaluation

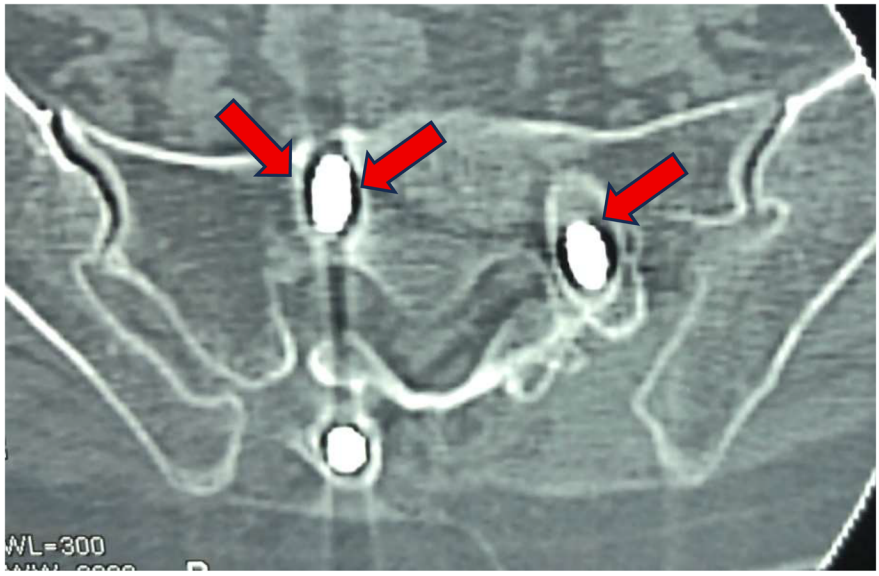


Figure 9. Patient excluded from the study due to the presence of a radiolucent rim of osteolysis around the S1 pedicle screws (arrows)

Table 1. Clinical outcomes (VAS and modified McNab scale) at T0, T1, and T2 in both treatment groups

Time point	VAS		Modified McNab scale improvement (%)	
	Group A	Group B	Group A	Group B
T0	7.5 (7.3 – 7.7)	7.4 (7.2 – 7.6)	0	0
T1	4.2 (4.0 – 4.4)	5.3 (5.1 – 5.5)	59.7 (47.4– 71.1)	25.0 (17.0 – 34.3)
T2	4.6 (4.4 – 4.8)	6.2 (6.0 – 6.4)	51.4 (39.0 – 63.6)	26.00 (18.0 – 35.1)

Notes: Group A: O_2–O_3 + physiotherapy; Group B: Pctharmacological therapy + physiotherapy.
Abbreviation: VAS: Visual Analog Scale.

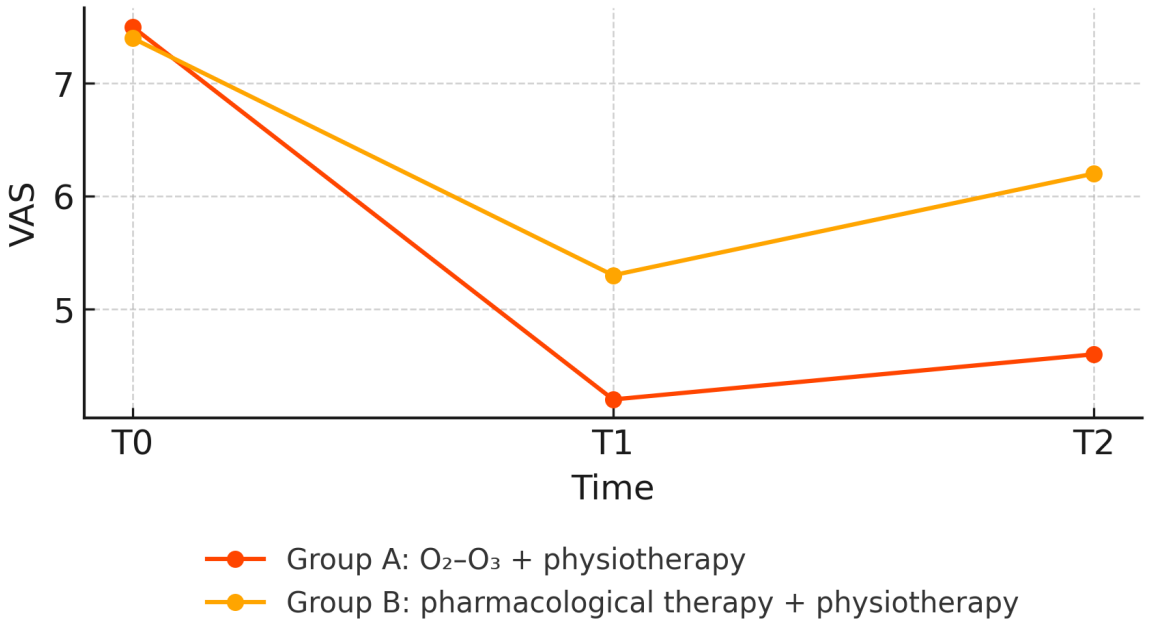


Figure 10. Perceived pain progression (Visual Analog Scale [VAS])

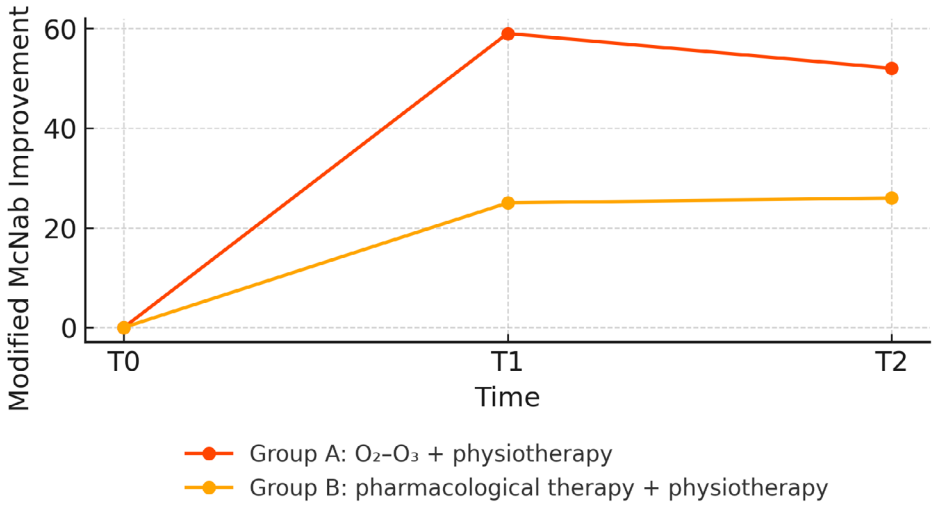


Figure 11. Percentage of functional improvement according to the modified McNab scale

molecule and modulates systemic redox homeostasis. This mechanism also accounts for the absence of hematologic toxicity (hemolysis < 1.2%) and the favorable impact on erythrocyte metabolism, including increased ATP and 2,3-diphosphoglycerate levels, which facilitate oxygen release to ischemic tissues. Additionally, O_2–O_3 therapy positively influences autophagy—a key process in maintaining cellular homeostasis—via Nrf2-mediated upregulation of P62 and inhibition of mammalian target of rapamycin (mTOR). This effect has been demonstrated in animal models and may contribute to tissue regeneration in chronic inflammatory conditions.[27-38]

In summary, O_2–O_3 therapy should not be considered merely a symptomatic intervention, but rather a systemic modulator exerting pleiotropic effects: restoring redox balance, regulating inflammation, improving tissue perfusion, and facilitating functional recovery. Its demonstrated efficacy in post-surgical spinal patients unresponsive to conventional approaches aligns well with the biochemical mechanisms described.[27-38]

This study is not without limitations. It was conducted retrospectively on real-world clinical data, without randomized allocation to treatment groups. This design, however, reflects the need to respect individualized therapeutic pathways already established at the time of clinical indication, with treatment decisions made by a multidisciplinary team according to each patient's specific characteristics. While the observational, non-randomized design and the absence of a placebo or rehabilitation-only control group preclude definitive causal inference, the application of strict eligibility criteria and the comparable baseline characteristics between groups help to mitigate potential selection bias.

Despite these constraints, the results are consistent and robust, strongly suggesting a tangible clinical benefit and providing a solid rationale for future prospective, randomized, controlled trials. Such studies, ideally with extended follow-up, will be essential to confirm these findings, further minimize bias, and better define the long-term stability and durability of therapeutic outcomes.

5. Conclusion

The collected data suggest that paravertebral administration of O_2–O_3 under CT guidance may represent an effective, safe, and durable therapeutic option for the management of chronic low back pain following spinal stabilization surgery. Compared to standard pharmacological therapy, O_2–O_3 treatment was associated with clinically significant superiority in both pain reduction and perceived functional improvement. Within the limitations of this observational study, O_2–O_3 therapy can be considered a promising integrative approach in the multimodal management of chronic post-surgical low back pain, particularly when conventional therapies prove ineffective or poorly tolerated. Future prospective, randomized, and controlled studies with long-term follow-up are warranted to further validate these findings and establish standardized protocols for their application in this specific clinical context.

Acknowledgments

None.

Funding

None.

Conflict of interest

The authors declare no conflicts of interest with respect to the research, authorship, and/or publication of this article.

Author contributions

Conceptualization: Matteo Bonetti, Mario Muto
Data curation: Matteo Bonetti, Mario Muto, Irene Volonghi
Formal analysis: Matteo Bonetti
Investigation: Matteo Bonetti, Michele Frigerio, Giannantonio Pellicanò, Alessio Zambello, Irene Volonghi, Mario Muto, Cosma Andreula
Methodology: Matteo Bonetti, Michele Frigerio, Cosma Andreula
Supervision: Mario Muto, Cosma Andreula
Visualization: Michele Frigerio
Writing–original draft: Matteo Bonetti, Federico Maffezzoni, Serena Miglio
Writing–review & editing: Matteo Bonetti, Federico Maffezzoni, Serena Miglio, Mario Muto, Giannantonio Pellicanò, Alessio Zambello, Irene Volonghi

Ethical approval and consent to participate

This retrospective observational study was conducted in accordance with the principles of the Declaration of Helsinki. Given the retrospective and observational design of the study, based on anonymized clinical data collected during routine clinical practice, no formal request for ethical committee approval was submitted. All patients provided written informed consent prior to inclusion in the study to undergo the planned treatments and to allow the use of their anonymized clinical data for research purposes.

Consent for publication

All patients provided written informed consent for publication of anonymized data and images included in this manuscript.

Availability of data

The datasets generated and/or analyzed during the current study are not publicly available due to patient confidentiality and applicable privacy regulations, but may be made available from the corresponding author on reasonable request.

References

1. Bonetti M, Fontana A, Cotticelli B, Volta GD, Guindani M, Leonardi M. Intraforaminal O(2)-O(3) versus periradicular steroidal infiltrations in lower back pain: randomized controlled study. *AJNR Am J Neuroradiol*. 2005;26(5):996-1000.

2. Czervionke LF. Lumbar intervertebral disc disease. *Neuroimaging Clin N Am*. 1993;3:465-485.

   doi: 10.1016/S1052-5149(25)00177-7

3. Al-Sebai MW, Al-Khawashki H. Spondyloptosis and multiple-level spondylolysis. *Eur Spine J*. 1999;8(1):75-77.

   doi: 10.1007/s005860050130

4. Theiss SM. Isthmic spondylolisthesis and spondylolysis. *J South Orthop Assoc*. 2001;10(3):164-172.

5. Bonetti M, Fontana A, Albertini F. CT-guided oxygen-ozone treatment for first degree spondylolisthesis and spondylolysis. *Acta Neurochir Suppl*. 2005;92:87-92.

   doi: 10.1007/3-211-27458-8_19

6. Cinotti G, Postacchini F, Fassari F, Urso S. Predisposing factors in degenerative spondylolisthesis. A radiographic and CT study. *Int Orthop*. 1997;21(5):337-342.

   doi: 10.1007/s002640050180

7. Rothman SL, Glenn WV Jr. CT multiplanar reconstruction in 253 cases of lumbar spondylolysis. *AJNR Am J Neuroradiol*. 1984;5(1):81-90.

8. Teplick JG, Laffey PA, Berman A, Haskin ME. Diagnosis and evaluation of spondylolisthesis and/or spondylolysis on axial CT. *AJNR Am J Neuroradiol*. 1986;7(3):479-491.

9. Ulmer JL, Mathews VP, Elster AD, King JC. Lumbar spondylolysis without spondylolisthesis: recognition of isolated posterior element subluxation on sagittal MR. *AJNR Am J Neuroradiol*. 1995;16(7):1393-1398.

10. Bonetti M, Frigerio M, Ottaviani GM, Pellicanò G, Muto M. The treatment with oxygen-ozone therapy of first-degree spondylolisthesis secondary to spondylolysis: observational study on 168 patients. *J Orthop.* 2023;15(2):68-76.

    doi: 10.69149/orthopedics/2023v15iss2_2

11. Davis RA. A long-term outcome analysis of 984 surgically treated herniated lumbar discs. *J Neurosurg*. 1994;80(3):415-421.

    doi: 10.3171/jns.1994.80.3.0415

12. Zhang Y, Ma Y, Jiang J, Ding T, Wang J. Treatment of the lumbar disc herniation with intradiscal and intraforaminal injection of oxygen-ozone. *J Back Musculoskelet Rehabil*. 2013;26(3):317-322.

    doi: 10.3233/BMR-130386

13. Bosscher HA, Heavner JE. Incidence and severity of epidural fibrosis after back surgery: an endoscopic study. *Pain Pract*. 2010;10(1):18-24.

    doi: 10.1111/j.1533-2500.2009.00311.x

14. Yeo J. Failed back surgery syndrome-terminology, etiology, prevention, evaluation, and management: a narrative review. *J Yeungnam Med Sci*. 2024;41(3):166-178.

    doi: 10.12701/jyms.2024.00339

15. Lewik G, Lewik G, Müller LS, von Glinski A, Schulte TL, Lange T. Postoperative epidural fibrosis: Challenges and opportunities - A review. *Spine Surg Relat Res*. 2023;8(2):133-142.

doi: 10.22603/ssrr.2023-0106

16. Tavares WM, de França SA, Paiva WS, Teixeira MJ. A systematic review and meta-analysis of fusion rate enhancements and bone graft options for spine surgery. *Sci Rep*. 2022;12(1):7546.

doi: 10.1038/s41598-022-11551-8

17. Shahzad H, Ahmad M, Singh VK, *et al.* Predictive factors of symptomatic lumbar pseudoarthrosis following multilevel primary lumbar fusion. *N Am Spine Soc J*. 2023;17:100302.

doi: 10.1016/j.xnsj.2023.100302

18. Peene L, Cohen SP, Kallewaard JW, *et al.* 1. Lumbosacral radicular pain. *Pain Pract*. 2024;24(3):525-552.

doi: 10.1111/papr.13317

19. Geudeke MW, Krediet AC, Bilecen S, Huygen FJPM, Rijsdijk M. Effectiveness of epiduroscopy for patients with failed back surgery syndrome: A systematic review and meta-analysis. *Pain Pract*. 2021;21(4):468-481.

doi: 10.1111/papr.12974

20. Huygen FJPM, Soulanis K, Rtveladze K, Kamra S, Schlueter M. Spinal cord stimulation vs medical management for chronic back and leg pain: A systematic review and network meta-analysis. *JAMA Netw Open*. 2024;7(11):e2444608.

doi: 10.1001/jamanetworkopen.2024.44608

21. Kurt E, Noordhof RK, van Dongen R, Vissers K, Henssen D, Engels Y. Spinal cord stimulation in failed back surgery syndrome: An integrative review of quantitative and qualitative studies. *Neuromodulation*. 2022;25(5):657-670.

doi: 10.1016/j.neurom.2021.11.013

22. Abbott AD, Tyni-Lenné R, Hedlund R. Early rehabilitation targeting cognition, behavior, and motor function after lumbar fusion: a randomized controlled trial. *Spine*. 2010;35(8):848-857.

doi: 10.1097/BRS.0b013e3181d1049f

23. Sakaguchi T, Gunjotikar S, Tanaka M, *et al.* Evaluation and rehabilitation after adult lumbar spine surgery. *J Clin Med*. 2024;13(10):2915.

doi: 10.3390/jcm13102915

24. Rushton A, Eveleigh G, Petherick EJ, *et al.* Physiotherapy rehabilitation following lumbar spinal fusion: a systematic review and meta-analysis of randomised controlled trials. *BMJ Open*. 2012;2(4):e000829.

doi: 10.1136/bmjopen-2012-000829

25. Cheng H, Liu J, Shi L, Hei X. The rehabilitation-related effects on the fear, pain, and disability of patients with lumbar fusion surgery: A systematic review and meta-analysis. *Neurospine*. 2023;20(1):278-289.

doi: 10.14245/ns.2245056.528

26. Özden F, Koçyiğit GZ. The effect of early rehabilitation after lumbar spine surgery: a systematic review and meta-analysis. *Egypt J Neurosurg*. 2024;39(1).

doi: 10.1186/s41984-024-00270-z

27. Ahmed SM, Luo L, Namani A, Wang XJ, Tang X. Nrf2 signaling pathway: Pivotal roles in inflammation. *Biochim Biophys Acta Mol Basis Dis*. 2017;1863(2):585-597.

doi: 10.1016/j.bbadis.2016.11.005

28. Bonetti M, Travagli V. Gaseous ozone or method of administration: Hunt for the culprit. *Am J Emerg Med*. 2021;41:256.

doi: 10.1016/j.ajem.2020.05.110

29. Cenci A, Macchia I, La Sorsa V, Sbarigia C, Di Donna V, Pietraforte D. Mechanisms of action of ozone therapy in emerging viral diseases: immunomodulatory effects and therapeutic advantages with reference to SARS-CoV-2. *Front Microbiol*. 2022;13:871645.

doi: 10.3389/fmicb.2022.871645

30. Cisterna B, Costanzo M, Lacavalla MA, *et al.* Low ozone concentrations differentially affect the structural and functional features of non-activated and activated fibroblasts in vitro. *Int J Mol Sci*. 2021;22(18):10133.

doi: 10.3390/ijms221810133

31. de Sire A, Marotta N, Ferrillo M, *et al.* Oxygen-ozone therapy for reducing pro-inflammatory cytokines serum levels in musculoskeletal and temporomandibular disorders: A comprehensive review. *Int J Mol Sci*. 2022;23(5):2528.

doi: 10.3390/ijms23052528

32. Galiè M, Covi V, Tabaracci G, Malatesta M. The role of Nrf2 in the antioxidant cellular response to medical ozone exposure. *Int J Mol Sci*. 2019;20(16):4009.

doi: 10.3390/ijms20164009

33. Goldman M. Cancer risk of low-level exposure. *Science*. 1996;271(5257):1821-1822.

doi: 10.1126/science.271.5257.1821

34. Hidalgo-Tallón FJ, Torres-Morera LM, Baeza-Noci J, Carrillo-Izquierdo MD, Pinto-Bonilla R. Updated review on ozone therapy in pain medicine. *Front Physiol*. 2022;13:840623.

doi: 10.3389/fphys.2022.840623

35. İnci H, İnci F. Effect of ozone therapy on neutrophil/lymphocyte, platelet/lymphocyte ratios, and disease activity in ankylosing spondylitis: a self-controlled randomized study. *Med Gas Res*. 2023;13(2):53-58.

doi: 10.4103/2045-9912.344981

36. Malatesta M, Cisterna B, Costanzo M. Cellular and molecular mechanisms of ozone therapy: Present knowledge and prospective applications. *Int J Mol Sci*. 2022;23(20):12586.

doi: 10.3390/ijms232012586

37. Martínez de Toda I, Ceprián N, Díaz-Del Cerro E, De la Fuente M. The role of immune cells in oxi-inflamm-aging. *Cells*. 2021;10(11):2974.

doi: 10.3390/cells10112974

38. Parzych KR, Klionsky DJ. An overview of autophagy: morphology, mechanism, and regulation. *Antioxid Redox Signal*. 2014;20(3):460-473.

doi: 10.1089/ars.2013.5371

39. Re L. Ozone in Medicine: A Few Points of Reflections. *Front Physiol*. 2022;13:842229.

doi: 10.3389/fphys.2022.842229

40. Scassellati C, Galoforo AC, Bonvicini C, Esposito C, Ricevuti G. Ozone: A natural bioactive molecule with antioxidant property as potential new strategy in aging and in neurodegenerative disorders. *Ageing Res Rev*. 2020;63:101138.

doi: 10.1016/j.arr.2020.101138

41. Serra MEG, Baeza-Noci J, Mendes Abdala CV, Luvisotto MM, Bertol CD, Anzolin AP. The role of ozone treatment as integrative medicine. An evidence and gap map. *Front Public Health*. 2023;10:1112296.

doi: 10.3389/fpubh.2022.1112296

42. Sun P, Xu W, Zhao X, *et al.* Ozone induces autophagy by activating PPARγ/mTOR in rat chondrocytes treated with IL-1β. *J Orthop Surg Res*. 2022;17(1):351.

doi: 10.1186/s13018-022-03233-y

43. Travagli V, Zanardi I, Bocci V. A realistic evaluation of the action of ozone on whole human blood. *Int J Biol Macromol*. 2006;39(4-5):317-320.

doi: 10.1016/j.ijbiomac.2006.03.024

44. Travagli V, Iorio EL. The biological and molecular action of ozone and its derivatives: State-of-the-art, enhanced scenarios, and quality insights. *Int J Mol Sci*. 2023;24(10):8465.

doi: 10.3390/ijms24108465

45. Ulasov AV, Rosenkranz AA, Georgiev GP, Sobolev AS. Nrf2/Keap1/ARE signaling: Towards specific regulation. *Life Sci*. 2022;291:120111.

doi: 10.1016/j.lfs.2021.120111

46. Viebahn-Haensler R, León Fernández OS. Ozone in Medicine. The low-dose ozone concept and its basic biochemical mechanisms of action in chronic inflammatory diseases. *Int J Mol Sci*. 2021;22(15):7890.

doi: 10.3390/ijms22157890

47. Zhang W, Feng C, Jiang H. Novel target for treating Alzheimer's diseases: Crosstalk between the Nrf2 pathway and autophagy. *Ageing Res Rev*. 2021;65:101207.

doi: 10.1016/j.arr.2020.101207

48. Magalhaes FN, Dotta L, Sasse A, Teixera MJ, Fonoff ET. Ozone therapy as a treatment for low back pain secondary to herniated disc: a systematic review and meta-analysis of randomized controlled trials. *Pain Physician*. 2012;15(2):E115-E129.

49. Rimeika G, Saba L, Arthimulam 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. *Eur J Radiol Open*. 2021;8:100389.

doi: 10.1016/j.ejro.2021.100389

50. Steppan J, Meaders T, Muto M, Murphy KJ. A metaanalysis of the effectiveness and safety of ozone treatments for herniated lumbar discs. *J Vasc Interv Radiol*. 2010;21(4):534-548.

doi: 10.1016/j.jvir.2009.12.393

51. Andreula CF, Simonetti L, De Santis F, Agati R, Ricci R, Leonardi M. Minimally invasive oxygen-ozone therapy for lumbar disk herniation. *AJNR Am J Neuroradiol*. 2003;24(5):996-1000.

52. Muto M, Andreula C, Leonardi M. Treatment of herniated lumbar disc by intradiscal and intraforaminal oxygen-ozone (O2-O3) injection. *J Neuroradiol*. 2004;31(3):183-189.

doi: 10.1016/s0150-9861(04)96989-1

53. Iliakis E, Valadakis V, Vynios DH, Tsiganos CP, Agapitos E. Rationalization of the activity of medical ozone on intervertebral disc a histological and biochemical study. *Riv Neuroradiol*. 2001;14(1_suppl):23-30.

doi: 10.1177/19714009010140s105

54. Rahimi-Movaghar V, Eslami V. The major efficient mechanisms of ozone therapy are obtained in intradiscal procedures. *Pain Physician*. 2012;15(6):E1007-E1008.

55. Lehnert T, Naguib NN, Wutzler S, *et al.* Analysis of disk volume before and after CT-guided intradiscal and periganglionic ozone-oxygen injection for the treatment of lumbar disk herniation. *J Vasc Interv Radiol*. 2012;23(11):1430-1436.

doi: 10.1016/j.jvir.2012.07.029

56. Splendiani A, Perri M, Conchiglia A, *et al.* MR assessment of lumbar disk herniation treated with oxygen-ozone diskolysis: the role of DWI and related ADC versus intervertebral disk volumetric analysis for detecting treatment response. *Neuroradiol J*. 2013;26(3):347-356.

doi: 10.1177/197140091302600316

57. Gallucci M, Limbucci N, Zugaro L, *et al.* Sciatica:

treatment with intradiscal and intraforaminal injections of steroid and oxygen-ozone versus steroid only. *Radiology*. 2007;242(3):907-913.

doi: 10.1148/radiol.2423051934

58. Perri M, Grattacaso G, Di Tunno V, *et al.* MRI DWI/ADC signal predicts shrinkage of lumbar disc herniation after O2-O3 discolysis. *Neuroradiol J*. 2015;28(2):198-204.

doi: 10.1177/1971400915576658

59. Borrelli E, Alexandre A, Iliakis E, Alexandre A, Bocci V. Disc herniation and knee arthritis as chronic oxidative stress diseases: The therapeutic role of oxygen ozone therapy. *J Arthritis*. 2015;04(03).

doi: 10.4172/2167-7921.1000161

60. Bonetti M, Zambello A, Leonardi M, Princiotta C. Herniated disks unchanged over time: Size reduced after oxygen-ozone therapy. *Interv Neuroradiol*. 2016;22(4):466-472.

doi: 10.1177/1591019916637356

61. Leonardi M, Simonetti L, Raffi L, Cenni P, Barbara C. Mini-invasive treatment of herniated disc by oxygen-ozone injection. *Interv Neuroradiol*. 2003;9(Suppl 2):75.

doi: 10.1177/15910199030090S211

62. Dall'Olio M, Princiotta C, Cirillo L, *et al.* Oxygen-ozone therapy for herniated lumbar disc in patients with subacute partial motor weakness due to nerve root compression. *Interv Neuroradiol*. 2014;20(5):547-554.

doi: 10.15274/INR-2014-10078

63. Pellicanò G, Martinelli F, Tavanti V, *et al.* The Italian Oxygen-Ozone Therapy Federation (FIO) study on oxygen-ozone treatment of herniated disc. *Int J Ozone Ther*. 2007;6:7-15.

64. Bonetti M, Fontana A, Martinelli F, Andreula C. Oxygen-ozone therapy for degenerative spine disease in the elderly: a prospective study. *Acta Neurochir Suppl*. 2011;108:137-142.

doi: 10.1007/978-3-211-99370-5_21

65. Alyan S, Zaghlol R, Mustafa SA. Efficacy of combined paravertebral ozone (O2O3) therapy with physiotherapy in patients with chronic mechanical low back pain. *Egypt Rheumatol Rehabil*. 2018;45(3):106-111.

doi: 10.4103/err.err_43_17

66. de Sire A, Agostini F, Lippi L, *et al.* Oxygen-ozone therapy in the rehabilitation field: state of the art on mechanisms of action, safety and effectiveness in patients with musculoskeletal disorders. *Biomolecules*. 2021;11(3):356.

doi: 10.3390/biom11030356

67. de Sire A, Parente A, Prestifilippo E, *et al.* Efficacy of oxygen-ozone therapy and proprioceptive neuromuscular facilitation on pain and disability in patients affected by acute or subacute low back pain: A randomized controlled trial. *J Back Musculoskelet Rehabil*. 2026;39(1):228-241.

doi: 10.1177/10538127251360867

68. Nuova FIO – Italian Federation of Oxygen-Ozone Therapy. *Aggiornamento delle linee guida e buone pratiche in ossigeno-ozonoterapia* [*Update of guidelines and best practices in oxygen-ozone therapy*]. Rome, Italy: Nuova FIO; 2024. Available from: https://nuovafio.it/aggiornamento-delle-linee-guida-e-buone-pratiche-in-ossigeno-ozonoterapia/ [Last accessed on May 8, 2026].

69. Ostelo RW, de Vet HC. Clinically important outcomes in low back pain. *Best Pract Res Clin Rheumatol*. 2005;19(4):593-607.

doi: 10.1016/j.berh.2005.03.003

70. Farrar JT, Young JP Jr, LaMoreaux L, Werth JL, Poole MR. Clinical importance of changes in chronic pain intensity measured on an 11-point numerical pain rating scale. *Pain*. 2001;94(2):149-158.

doi: 10.1016/S0304-3959(01)00349-9

71. Copay AG, Glassman SD, Subach BR, Berven S, Schuler TC, Carreon LY. Minimum clinically important difference in lumbar spine surgery patients: a choice of methods using the Oswestry Disability Index, Medical Outcomes Study questionnaire Short Form 36, and pain scales. *Spine J*. 2008;8(6):968-974.

doi: 10.1016/j.spinee.2007.11.006