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Safety of Intra-Articular Oxygen-Ozone Therapy Compared to Intra-Articular Sodium Hyaluronate in Knee Osteoarthritis: A Randomized Single Blind Pilot Study

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Abstract

Objective: Osteoarthritis (OA) is a chronic degenerative musculoskeletal disease and one of the leading causes of chronic musculoskeletal pain, often affecting the knee. Despite intra-articular (IA) injectable hyaluronan (HA) preparations are widely used in the treatment of this debilitating condition, only a few data about their efficacy have been reported so far. Therefore, the use of HA in OA management is not universally recommend in clinical practice. Oxygen-ozone (O_2O_3) therapy can be employed in several pain-related conditions and diseases. However, both its efficacy and safety for IA therapy in knee OA have never been explored. Here, we evaluated the reliability of knee IA therapy with O_2O_3 compared to IA HA in chronic knee OA.

Methods: A total of 42 consecutive chronic OA patients with radiological diagnosis of knee OA were prospectively enrolled in this single-blind, controlled study. After randomization, all patients underwent IA therapy with O_2O_3 or HA (q1wk) for 4 weeks, with additional 4 weeks of follow-up. Examination of the adverse events occurred during the whole study was performed. To measure knee function and pain, visual analogue scale (VAS), Oxford knee questionnaire (OKQ), and 12-item short form survey (SF-12) were administered. EuroQol five dimensions questionnaire (EQ-5D) was used to assess patients' quality of life.

Results: No significant difference in adverse events occurrence was observed. Knee IA treatment with O_2O_3 showed shorter reduction of pain compared to IA HA. VAS score decreased in both groups during the treatment period ($p<0.001$), while OKQ score significantly increased ($p<0.001$). SF-12 and EQ-5D scores were comparable between the two groups of patients.

Conclusions: Despite IA administration of O_2O_3 and hyaluronan are comparable treatments in knee OA both in terms of safety and quality of life improvement, the latter shows longer times of pain reduction.

Keywords: Chronic degenerative musculoskeletal disease; Osteoarthritis; Osteophytes

Introduction

Osteoarthritis (OA) is a chronic degenerative musculoskeletal disease affecting approximately 10% of people older than 55-year-old [1]. This condition represents the most common rheumatic disease and one of the leading causes of joints' debilitation [1]. Regrettably, the specific aetiology of this condition is poorly understood. OA is pathologically characterized by the gradual loss of articular cartilage, formation of osteophytes, subchondral bone remodelling, and joints' inflammation [2,3]. Knee, hip, interphalangeal, and spine joints are the most commonly involved anatomical sites [3]. The symptoms of OA are rather severe, being characterized by deep articular pain and progressive loss of function, with subsequent disability and significant burden on the healthcare system [4]. At present, therapeutic strategies for OA include both pharmacological and non-pharmacological interventions, such as oral non-steroidal anti-inflammatory drugs (NSAIDs), and intraarticular (IA) injections of corticosteroids, viscosupplements, blood-derived products, as well as patients' education, physical exercise, acupuncture and electromagnetic therapy, respectively [4].

Hyaluronic acid (HA), also known as hyaluronan, is a natural component of the synovial fluid and the cartilage matrix. Preparations containing this endogenous glycosaminoglycan can be obtained either from rooster combs and bacterial fermentation [5]. The viscous IA-injectable forms of HA are currently considered useful tools to restore the viscoelastic properties of the synovial fluid. Among them, sodium hyaluronate (the sodium salt of hyaluronan), hylan G-F 20, and high-molecular-weight hyaluronan have gained Food and Drug Administration (FDA) approval for the treatment of articular diseases. To date, there are several lines of evidence to suggest that such

preparations could also lead to a reduction of the synovial inflammation [6], protection against cartilage erosion [7], and promotion of intra-articular hyaluronan production [8]. IA HA is currently considered a safe and relatively well-tolerated procedure. Indeed, the most common adverse event described is represented by mild and transient inflammatory reaction or flare at the injection site [9,10]. It should be noted that these events occur more frequently with chemically cross-linked compounds (e.g. hylan G-F 20), compared to non-chemically modified products, such as sodium hyaluronate [10]. Moreover, the cross-linked hyaluronan seems to be associated with an increased incidence of pseudo-septic arthritis, also called severe acute inflammatory reaction (SAIR) [11]. Regrettably, the results provided by clinical trials on the use of HA in OA are somewhat contradictory. Therefore, the Osteoarthritis Research Society International (OARSI) [12], American Academy of Orthopaedic Surgeons [13], and American College of Rheumatology [14] do not recommend HA for the treatment of either knee or multiple-joints OA. At present, the use of HA in OA patients is considered a second-line approach in patients with a unsatisfactory response to acetaminophen and/or NSAIDs [14].

Oxygen-ozone (O_2O_3) is a mixture of Oxygen (O_2) and Ozone (O_3) that can be used in the treatment of several painful conditions, such as low back pain and lumbar disc herniation [15-18]. This gas combination is produced from pure O_2 passing through a high-voltage gradient (5-13 mV) in a medical generator. The analgesic and anti-inflammatory effects of O_2O_3 seem to be due to the O_3 intrinsic chemical properties. Indeed, this unstable allotropic form of O_2 can act directly on the proteoglycans of the nucleus pulposus [16-18]. During the past decade, a randomized controlled trial (RCT) on patients affected by chronic and acute low-back pain showed comparable effects of computed tomography (CT)-guided intra-foraminal O_2O_3 infiltration and peri-radicular steroid infiltrations [19]. At IA level, Chen et al. showed that the synovial levels of TNF-α were reduced after O_3 injection in rats affected by rheumatoid arthritis [20]. Furthermore, Al-Jaziri et al. treated 220 patients with spine or joints osteoarthritis with an IA or intramuscular paravertebral O_2O_3, observing a significant pain reduction after 12 sessions [21]. More recently, a RCT comparing IA injection of platelet-rich plasma (PRP), HA, and O_2O_3 in knee OA patients, showed comparable effects on pain reduction among the three approaches after 1 month of treatment [22]. Despite these charming data, the conundrum of the efficacy and safety of IA O_2O_3 compared to IA HA therapy remains unsolved.

Considering the lack of recommendation of IA HA use in OA and its modest efficacy for pain relief [23], further possible interventions to treat OA should be explored. On the other hand, only few studies attempted to address the safety and efficacy of IA O_2O_3 therapy for these patients. In this scenario, the purpose of this study was to evaluate the reliability of IA injection of O_2O_3 compared to HA in chronic knee OA patients. To address this aim, we sought i) to evaluate the safety of the two procedures through the monitoring of the adverse events, ii) to compare their specific degrees of pain reduction, and iii) to define the differences between the two treatments in terms of patients' health status and quality of life.

Methods

Study population

This was a randomized, single-blind, controlled study. In total, 42 chronic knee OA patients were consecutively enrolled at the Physical and Rehabilitative Medicine Unit of the University Hospital in Novara, Italy from December 2014 to June 2015. Inclusion criteria were defined as follows: age range 60-85; radiological diagnosis of knee OA with a grade II or III according to the Kellgren's classification [24]; ineffective previous rehabilitative treatment and/or oral NSAIDs, corticosteroid, and analgesic drugs; no contraindication for IA treatment. Patients showing infection in the injection area, knee ligaments' lesions, knee arthroplasty, as well as with history in the previous year of knee surgery, femur or tibia/fibula fractures, IA corticosteroid therapy, were excluded from this study. In addition, clinical history of diseases that could be worsened by O_2O_3 (i.e. hyperthyroidism, thrombocytopenia, myocardial infarction, bleeding disorders) and established sensitivity to HA were considered exclusion criteria. This study was conducted according to the Declaration of Helsinki, with pertinent national and international regulatory requirements. All patients provided written informed consent and were free to withdraw from the study at any time.

Study design

All patients enrolled in this study quit any kind of anti-inflammatory and/or analgesic oral treatments and underwent 1-week-wash-out from oral analgesic drugs and NSAIDs. Two study arms were defined by the IA therapeutic injection of O_2O_3 (Group 1) and IA HA (Group 2) in the knee joint. The therapy for each patient was administered once per week (q1wk), for 4 consecutive weeks, defined as t0, t1, t2, and t3, respectively. An additional follow-up visit 4 weeks after the final (fourth) injection was performed (t4). The examiner was unaware of patients' allocation. IA injections were executed in supine position with knee flexed at 90°C, in sterile conditions, including skin cleanse and double disinfection with iodopovidone 7.5%. In both groups, (O_2O_3 and HA) 0.7 × 32 mm (22G) sterile needles were used. The injection approach was anterior, lateral to rotula tendon and between the inferior margin of condilus-femoralis lateralis and superior margin of tibial plateau. Needles were always substituted after drawing up the drug. No pre-medication or anaesthesia was used. The study flow-chart is depicted in (Figure 1).

Due to the short half-life of O3 (~45 minutes at 20°C), O_2O_3 was generated freshly before each medical examination of all patents in Group 1. For this, an Ozonline E80 generator (Eco3 s.n.c., Brandizzo, TO, Italy) connected to a pure O_2 source was employed in-house. Briefly, the ozone generator uses O2 through high-voltage tubes with outputs values of 5% O_2O_3 ranging from 4,000 to 14,000 litres. For IA knee injections, a concentration of 20 mcg/ml was selected. Due to O_3 instability which starts decaying after 20 seconds, an injection time of 15 seconds was used, as previously described [15]. Patients in Group 2 received IA HA, administered via a pre-filled syringe device (Hyalgan®, Fidia Farmaceutici s.p.a., Abano Terme, PD, Italy,) containing a high-molecular weight (500,000-730,000 Da) fraction of purified natural sodium hyaluronate in buffered physiological sodium chloride (pH=6.8-7.5). The viscous solution was injected into knee joint.

Outcome measurement

Treatment safety was examined through anamnestic report at each time-point (t1-4) of any adverse event occurred after the first visit (t0). To assess the global level of subjective pain in the target joint, continuous 100-mm Visual Analogue Scale (VAS) was adopted [25], while the Oxford Knee Questionnaire (OKQ) [26,27] and the 12-Item Short Form Health Survey (SF-12) [28,29] were used to assign specific scores to knee function and pain. Treatment effect on participants' quality of life was also evaluated at t0 and t4 using the European

Quality of Life Questionnaire (EuroQoL; also known as EQ-5D) [30,31].

Statistical methods

Patients were allocated to each of the treatment arms using a digitally-generated randomization algorithm, with 1:1 distribution and no blocks. Because of the small sample size, we assumed a non-Gaussian distribution of the considered variables. Differences between each variable in the O_2O_3 and hyaluronan groups have been evaluated with Friedman's analysis of variance (ANOVA). Dunn post hoc comparison was used to identify significant differences between mean values. Differences between single variables in different groups were evaluated with the Mann–Whitney U-test. A type I error (alpha) level of 0.05 was chosen, and the Bonferroni correction for multiple comparisons was applied considering four variables, which resulted in a new alpha-error level of 0.013. All statistical analyses were performed using the GraphPad Prism package, version 6.0 (GraphPad Software, Inc., San Diego, CA, USA).

Results

A total of 42 patients have been enrolled in the study. No drop outs or withdrawal of patients enrolled have been registered during the whole study period. Anamnestic and demographical characteristics are shown in Table 1.

	O_2O_3 Group (n=22)	HA Group (n=20)	p
Age (Years)	70.3 ± 6.5	70.7 ± 5.4	ns
Sex (F/M)	16/6	13/7	ns
NSAIDs and/or CS before enrollment (Y/N)	5/17	2/18	ns
Body Mass Index (Kg/m^2)	27.1 ± 1.9	26.8 ± 1.7	ns
Kellgren-Lawrence Grade	II n=13 III n= 7	II n=14 III n=6	ns

Abbreviations: O_2O_3: Oxygen-Ozone; HA: Hyaluronic Acid; CS: oral corticosteroids; SD: standard deviations. *Group differences were analyzed by either χ^2 test or Mann-Whitney U test.

Table 1: Anamnestical and Demographical characteristics of patients enrolled. Data are presented as means ± SD.

No significant differences in adverse events occurrence were observed between the two groups, suggesting that IA O_2O_3 and IA HA are similarly safe therapies. In Group 1, two patients reported adverse events in the first two minutes after treatment, specifically swelling and joint heaviness. Furthermore, three Group 2 patients complained self-limiting pain and encumbering sensation following IA injection. All adverse events were self-limiting in 24-48 hours after treatment.

VAS scores showed similar trends in both groups, with a statistically significant reduction between t0 and t3 ($p<0.001$) and between t0 and t4 ($p<0.01$). Interestingly, the only differences in VAS scores among the two groups emerged at the follow-up visit (t4) with a statistically significant lower scores in HA group compared to O_2O_3 group ($p<0.001$), as shown in (Figure 2). All patients belonging to the HA group (Group 2) showed statistically significant variations between either t0-3 ($p<0.01$) and t0-4 ($p<0.0001$), while in Group 1 (O_2O_3) these variations were restricted to the only t0-3 timeframe ($p<0.001$), No differences between the two groups have been observed at any evaluation, regarding OKQ scores.

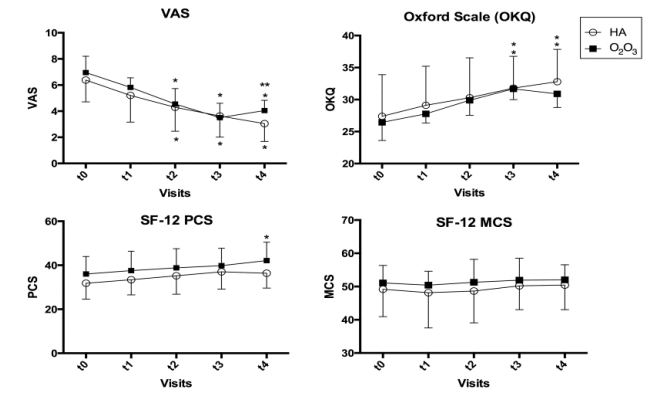

Figure 2: Figure 2: VAS, OKQ and SF-12 modifications at each study visit. * t0 *vs.* t1,t2,t3,t4 $p<0.013$; ** O_2O_3 *vs.* HA $p<0.013$.

SF-12 MCS scores did not show any statistically significant modification at any evaluation in and among both O_2O_3 and HA groups. On the contrary, SF-12 PCS scores showed a statistically significant increase in HA group between t0 and t4 ($p<0.01$). However, no significant variation has been found at any evaluation between the two groups (Figure 2). EQ-5D score comparison between t0 and t4 showed a statistically significant difference in HA group only ($p<0.001$). However, there were no statistically significant differences between groups at both t0 and t4, as shown in (Figure 3).

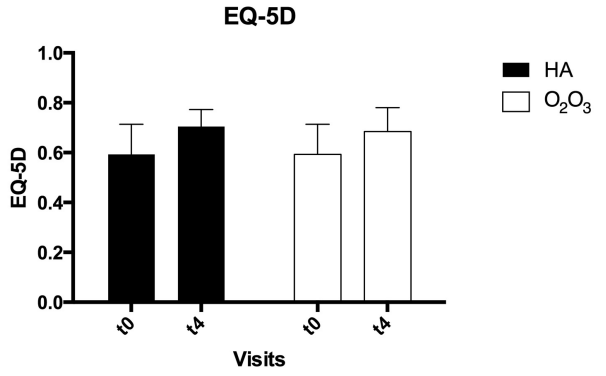

Figure 3: EQ-5D modifications between t0 and t4.

Discussion

In this study, we have provided evidences on the safety of one-month weekly IA O_2O_3 treatment compared to IA HA, for knee OA. Furthermore, our results suggest that IA HA treatment have a prolonged efficacy, in terms of pain reduction, compared to IA O_2O_3 in knee OA.

In our cohort, only two patients treated with O_2O_3 reported self-limiting adverse events during 8 weeks, while the side effects of IA HA affected three patients. Specifically, in the O_2O_3 group, adverse events consisted in self-limiting pain and encumbering sensation resuming spontaneously a few minutes after treatment, while in HA group, swelling and joint heaviness/encumbering sensation were observed immediately after treatment. These results constitute the proof-of-principle of the similar safety of one-month weekly IA O_2O_3 injection for knee OA, compared to HA, confirming previous reports [21,22].

Importantly, the IA O_2O_3 treatment determined a statistically significant reduction of pain measured with VAS scale at any evaluation. This reduction, showed no statistical difference at any evaluation compared to HA group, except for the visit in the follow-up period (t4), 1 month after the end of the treatment. Importantly, VAS score showed a decrease at t4 in all knee OA patients that were treated with IA HA, while it increased in the O_2O_3 group. This data seems to suggest a prolonged efficacy after the end of the treatment protocol of HA, unlike in O_2O_3. Taken together, our results provide further credence to those obtained by Duymus et al. with a similar protocol of IA O_2O_3 compared to a single IA HA administration [22]. Regarding the other secondary outcome variables (OKQ, MCS and PCS), the O_2O_3 treatment showed comparable scores to HA improvements without any statistically significant difference between the two groups.

At present, the precise mechanism of action of O_2O_3 has not been fully clarified. It should be noted, however, that there are several lines of evidence to suggest that the pharmacological effect of this substance is likely to be achieved through physiological mechanisms involved in immunomodulation [32], anti-inflammatory activity [33], and analgesia [34]. All these biologic effects are mainly related to a cytokine modulation activity, with the inhibition of pro-inflammatory prostaglandins, and stimulation of pro-inflammatory cytokines antagonists and immunosuppression [15-18].

The clinical use of O_2O_3 has been implemented over the past few years in the treatment of different pathological conditions such as lumbar disc herniation, healing of refractory ulcers, viral hepatitis and other conditions [15,17,18]. Promising results were obtained by Chen et al. for the IA treatment with O_2O_3 in a rat murine model of RA [20]. In this study, the authors used several ozone concentrations, ranging from 10 to 50 ug/ml. Intriguingly, the optimal O_2O_3 concentration suggested by the authors is 40 ug/ml. Indeed this posology shows the best results both in terms of hind paw thickness and reduction of TNF-a, TNF-R1, and TNF-R2 in the serum and synovia. As a result, synovial cells proliferation is inhibited and apoptosis increased. To reduce the risk of toxicity, we have decided to administer IA O_2O_3 with a concentration of 20 ug/ml, according to previous data obtained in humans. Thus, a possible toxicity of concentrations of O_2O_3 greater than 60 mcg/ml was previously reported [15]. This major side effect is probably due to the superoxide dismutase, catalase and glutathione peroxidase impairment, leading to a possible degradation of the cell membrane. Of note, data about IA O_2O_3 treatment in humans are currently scarce. Further clinical studies, coupled with functional validations of data are warranted to elucidate the operational ramification of this issue. On the other hand, a large-cohort prospective clinical study with O_2O_3 administration [21], left many concerns about treatment safety, precise mechanisms underlying the therapeutic effects, optimal O_2O_3 concentrations and clinical efficacy. To date, only one randomized clinical trial investigated the safety and efficacy of IA O_2O_3 treatment for knee OA. Despite the encouraging results, the authors compared a once IA HA treatment versus four IA O_2O_3 treatments and two IA PRP treatments, with consequent possible bias related to different IA injective protocols. Notably, these data are similar to those showed in the present work, with similar efficacy rates in pain reduction of O_2O_3 and HA in knee OA patients, and a decrease of O_2O_3 in the long-term follow-up compared to HA [22]. Thus, data emerging from our study, although limited by the relatively small sample size, are the first comparing two identical protocols (i.e. 4 weekly injection for one month) of IA O_2O_3 and IA HA treatment, providing novel insights about a possible clinical use of IA O_2O_3 therapy in knee OA.

In this study, we only observed two self-limiting adverse events of the duration of few minutes characterized by local pain and swelling on more than 84 total IA O_2O_3 procedures. These results, even though not directly comparable, are in line with those obtained in other studies of ozone treatment for spinal disc herniation [15], suggesting the overall safety of this approach. It has been recently reported only one case of fatal septic shock after intramuscular-paravertebral O_2O_3 injection [35]. However, this unique occurrence was probably due to inadequate asepsis during the invasive manoeuvre, easily preventable using a sterile procedure, as suggested by other authors [15]. On the other hand, the most common adverse event associated with viscosupplementation is an inflammatory reaction, or flare, at the injection site characterised by pain and swelling [2,4,5,8,10]. These reactions are typically mild, and often self-limiting [8]. However, the cross-linked hyaluronan could be associated with an increased incidence of SAIR, clinically distinct from the local inflammatory reactions or flares associated with IA injections [36]. Interestingly, SAIR is not usually associated with naturally-derived sodium hyaluronans, highliting a possible link with chemical modification of the hyaluronan molecule [37-40].

This study has several limitations. First, we acknowledge that the small sample size of the analysed cohort prevent any robust data analyses about O_2O_3 treatment efficacy compared to HA. Second, we

are aware of the fewer number of consecutive ozone treatments compared to the usual protocols. However, we chose to perform only four IA O_2O_3 treatments in order to have a more comparable protocol with IA HA.

Conclusion

Despite these intrinsic limitations, our work suggests that IA O_2O_3 treatment is comparable in terms of safety to IA HA in chronic knee OA. Considering that this is the second randomized study performed with IA O_2O_3 in knee OA patients, our results should be taken cautiously and need further validations on larger cohorts. This would allow a comprehensive evaluation not only of the safety but also efficacy of pain reduction, functional outcome, and quality of life modifications. Lastly, considering the lack of evidence and practice guideline recommendations of IA HA use in knee OA, a cost-effectiveness analysis would be needed due to the relatively low cost of IA O_2O_3 therapy in terms of medication, procedure and personnel for knee OA treatment.

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References

1. Richmond J, Hunter D, Irrgang J, Jones MH, Snyder-Mackler L, et al. (2010) American Academy of Orthopaedic Surgeons clinical practice guideline on the treatment of osteoarthritis (OA) of the knee. J Bone Joint Surg Am 92: 990-993.
2. Foti C, Cisari C, Carda S, Giordan N, Rocco A, et al. (2011) A prospective observational study of the clinical efficacy and safety of intra-articular sodium hyaluronate in synovial joints with osteoarthritis. Eur J Phys Rehabil Med 47: 407-415.
3. Martel-Pelletier J, Boileau C, Pelletier JP, Roughley PJ (2008) Cartilage in normal and osteoarthritis conditions. Best Pract Res Clin Rheumatol 22: 351-384.
4. Ayhan E, Kesmezacar H, Akgun I (2014) Intraarticular injections (corticosteroid, hyaluronic acid, platelet rich plasma) for the knee osteoarthritis. World J Orthop 5: 351-361.
5. Bagga H, Burkhardt D, Sambrook P, March L (2006) Longterm effects of intraarticular hyaluronan on synovial fluid in osteoarthritis of the knee. J Rheumatol 33: 946-950.
6. Amiel D, Toyoguchi T, Kobayashi K, Bowden K, Amiel ME, et al. (2003) Long-term effect of sodium hyaluronate (Hyalgan) on osteoarthritis progression in a rabbit model. Osteoarthritis Cartilage. 11: 636-43.
7. Ghosh P (1994) The role of hyaluronic acid (hyaluronan) in health and disease: interactions with cells, cartilage and components of synovial fluid. Clin Exp Rheumatol 12: 75-82.
8. McArthur BA, Dy CJ, Fabricant PD, Valle AG (2012) Long term safety, efficacy, and patient acceptability of hyaluronic acid injection in patients with painful osteoarthritis of the knee. Patient Prefer Adherence. 6: 905-910.
9. Campbell KA, Erickson BJ, Saltzman BM, Mascarenhas R, Bach BR Jr, et al. (2015) Is local viscosupplementation injection clinically superior to other therapies in the treatment of osteoarthritis of the knee: a systematic review of overlapping meta-analyses. Arthroscopy 31: 2036-2045.e14.
10. Divine JG, Shaffer MD (2011) Use of viscosupplementation for knee osteoarthritis: an update. Curr Sports Med Rep 10: 279-284.
11. Goldberg VM, Coutts RD (2004) Pseudoseptic reactions to hylan viscosupplementation: diagnosis and treatment. Clin Orthop Relat Res 419: 130-137.
12. McAlindon TE, Bannuru RR, Sullivan MC, Arden NK, Berenbaum F, et al. (2014) OARSI guidelines for the non-surgical management of knee osteoarthritis. Osteoarthritis Cartilage 22: 363-88.
13. Richmond J, Hunter D, Irrgang J, Jones MH, Levy B, et al. (2009) Treatment of osteoarthritis of the knee (nonarthroplasty). J Am Acad Orthop Surg 17: 591-600.
14. Hochberg MC, Altman RD, April KT, Benkhalti M, Guyatt G, et al. (2012) American College of Rheumatology 2012 recommendations for the use of nonpharmacologic and pharmacologic therapies in osteoarthritis of the hand, hip, and knee. Arthritis Care Res 64: 465-474.
15. Paoloni M, Di Sante L, Cacchio A, Apuzzo D, Marotta S, et al. (2009) Intramuscular oxygen-ozone therapy in the treatment of acute back pain with lumbar disc herniation: a multicenter, randomized, double-blind, clinical trial of active and simulated lumbar paravertebral injection. Spine 34: 1337-1344.
16. Murphy K, Elias G, Steppan J, Boxley C, Balagurunathan K, et al. (2016) Percutaneous treatment of herniated lumbar discs with ozone: investigation of the mechanisms of action. J Vasc Interv Radiol 27: 1242-1250.
17. Bocci V, Borrelli E, Zanardi I, Travagli V (2015) The usefulness of ozone treatment in spinal pain. Drug Des Devel Ther 9: 2677-2685.
18. Zanardi I, Borrelli E, Valacchi G, Travagli V, Bocci V (2016) Ozone: a multifaceted molecule with unexpected therapeutic activity. Curr Med Chem 23: 304-314.
19. Bonetti M, Fontana A, Cotticelli B, Volta GD, Guindani M, et al. (2005) Intraforaminal O2–O3 versus periradicular steroidal infiltrations in lower back pain: randomized controlled study. Am J Neuroradiol 26: 996-1000.
20. Chen H, Yu B, Lu C, Lin Q (2013) The effect of intra-articular injection of different concentrations of ozone on the level of TNF-a, TNF-R1, and TNF-R2 in rats with rheumatoid arthritis. Rheumatol Int 33: 1223-1227.
21. Al-Jaziri AA, Mahmoodi SM (2008) Painkilling effect of ozone-oxygen injection on spine and joint osteoarthritis. Saudi Med J 29: 553-557.
22. Duymus TM, Mutlu S, Dernek B, Komur B, Aydogmus S, et al. (2016) Choice of intra-articular injection in treatment of knee osteoarthritis: platelet-rich plasma, hyaluronic acid or ozone options. Knee Surg Sports Traumatol Arthrosc pp. 1-8.
23. Hunter DJ (2015) Viscosupplementation for osteoarthritis of the knee. N Engl J Med 372: 1040-1047.
24. Petersson IF, Boegård T, Saxne T, Silman AJ, Svensson B (1997) Radiographic osteoarthritis of the knee classified by the Ahlbäck and Kellgren & Lawrence systems for the tibiofemoral joint in people aged 35-54 years with chronic knee pain. Ann Rheum Dis 56: 493-496.
25. Scott J, Huskisson EC (1976) Graphic representation of pain. Pain 2: 175-184.
26. Dawson J, Fitzpatrick R, Murray D, Carr A (1998) Questionnaire on the perceptions of patients about total knee replacement. J Bone Joint Surg Br 80: 63-69.
27. Padua R, Zanoli G, Ceccarelli E, Romanini E, Bondì R, et al. (2003) The Italian version of the Oxford 12-item Knee Questionnaire-cross-cultural adaptation and validation. Int Orthop 27: 214-216.
28. Ware J Jr, Kosinski M, Keller SD (1996) A 12-Item Short-Form Health Survey: construction of scales and preliminary tests of reliability and validity. Med Care 34: 220-233.
29. Gandek B, Ware JE, Aaronson NK, Apolone G, Bjorner JB, et al. (1998) Cross-validation of item selection and scoring for the SF-12 Health Survey in nine countries: results from the IQOLA Project. International Quality of Life Assessment. J Clin Epidemiol 51: 1171-1178.
30. EuroQol Group (1990) EuroQol - a new facility for the measurement of health-related quality of life. Health Policy 16: 199-208.
31. Hurst NP, Jobanputra P, Hunter M, Lambert M, Lochhead A, et al. (1994) Validity of EuroQol - a generic health status instrument - in patients with rheumatoid arthritis. Economic and Health Outcomes Research Group. Br J Rheumatol 33: 655-662.
32. Bocci V (1994) Autohaemotherapy after treatment of blood with ozone. A reappraisal. J Int Med Res 22: 131-144.

33. Andreula CF, Simonetti L, De Santis F, Agati R, Ricci R, et al. (2003) Minimally invasive oxygen-ozone therapy for lumbar disk herniation. AJNR Am J Neuroradiol 24: 996-1000.

34. Bocci V, Luzzi E, Corradeschi F, Paulesu L, Di Stefano A, et al. (1993) Studies on the biological effects of ozone: III, an attempt to define conditions for optimal induction of cytokines. Lymphokine Cytokine Res 12: 121-126.

35. Gazzeri R, Galarza M, Neroni M, Esposito S, Alfieri A (2007) Fulminating septicemia secondary to oxygen-ozone therapy for lumbar disc herniation: case report. Spine 32: 121-123.

36. Owen DS (2001) Diagnostic tests and procedures. Aspiration and Injection of Joints and Soft Tissue. In: Ruddy S, Harris ED, Sledge CB, editors (2001) Kelly's textbook of rheumatology. Sixth edition, W.B. Saunders, Philadelphia.

37. Chen AL, Desai P, Adler EM, Di Cesare PE (2002) Granulomatous inflammation after Hylan G-F 20 viscosupplementation of the knee : a report of six cases. J Bone Joint Surg Am 84: 1142-1147.

38. Pullman-Mooar S, Mooar P, Sieck M, Clayburne G, Schumacher HR (2002) Are there distinctive inflammatory flares of synovitis after hylan GF intra-articular injections? J Rheumatol 29: 2611-2614.

39. Puttick MP, Wade JP, Chalmers A, Connell DG, Rangno KK (1995) Acute local reactions after intraarticular hylan for osteoarthritis of the knee. J Rheumatol 22: 1311-1314.

40. Rees JD, Wojtulewski JA (2001) Systemic reaction to viscosupplementation for knee osteoarthritis. Rheumatology (Oxford) 40: 1425-1426.