

Review

CONSENSUS CONFERENCE: USE OF OZONE IN THE CONTEXT OF REGENERATIVE MEDICINE. RECOMMENDATIONS FROM THE ITALIAN SOCIETY OF MULTI-SPECIALIST REGENERATIVE MEDICINE AND SURGERY (SIMCRI)

Running head: Consensus Conferences SIMCRI Ozone

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ABSTRACT

This paper summarizes the final pronouncement of the Consensus Conference on “Use of Ozone in Regenerative Medicine” and discusses the integration of ozone therapy (OT) in clinical practice based on current scientific knowledge and clinical experience. Organized by the Italian Society of Multi-Specialist Regenerative Medicine and Surgery (SIMCRI), the consensus meeting brought together expert clinicians and researchers to explore OT's mechanisms, indications, contraindications, clinical protocols, and potential adverse reactions. Key aspects addressed included evaluating the effectiveness of OT in vulnology and for degenerative joint disorders and its combination with Platelet Rich Plasma (PRP). Efficacy assessment criteria were based on evidence-based medicine. The consensus concluded that local or systemic OT is both safe and effective, particularly for challenging ulcers and some degenerative osteoarticular conditions. Additionally, combining PRP with ozone may enhance reparative processes without adverse effects. Ozone's ability to improve circulation, reduce oxidative stress and inflammation, and counteract hypoxia complements PRP in tissue regeneration. However, while these findings are promising, the available literature remains limited and of low evidence quality. The consensus recommends further multidisciplinary studies to establish OT as a clinical practice, especially for conditions like diabetic foot, where combined ozone and PRP therapies could provide significant therapeutic benefits.

KEYWORDS: *ozone therapy, regenerative medicine, consensus conference, platelet-rich plasma*

INTRODUCTION

This document aims to serve as a comprehensive reference point for professionals engaged in the rapidly evolving field of Regenerative Medicine and Oxygen-Ozone Therapy (OOT). As this discipline is relatively young, it is in a constant state of dynamic development (1). Therefore, this text should not be seen as definitive but rather as a clear snapshot of the current "state of the art". It provides a foundation for further progress, fostering growth and integration within the broader medical community and its related specialties.

In recent years, Regenerative Medicine, particularly through the use of non-transfusional blood components (NTBC) and OOT, has seen significant advancement and widespread application across a variety of clinical contexts (2). Studies have demonstrated that OOT offers a range of benefits, including germicidal, anti-inflammatory, antioxidant, analgesic, and immunomodulatory properties (3-6). The combination of these two therapies holds great promise, driven

by two hypothesised physiopathological mechanisms: 1) preparing the microenvironment for the application of regenerative therapies; 2) stimulating the release of growth factors from platelets (7).

The Consensus Conference (CC) focuses on two primary groups of pathologies: 1) Vulnology (e.g., diabetic ulcer, chronic ulcers, and difficult wounds); 2) degenerative musculoskeletal conditions (e.g., discogenic lumbago and gonarthrosis).

It is essential to note that, for all diseases within the scope of regenerative medicine, including those discussed here, two key physiopathological issues remain unresolved and open for debate: a) the incomplete understanding of the physiological mechanisms underlying "natural" tissue regeneration, as well as regeneration induced by regenerative medicine through NTBC or OOT; b) the effects that OOT may have when applied alongside specific regenerative medicine procedures, such as PRP treatment.

Consequently, this document draws on a thorough review of the existing literature to identify relevant publications that either support the use of OOT in the clinical conditions under examination or the combined application of OOT and Regenerative Medicine procedures, such as PRP. The aims of the CC were to establish a structured, evidence-based approach to integrating OOT with regenerative treatments, ultimately advancing the field in a scientifically rigorous manner.

MATERIALS AND METHODS

The methodology for developing the recommendations in the context of OOT and regenerative medicine CC follows the principles of Evidence-Based Medicine (EBM) and aligns with the Italian National System for Guidelines (INSG) (8). Below is an overview of the process and its rigorous framework:

- 1) systematic literature reviews: based on the PRISMA guidelines, ensuring methodological rigor;
- 2) emphasizes the inclusion of systematic reviews, RCTs, and high-quality observational studies;
- 3) multidisciplinary and institutional discussions: recommendations are evaluated and discussed in multidisciplinary forums and institutional settings to ensure clinical relevance and broad acceptance;
- 4) evidence assessment: focuses on the statistical validity, clinical relevance, dissemination, and real-world applicability of selected studies. Evidence quality and recommendation strength are derived using adapted frameworks like those of the US Preventive Services Task Force and Centre for Evidence-Based Medicine, Oxford;
- 5) conflict of interest transparency: all contributors signed declarations of the absence of conflicts of interest, ensuring unbiased recommendations.

The Levels of evidence and grades of recommendation were then established by the following criteria:

- 1) level A: strong scientific evidence from systematic reviews with RCTs or homogenous cohort/case-control studies. Implication: clinical benefits substantially outweigh risks;
- 2) level B: moderate-quality evidence from individual RCTs or well-conducted observational studies. Implication: clinical benefits outweigh risks;
- 3) level C: lower-quality evidence based on expert opinion, case reports, or physiological/laboratory research. Implication: Clinical benefits and risks are approximately equal;

The recommendations aim to ensure high-quality evidence synthesis, clear grading based on the strength of evidence, and benefit-risk balance, as well as adherence to international standards (PRISMA, EBM guidelines) and INSG.

This structured approach facilitates the development of actionable, evidence-based recommendations while maintaining transparency and minimizing bias. The methodology for conducting the CC followed a structured approach to ensure comprehensive evaluation and decision-making was based on the following points:

- 1) Establishment of working groups. Two groups were formed, each focused on specific areas: the medical-technical group on vulnology, addressing the use of OOT and Regenerative Medicine in wound care. the medical-technical group on orthopaedics focuses on similar applications in orthopaedics. Each group developed preliminary guidelines and recommendations, which were shared with the jury before the final meeting.
- 2) Jury composition and coordination: the jury consisted of two representatives from each working group, led by Dr. Gianfranco Francioni. The jury reviewed documents and prepared for deliberations ahead of the meeting, scheduled for 20 September 2024 in Rome.
- 3) Consensus Conference phases. First phase: experts presented evidence related to their fields. All stakeholders, including the coordination panel, technical-scientific committee, and the jury, participated in discussions. Second

Phase: The jury formulated recommendations based on a pre-prepared draft document. A writing committee proposed recommendation texts, which were reviewed, discussed, and voted upon.

- 4) Voting procedure. Recommendations were divided into two parts for voting: The text of the recommendation and the strength of the recommendation (weak/strong). Votes were collected anonymously via an online poll, with results calculated as the percentage of agreement. A recommendation was accepted if at least 75% approval was achieved for both the text and its strength. The process followed the Delphi method, promoting iterative discussion and consensus-building.
- 5) Bibliographic databases consulted: MEDLINE/PubMed, SciELO, LILACS, PAHO, EMBASE, ZOTERO ISCO3, WHO International Clinical Trials Registry Platform, and NIH. U.S. National Library of Medicine. The documents reviewed were published between 1980 and 2024, in English, Russian, or Spanish, and included: original articles, published theses, clinical reports, ongoing clinical trials, and bibliographic reviews. The exclusion criteria were the lack of free access to complete text due to financial constraints and/or, studies presenting inadequate scientific evidence.

RESULTS

Evidence on the topic of vulnology - difficult wounds

Ulcers pose a significant challenge to global health, transcending medical concerns to become a societal and policy issue. Over the past two decades, studies have documented a steady rise in their prevalence, partly due to the aging population and the increased prevalence of lifestyle-related risk factors such as smoking, obesity, and diabetes (9).

It is estimated that ulcers affect approximately 80 million people worldwide, or about 1% of the global population. Among individuals aged 60 and older, the incidence rises markedly, approaching 5% (10,11). In Italy, an estimated 2 million people suffer from ulcers, resulting in a loss of about 450,000 workdays annually. Economically, chronic varicose ulcers in developed nations accounted for 1% of GDP as early as 2001, with annual costs of 1-3 billion euros. Current projections suggest that the economic burden of treating chronic ulcers may rise from \$18 billion in 2021 to \$30 billion by 2030 (12).

A notable characteristic of these wounds is their cyclical nature, with periods of healing followed by recurrence. This dynamic contributes to high recurrence rates of 18-28% within 12 months of healing (13).

Use of PRP in difficult wounds

Recent investigations into the application of PRP (platelet-rich plasma) for treating challenging wounds, including systematic reviews, meta-analyses, and randomized controlled trials, highlight significant findings. Studies consistently show that PRP is associated with a higher rate of complete wound healing and shorter healing times compared with controls (14-20). However, reductions in ulcer size achieved with PRP have not consistently shown significant differences compared with conventional treatments. These findings underscore the potential of PRP as an effective therapy for skin ulcers, particularly diabetic foot ulcers (DFUs), while affirming its safety profile (21).

Given these observations, PRP appears to enhance chronic wound healing without increasing the risk of infection or adverse events. However, large-scale, standardized, long-term clinical trials are necessary to conclusively confirm its efficacy and safety. More comprehensive reporting on preparation methods and administered platelet doses is also essential to ensure the reproducibility of results. In practice, PRP has become a regular component of treatment protocols for various types of skin ulcers, with its use expanding in both public and private healthcare settings.

Use of ozone in difficult wounds

In managing difficult wounds, OOT is applied in various forms, gas, ozonated water, and ozonated oil, primarily via local administration. Occasionally, it is combined with systemic approaches. Numerous clinical studies have explored the effects of OOT on chronic ulcers (22-27), DFUs (2, 28-43), and infected burn ulcers (44-48).

Selected scientific articles categorised by evidence level

Meta-analysis: a comprehensive search of The Cochrane Library, PubMed, Ovid Embase, Web of Science, and the Chinese Biomedical Literature Database included randomised controlled trials (RCTs) involving patients with chronic wounds.

Bias risk was assessed using the Cochrane Risk of Bias Tool. Results, grouped by wound type, showed that OOT, administered through topical methods (e.g., ozone gas bag, ozonated oil, ozonated water washing) or systemic

methods (auto-blood transfusion, rectal insufflation), significantly accelerated wound area reduction (standardised mean difference SMD = 66.54%, 95% CI = (46.18–86.90), $P < 0.00001$) and reduced amputation rates (RR = 0.36, 95% CI = (0.24–0.54), $P < 0.00001$) for diabetic foot ulcers, compared with standard therapy. However, no significant difference in complete healing rates was observed during hospital stays. No adverse events were reported. The efficacy of OOT for other chronic wound types remains uncertain due to insufficient reliable data. Further large-scale RCTs are required to confirm its efficacy and safety (48).

Systematic review: of 326 articles reviewed, 17 were selected after applying exclusion criteria. The study aimed to evaluate OOT's safety and efficacy in dermatological conditions. Findings suggest OOT is effective in the short term, particularly for ulcers and scars, but long-term safety requires further investigation (4).

Systematic review: a review of 44 articles identified seven studies on OOTs' role in venous ulcers. The conclusions indicate that OOT reduces infection, amputation, and hospitalisation rates, thereby promoting quicker healing times. However, its safety and efficacy need to be validated by high-quality studies (25).

Systematic review: from 12 selected articles, nine met the inclusion criteria. The study examined OOT's potential in chronic wound management, concluding that while positive effects were noted, standardisation of application methods is necessary to optimise dosing (49).

Prospective study: a trial with 54 patients demonstrated that local OOT reduced wound size and pain intensity in venous ulcers (50).

RCT: a trial with 47 patients showed systemic ozone therapy improved wound healing, oxidative indices, and reduced hospital stays in DFUs (51).

RCT: a study with 50 participants found that OOT significantly accelerated wound healing and increased expression of VEGF, TGF, and PDGF, highlighting its role in stimulating growth factor signaling (31).

Prospective observational study: in 162 diabetic foot ulcer patients, topical ozone accelerated granulation tissue formation, reduced inflammation, improved wound margins, and shortened hospital stays. OOT is increasingly adopted in chronic wound care, demonstrating efficacy in reducing infection rates and healing times. However, variability in protocols and lack of long-term data necessitate further research to establish standardised, evidence-based guidelines (52).

Prospective observational comparative cohort study: a study involving 162 patients assessed the safety and efficacy of topical ozone gas for the management of DFUs. The participants were divided into two groups: 81 patients received standard treatment (debridement, cleaning, Betadine, and bandaging), while the other 81 received the same regimen supplemented with topical ozone. The article concluded that topical ozone therapy accelerated exudate clearance, promoting faster granulation tissue formation and improved wound edge healing. Ozone therapy facilitated the reduction of microbial colonisation and inflammation. Patients receiving OOT experienced a significant reduction in hospital stay duration. This study supports the potential of ozone therapy as an adjunctive treatment for DFUs, highlighting its benefits in promoting healing and reducing treatment time (39).

Use of PRP activated with ozone in difficult wounds

Randomised controlled trial: this study evaluated the effects of autologous PRP activated by either CaCl_2 or medical ozone on wound healing in 90 high-risk pregnant women undergoing caesarean section. Participants were divided into three groups: 1) CaCl_2 Group: Received autologous PRP and CaCl_2 -activated fibrin gel at the wound site; 2) ozone Group (O): received autologous PRP and ozone-activated fibrin gel at the wound site; 3) Control Group (P): did not receive PRP.

Wound healing and complications were assessed on postoperative days 1, 7, and 21 using the VAS (Visual Analogue Scale) for pain assessment. REEDA Scale: is a 0-15 descriptive scale evaluating redness, oedema, ecchymosis, discharge, and wound-edge approximation, where lower scores indicate better healing. Vancouver Healing Scale (VSS): was used for scar healing. Results: pain (VAS): the ozone group reported significantly less pain compared to the control group on days 7 and 21; healing (REEDA): the ozone group demonstrated significantly lower scores compared to the CaCl_2 group on days 1 and 7, and compared to the control group on all days; scar healing (VSS): both the ozone and CaCl_2 groups showed better results than the control group on all days.

Ozone-activated PRP was more effective than CaCl_2 -activated PRP and standard care in promoting wound healing and reducing complications in high-risk patients, potentially preventing infections in caesarean section wounds (53).

Case Report: A 65-year-old female with chronic venous insufficiency and a phlebostatic ulcer on her lower limb was treated with ozone-activated PRP-calcium chloride and fibrin membranes. The patient received five applications, with visible improvements in re-epithelialisation observed after the second session.

Complete ulcer healing was achieved six weeks post-treatment, with no adverse effects and reductions in pain and improved limb mobility reported. These findings highlight the therapeutic potential of ozone-activated PRP in enhancing wound healing in various clinical settings. Further trials are recommended to establish standardised protocols and confirm long-term benefits (54).

Evidence on the topic joints disorders

The global prevalence of osteoarthritis (OA) has more than doubled over recent decades, rising by 113.25% from 247.51 million cases in 1990 to 527.81 million in 2019, reflecting an average annual increase of 0.12%. Among the various forms of OA, knee osteoarthritis is the most significant contributor. The prevalence of knee OA increases with age and is higher in women. This trend has resulted in a growing number of individuals experiencing knee pain and functional limitations, many of whom are likely candidates for knee replacement surgeries in the future (55).

In Italy, the impact of knee OA is starkly illustrated by the increase in knee replacement procedures, which rose from 9,632 in 2019 to 27,156 in 2021. Between 2007 and 2021, a total of 255,709 knee prosthesis interventions were performed (56). The anticipated rise in OA prevalence underscores the urgent need for enhanced primary and secondary prevention strategies, alongside early interventions such as regenerative medicine. These measures could mitigate the clinical, social, and economic burdens posed by OA on healthcare systems. Additionally, addressing modifiable risk factors such as obesity, injury prevention, and excessive joint loading is essential. Educational initiatives should aim to increase awareness of disease progression, consequences, early treatment options, and rehabilitation strategies (55).

Symptomatic intervertebral disc degeneration affects an estimated 403 million individuals worldwide, equivalent to approximately 5.5% of the global population. The lifetime risk of experiencing discogenic low back pain (DLBP) is around 30%. A majority (60–90%) of individuals with DLBP respond positively to non-surgical interventions. However, for those requiring surgical treatment, the recurrence rate of DLBP is approximately 9.1%, with 38% of these recurrences occurring within the first-year post-surgery (57, 58).

Evidence of the use of PRP, ozone, and ozone-activated PRP in knee OA and evidence of the use of PRP in knee OA

Meta-analysis: this meta-analysis adhered to Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines for systematic reviews and meta-analyses. The literature search included databases such as Web of Science, Embase, Cochrane Library, PubMed, and Wanfang, focusing on RCTs evaluating the efficacy of corticosteroids (CSC), PRP, hyaluronic acid (HA), and combination therapies for managing knee OA. Bias was assessed using Cochrane tools (v1.0), and the study employed the VAS, Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC), and treatment-related adverse events as primary outcome measures. A network meta-analysis was conducted using STATA17 and a Bayesian stratified random-effects model.

Results: a total of 35 studies encompassing 3104 participants were analysed. Short-term Outcomes (3 months): PRP exhibited the best WOMAC scores, followed by PRP + HA, HA, placebo, and CSC. In terms of VAS scores, PRP + HA was superior, followed by PRP, CSC, HA, and placebo.

Mid-term Outcomes (6 months): WOMAC scores favoured PRP, CSC, HA, and placebo equally. PRP + HA was the most effective for VAS scores.

Long-term Outcomes (12 months): PRP had the best WOMAC scores, followed by PRP + HA, HA, placebo, and CSC. PRP also showed the best VAS scores, with PRP + HA ranking second.

Safety: none of the therapies demonstrated a significant increase in treatment-related adverse events compared to placebo, confirming their safety profiles. This study highlights the superior efficacy of PRP and PRP + HA in improving function and alleviating pain at 3, 6, and 12 months of follow-up in OA patients. Furthermore, CSC, HA, PRP, and combination therapies did not show any notable increase in adverse events compared to placebo, supporting their safety for clinical use (59).

Evidence of the use of ozone in knee OA

The studies provide a detailed picture of the current understanding of intra-articular OOT in knee OA, with varying levels of evidence and conclusions.

Meta-analysis: this meta-analysis included six RCTs comparing intra-articular ozone to HA for knee OA. The primary outcome (VAS for pain) did not show significant differences between groups, although overall WOMAC scores favoured HA slightly. However, no specific time points showed a meaningful statistical advantage for either therapy. The study concluded that ozone therapy's effects were similar to those of HA, suggesting it acts more as a placebo than as a

true disease modifier. Conclusion: Ozone may provide symptomatic relief comparable to HA but lacks long-term evidence as a disease-modifying agent (60).

Meta-analysis: a systematic review of 10 studies highlighted the effectiveness of OOT for chronic OA pain, with a pooled SMD of -28.551, indicating significant pain reduction. The study emphasised a statistically significant impact, with no serious adverse events reported. Conclusion: This study supported the potential of ozone therapy to manage chronic pain effectively in knee OA, although long-term safety and standardisation remain lacking (61).

Meta-analysis: a smaller analysis of five RCTs with 428 patients showed mixed outcomes. Ozone performed slightly better than control injections (HA, dextrose, or air) in the short term (first month), but its benefits decreased over longer follow-up periods (3–6 months), favouring controls. Adverse events were similar between groups. Conclusion: While ozone can be effective for short-term management, its long-term efficacy is lower than that of other interventional treatments (62).

Systematic review: this review of 10 studies involving 400 patients treated with ozone concluded that intra-articular ozone provided more effective short-term pain relief than placebo and was comparable to other interventions. However, many studies were at high risk of bias, highlighting the need for higher-quality research. Conclusion: Ozone appears effective for short-term symptom relief, but its long-term efficacy and methodological robustness need confirmation (63).

Non-RCT study: an observational study at Ankara University that enrolled 94 patients receiving intra-articular ozone injections. Significant reductions in pain scores and analgesic use were noted over three months, with high patient satisfaction (70.2%). No side effects were reported. Conclusion: Ozone injections significantly reduced pain in stage 2–3 knee OA over 3 months, but uncertainties remain regarding dosing, session frequency, and long-term effects (64).

Key takeaways across studies: ozone therapy provides effective short-term symptom relief in knee OA, particularly for pain reduction; comparative outcomes: its efficacy is comparable to HA and other interventions in the short term but may decrease over longer follow-up periods; safety profile: consistent across studies, OOT is associated with minimal adverse effects, highlighting its safety; research gaps: Long-term efficacy, standardisation of treatment protocols, and better-quality RCTs are needed to establish its role in managing OA.

These findings suggest that intra-articular OOT can be considered a safe and effective short-term treatment option for mild to moderate knee OA, though its role as a long-term therapeutic modality remains uncertain.

Evidence of the use of PRP plus ozone in knee OA

Randomised Controlled Trial. This study retrospectively analysed data from patients who received either PRP alone ($n = 45$) or a combined PRP and ozone treatment ($n = 35$). Patients were evaluated using VAS for pain and the WOMAC to assess physical function and joint health. Both treatment groups demonstrated significant reductions in post-treatment VAS and WOMAC scores at months 1, 3, and 6 when compared to pre-treatment levels ($p < 0.001$).

Notably, the combined PRP + ozone group exhibited significantly lower VAS scores and total WOMAC scores at month 3 post-treatment compared to the PRP-only group. Additionally, the PRP + ozone group experienced reduced pain at day 10 and lower hyperinflammation at the injection site compared with the PRP-alone group.

The combined treatment of PRP and ozone showed overall efficacy comparable to that of PRP alone in managing OA symptoms. However, the addition of ozone appeared to enhance recovery rates and minimise post-injection pain, suggesting potential advantages for certain patient groups (65).

Randomised Controlled Trial. This study included 102 patients diagnosed with knee OA based on the American College of Rheumatology criteria. Participants were randomised into three groups, each containing 34 patients: 1) Ozone Group: Treated with intra-articular ozone injections (10 $\mu\text{g/mL}$, 5 mL), administered twice weekly for six weeks (12 injections). 2) Combination Group (PRP + Ozone): Received intra-articular injections of PRP with ozone (10 $\mu\text{g/mL}$, 5 mL) once weekly for three weeks (3 injections). 3) PRP Group: Treated with PRP injections alone, once weekly for three weeks (3 injections).

VAS scores were recorded to assess pain levels, and participants were included only if baseline scores were 5 or higher. The Clinical Global Impression Scale was used to evaluate the overall strength of improvement. The combination of PRP + ozone yielded superior outcomes compared to PRP or ozone monotherapy. The combined treatment group demonstrated the greatest improvements in pain reduction and overall clinical outcomes, suggesting synergistic effects of the two interventions. This approach may provide a more effective option for patients requiring greater pain relief and functional improvement (66).

Evidence of the use of PRP, ozone, and ozone-activated PRP in DLBP pain; evidence of the use of PRP in DLBP pain

Meta-Analysis: this meta-analysis included 6 studies (3 RCTs, 3 prospective single-arm studies) investigating PRP for DLBP. Pain scores and functional outcomes were measured up to 6 months post-treatment. Findings: pain scores decreased significantly at 1, 2, and 6 months (SMD: -1.04 to -1.42, $P < .05$), and Oswestry Disability Index (ODI) scores improved by >30% and >50% in significant portions of patients at 2 and 6 months. No adverse reactions were reported. Conclusion: PRP intradiscal injections are effective and safe for DLBP, providing sustained pain relief and functional improvements for at least 6 months. However, more high-quality studies are needed to strengthen these findings (67).

Meta-Analysis: this meta-analysis pooled data from 3 RCTs involving 131 patients with DLBP treated with PRP. Findings: PRP significantly reduced pain (mean difference: -1.47, $P < 0.0001$) and increased the proportion of patients experiencing >50% pain relief at 3 months (RR: 4.14, $P < 0.00001$). Patient satisfaction was notably higher, with no reported increase in adverse events ($P = 0.07$). Conclusion: PRP demonstrated significant pain reduction and improved patient satisfaction compared to controls, without increased risks (68).

Systematic Review: A total of 37 studies were reviewed, and 12 met the inclusion criteria, focusing on PRP and other intradiscal biologic agents. Findings: PRP success rates were 54.8% (CI: 40%-70%), but the evidence quality was very low due to methodological flaws in many studies. Conclusion: While PRP showed potential in DLBP management, the current evidence is of low quality, and robust RCTs are necessary for definitive conclusions (69).

Summary of evidence and recommendations: PRP shows moderate effectiveness (grade B evidence) in managing pain and disability in DLBP, with improvements sustained over several months. The current data indicate PRP is safe, with no significant adverse events reported across studies. Despite positive results, the quality of evidence remains inconsistent, necessitating larger, well-designed RCTs to confirm efficacy and standardise protocols for DLBP treatment.

Evidence of the use of OOT in DLBP

Meta-Analysis: reviewed 45 studies on percutaneous O₂/O₃ injections (image-guided and non-image-guided) for DLBP and sciatica from 1980–2020. The O₂/O₃ mixture (10–40 µg/mL) was administered alone or combined with other treatments (e.g., steroids, anti-inflammatory drugs). Image guidance (fluoroscopy or CT) was used in 99% of cases. Findings: Image-guided O₂/O₃ injections were more effective for pain reduction (effect size: 4.48, $p < 0.0001$) compared to non-image-guided procedures (effect size: 3.17, $p < 0.0001$). Conclusion: OOT is a minimally invasive and cost-effective option for DLBP resistant to conservative treatments. Image-guided procedures show better results but require further validation in high-quality studies (70).

Meta-Analysis: included 6 RCTs comparing OOT with other interventions for DLBP. Findings: OOT provided superior pain relief at six months (RR = 2.2, $p < 0.00001$) compared to steroids or placebo. However, biases in study designs limit the reliability of these results. Conclusion: While OOT appears effective for sustained pain relief, additional well-designed RCTs are necessary (71).

Systematic Review: reviewed 15 studies (2,597 patients) on OOT for DLBP and herniated disc. Findings: OOT was generally more effective than control treatments, including steroids, placebo, and even surgery (microdiscectomy). Despite these positive outcomes, most studies were of low quality and showed significant bias. Conclusion: OOT is a safe alternative with promising results for short- and medium-term outcomes. High-quality studies are required to determine long-term efficacy and safety (72).

Systematic Review: analyzed 7 studies comparing OOT with other treatments (e.g., steroids, microdiscectomy) for DLBP. Findings: OOT showed similar or better pain and functional outcomes. Adverse effects were rare and generally minor. Conclusion: despite limited and poor-quality studies, OOT demonstrates potential for DLBP and herniated disc. Improved research designs are essential for more robust conclusions (73).

Meta-Analysis: Pooled 12 studies with over 8,000 patients. Findings: OOT demonstrated similar efficacy to surgical discectomy for pain and functional improvement but with lower complication rates (0.064%) and faster recovery. Conclusion: OOT is effective and extremely safe, with a significantly better safety profile than surgical alternatives (74).

Summary and Recommendations: Efficacy: OOT consistently shows pain relief and functional improvement in DLBP and herniated disc across studies. Image-guided procedures appear to enhance therapeutic outcomes compared to non-image-guided methods. Safety: adverse effects are rare and minor, supporting OOT as a safe alternative to invasive procedures like surgery. Limitations: most studies suffer from methodological flaws, high bias risk, or poor quality. Standardized protocols and rigorous research are essential to establish definitive conclusions. Clinical implication: OOT is a viable option for patients unresponsive to conservative treatments, with evidence supporting its use for short- to medium-term outcomes. However, its role relative to surgery and other minimally invasive methods requires further exploration.

Evidence of the use of PRP plus OOT in DLBP

Prospective Non-Randomized Study: 60 patients (ages 35–82) with severe lumbosciatica (VAS: 7–8) underwent foraminal and facet injections of ozone (10 µg/mL, 10 mL) combined with Concentrated Growth Factors (CGF), 2 mL and sacro-hiatal injections of ozone (15 µg/mL, 15 mL) plus CGF (1 mL). Outcomes were assessed using clinical monitoring, VAS scores, and MRI imaging. Results: Efficacy: 90% of patients achieved complete pain resolution (VAS: 0–2) by 4–6 months. MRI findings indicated disc rehydration and herniation resolution. Adverse Effects: Mild, transient headache (3.3%) and one case of pneumocephalus (1.7%) resolved within 48 h. Conclusion: Demonstrates the effectiveness and safety of combined therapy for severe lumbosciatica, with improvements in both pain relief and imaging outcomes (75).

Case Reports: analysis of 50 clinical cases using PRP activated with ozone (PRP-O3) on lumbar and cervical spine. Findings: regenerative potential: the technique enhanced early analgesia and posterior joint regeneration, combining the biological effects of PPP and the antiseptic properties of ozone. Safety and efficacy: outpatient, minimally invasive procedure yielded satisfactory results in 85% of cases. The therapy also allowed for repeat treatments or additional therapeutic options if needed. Conclusion: PRP-O3 is a promising, low-risk intervention with regenerative and analgesic effects, offering a viable option for lumbar and cervical spine pathologies (76).

Summary and clinical Implications, both ozone combined with PRP and ozone-activated PRP demonstrate potential for synergistic effects, including enhanced regenerative capacity, pain relief, and imaging-based disc recovery. Safety profile: adverse events were rare and minor, reinforcing the minimally invasive nature and safety of these therapies. Efficacy: both studies reported high success rates, with up to 90% improvement in pain scores and MRI-based recovery indicators. These findings highlight the need for larger-scale RCTs to validate these preliminary outcomes and establish standard treatment protocols. These therapies represent innovative approaches in regenerative medicine, offering less invasive alternatives to traditional surgical interventions for spine-related pathologies.

DISCUSSION

Critical appraisal of the evidence for PRP and ozone therapies in difficult wounds

Evidence for the use of PRP (grade of evidence: grade B). Recent meta-analyses and systematic reviews suggest that PRP can enhance the healing of chronic wounds, especially DFUs, compared to conventional therapies. Benefits include improved rates of complete ulcer healing and the absence of treatment-related adverse events. Limitations: to strengthen the evidence base, the following are needed: Large-scale, standardised, long-term clinical studies and unified, standardised PRP preparation protocols to minimise variability across studies.

Evidence for OOT (grade of evidence: grade B). Systematic reviews and meta-analyses report that OOT offers significant benefits: reduced amputation rates, faster reduction in wound size, and decreased infection rates and hospitalisation times. Limitations: Current evidence requires further validation through high-quality RCTs. No adverse events associated with OOT have been reported, but further research is needed to confirm its safety profile.

Evidence for ozone-activated PRP (grade of evidence: grade C). The data supporting the combined use of PRP activated with ozone for difficult wounds remains limited, though it presents a promising therapeutic avenue. Preliminary findings suggest that the Safety of the PRP activation method using ozone is acceptable and has the potential to enhance wound healing. Limitations: current studies are too scarce to establish efficacy conclusively. Additional high-quality RCTs are essential to validate the approach (Table I).

Table I. Summary of the grade of evidence.

Area	Condition	Ozone	PRP	Ozone + PRP
Vulnology	Chronic wounds	B	B	C
	DFU	B	B	C
Joint disorders	Gonarthrosis	B	B	C
	Lumbosciatica	B	B	C

For the grade of evidence criterium see Materials and Methods. **DFU**: diabetic food ulcer; **PRP**: Platelet Rich Plasma. Percentage of agreement according to the jury vote: partially Against 6 %; favorable 31%; very favorable 63%. The strength of the recommendation was considered weak at 6% or strong at 94%.

Critical appraisal of the evidence for PRP and OOT in knee OA and DLBP

Recent meta-analyses and systematic reviews indicate that PRP has grade B evidence for the management of gonarthrosis. Studies demonstrate PRP's efficacy in improving joint function and alleviating pain at 3-, 6-, and 12-month post-treatment. PRP is generally well tolerated, with no significant increase in adverse events compared with controls. However, these promising outcomes require validation through larger, standardised, and long-term clinical trials to confirm their durability and cost-effectiveness.

For herniated disc-associated lumbosciatica, the evidence also supports a grade B level, showing PRP's significant role in pain relief and enhanced patient satisfaction. PRP has demonstrated safety, with no increased adverse events reported in existing trials. The mechanism likely involves its regenerative and anti-inflammatory effects on nerve root irritation and disc pathology. As with gonarthrosis, more rigorous, standardised research is needed to establish PRP as a mainstream therapeutic option.

Combined therapy in gonarthrosis and lumbosciatica: The current literature on PRP combined with OOT for these conditions is limited (grade C). Preliminary findings suggest this approach could be a promising therapeutic avenue, particularly in leveraging the regenerative properties of PRP and the anti-inflammatory and oxygenating effects of OOT. Available data indicate no adverse events, supporting the safety of ozone-activated PRP.

However, the evidence is insufficient to recommend this combination as a standard treatment due to the lack of high-quality RCTs. Future research should focus on assessing the efficacy and exploring the mechanistic synergy between PRP and OOT in large, diverse patient populations.

Both PRP alone and in combination with OOT hold significant potential in managing gonarthrosis and lumbosciatica. While PRP shows consistent grade B evidence, combined therapy remains an intriguing hypothesis that warrants further robust investigation.

Recommendations and research gaps; clinical integration of OOT for DFUs

Recommendation: Incorporate OOT as an integrative therapy for neuroinfected diabetic foot via local and systemic applications. Obtain patient consent prior to initiating therapy.

Implementation: Adhere to international (ISCO3) (77) and national (Nuova FIO, AIRO, CIO3) guidelines. Use certified ozone generators and recognized protocols. Train medical personnel in safe and effective application methods. Foster clinical studies and encourage result dissemination in scientific journals.

Research and application of ozone-activated PRP for difficult ulcers. Recommendation: Expand laboratory and clinical studies on ozone-activated PRP for the management of difficult ulcers. Prioritize high-quality, double-blind RCTs. Treatments should require explicit patient consent as they remain experimental.

Research on PRP-ozone for lumbosciatica and gonarthrosis. Recommendation: Promote research into PRP activated with ozone for lumbosciatica and gonarthrosis. Conduct double-blind RCTs to strengthen evidence. Ensure patient consent for experimental treatments.

Continuous training in OOT and regenerative medicine. Recommendation: Develop training programs through master's courses offered by scientific associations and universities. Focus on the latest efficacy data and correct administration techniques.

International Collaboration and Evidence Review. Recommendation: SIMCRI should collaborate with international scientific associations to refine protocols. Conduct a reassessment of the scientific evidence in 5 years.

These recommendations emphasize OOT's potential as a minimally invasive, cost-effective, and safe approach for managing chronic and refractory conditions like DFUs, herniated disc-induced sciatica, and gonarthrosis. They also stress the importance of standardized protocols, rigorous clinical trials, and ongoing education for healthcare providers to optimize patient outcomes and foster global collaboration in regenerative medicine.

CONCLUSIONS

Local or systemic administration of OOT is a safe and effective treatment for difficult ulcers and some degenerative osteoarticular conditions. Combining PRP activated with OOT shows promising synergy, enhancing tissue repair by improving local circulation and oxygenation. This complements PRP's growth factor delivery without reported

adverse effects. Multidisciplinary studies are encouraged to incorporate OOT as an integrative therapy, particularly for conditions like DFUs, using both local and systemic techniques.

While preliminary findings are promising, the literature remains limited and of low evidence quality. Further high-quality RCTs are essential. SIMCRI aims to promote and support clinical trials on OOT, adhering to national and international protocols, including advanced therapeutic protocols such as OOT for Peripheral Artery Disease (PAD).

In gonarthrosis and lumbosciatica due to a herniated disc, OOT combined with PRP demonstrates significant pain reduction and improved outcomes compared to PRP or ozone alone. Despite existing evidence (Level C) supporting the separate efficacy of OOT and PRP, further studies are needed to clarify the mechanisms and optimise combined protocols.

Research Recommendations: Evaluate whether OOT-activated PRP is more effective than standalone treatments. Define optimal protocols, including dosages and administration methods. Investigate molecular synergies, including ozone's role in platelet degranulation and growth factor release. Explore gene expression and protein synthesis changes after OOT-PRP combination therapy.

These findings highlight the need for further exploration to standardise and validate the combined use of OOT and PRP in clinical settings.

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