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Research article

Ozone therapy vs. classical treatment in musculoskeletal disorders

Maricela Dragomir [1], Claudiu Mereuță [2,*], Carmen Gheorghe [3]

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1 Assistant Professor, Doctoral School of Socio-Humanities Sciences, "Dunarea de Jos" University of Galati (Romania), e-mail: maricela.dragomir@ugal.ro;
2 Prof. dr. habil. "Dunarea de Jos" University of Galați (Romania), e-mail: claudiu.mereuta@ugal.ro;
3 Lect. dr. "Dunarea de Jos" University of Galați (Romania), e-mail: carmen.gheorghe@ugal.ro

* Correspondence: claudiu.mereuta@ugal.ro

Abstract: With aging and weight gain, the population is significantly overweight and faces additional pressure on vertebral and peripheral joints (ankles, knees, hips, shoulders), which affects muscles, tendons, ligaments, and nerves. Muscle contraction, translated by pain, sends the patient to the doctor. Lumbar and cervical spine disorders occur in 70-80% of the world's population during their lifetime. They are a major problem because they reduce the quality of life and increase the costs borne by health services and patients. There are multiple therapeutic options, both classic medication and alternative treatment, to combat pain for a longer or shorter period. Relief or suppression of pain, the dominant symptom in the manifestations of degenerative arthritic diseases, is an important goal of treatment, ultimately improving the quality of life. Patients, for various reasons, frequently seek alternative therapies, especially to surgical treatment. Finding the most comprehensive treatment is often a challenge. If we consider the benefit that a treatment (therapy) should bring to the health condition, then an individualized therapeutic plan must be chosen. A retrospective study on the effects of ozone and platelet-rich plasma (PRP) treatment compared to classic medication shows the role of ozone therapy in pain relief and the beneficial effect of this therapy, especially in combination with PRP, over a longer period.

Keywords: arthrosis, spine, pain, ozone therapy, PRP

1. Introduction

We conducted a clinical study comparing two or even three therapies (classical drug treatment, ozone therapy and platelet-rich plasma) to highlight the effectiveness of each of them and to add a further argument in support of the idea that the patient's well-being must always come first and, to this end, the most appropriate therapy must be administered in the case and at the time. Whether we are talking about spinal or knee osteoarthritis pain, we wanted to see whether or not ozone treatment brings improvements compared to other treatments currently used. In this study, subcutaneous and deep intramuscular infiltrations were administered (if we are referring to degenerative diseases of the spine) and subcutaneous and intra-articular infiltrations (if we are referring to knee osteoarthritis).

Oxygen-ozone therapy (OOT) is a complementary, sometimes alternative, minimally invasive treatment that involves the safe administration [1] of an oxygen-ozone gas mixture (ozone concentrations between 6 and 20 μg/ml) using different routes to treat certain conditions.

Ozone (O3) is an allotropic form of oxygen with three atoms in the molecule, covalently linked, forming a resonant structure (the three oxygen atoms share their electrons equally and are linked by an alternating single and double bond).

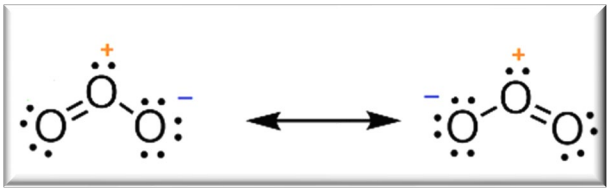


Figure 1. The resonance structure of the ozone molecule

At the concentrations used in medical practice, ozone has multiple effects on the human body:

Ozone (O_3, a molecule with three oxygen atoms) at the concentrations used has multiple properties:

- improves the rheological properties of blood [2,3];
- analgesic [1,4];
- stimulates the production of antioxidant enzymes [2,5];
- improves blood circulation by increasing cellular oxygenation, resulting in decreased ischemia [5–7];
- removes substances that cause pain by decreasing leukotriene B4, derived from arachidonic acid, involved in mediating inflammation [8];
- muscle relaxant, increases joint mobility [9];
- anti-inflammatory [10], succeeding by eliminating water from the herniated disc in reducing nerve compression and, consequently, the neurological deficit secondary to it [11–14];
- immune role by activating macrophages [15];
- increases quality of life [10,15–17].

Several authors have studied ozone therapy's role in treating back pain [1,11,18] or knee pain [19–21]. Studies focused on OOT alone [13,22] or in combination with corticosteroids [14,19], especially in intradiscal administration [23–25] to see if the effect, in the two situations, is comparable. In knee osteoarthritis, the most common knee condition, the effect of intra-articular administration of OOT [26] compared to placebo [21,27], hyaluronic acid [19,28], corticosteroids [19,29] or with platelet-rich plasma [30]. Although they all consider the results obtained to be gratifying, improving joint movements and the visual analog pain scale (VAS) [31] by suppressing inflammation mediators [27] and activating the antinociceptive system ([32], all agree that further studies are welcome [33,34].

Few comparative studies between OOT and usual analgesics or nonsteroidal anti-inflammatory drugs exist [35], but many authors mention that OOT is safe [6,36] and preferable to anti-inflammatory medication, which has numerous adverse effects or does not change the primary state; the effect is reduced over time [27].

In osteoarthritis of the knee and low back pain due to a herniated disc, there is high-level evidence for OOT. According to the Scottish Intercollegiate Guidelines Network (SIGN), and GRADE, the recommendation level of ozone therapy is "B", as are other drugs and techniques used in pain therapy centers [36]. Depending on the pathology, several ways of administering ozone are used in medical practice. Thus, ozone can be administered locally (through water, ozonated creams and oils, sauna and ozone baths, bags and cups) or systemically (infusions, infiltrations, blood ozonation like major autohemotherapy and minor autohemotherapy).

In our study, we chose to administer the therapy in the form of local infiltrations (subcutaneous, intramuscular, intra-articular), following the rapid effect on pain, the easy administration and the low cost of the procedure.

Contraindications to ozone therapy are few and boil down to one absolute contraindication, glucose-6-phosphate dehydrogenase (G-6-PHD) deficiency, a condition known as favism. Relative contraindications refer to thyrotoxic and hypertensive crises, as well as recent haemorrhages [5,36,37].

The lack of adverse reactions, when following the protocols, recommends using this route in patients allergic to analgesics and nonsteroidal anti-inflammatory drugs (NSAIDs) and in those with a history of upper gastrointestinal bleeding or other relatively recent haemorrhages, where anti-inflammatory medication is absolutely contraindicated [38,39].

Biological therapy using autologous plasma preparation, platelet-rich plasma (*PRP)* is minimally invasive, risk-free, and addresses an active population segment (18-50 years) with stage I-III knee osteoarthritis [40–43].

We chose PRP for its known beneficial effects:

- inflammation decrease, by reducing IL-17 , COX-1, COX-2 [44]
- pain relief [45]
- increased joint mobility [46]
- decreased WOMAC score at 6 and 12 months compared to hyaluronic acid [43,44,46]
- contains transforming growth factor β, with a role in chondrocyte proliferation and extracellular matrix synthesis [45–47].

2. Materials and Methods

The study methodology included a general study protocol and a specific protocol for each condition, monitoring the evolution of pain and joint mobility. The clinical trial was a retrospective, comparative group study and took place between 2014-2016 in two medical centers:

- "Dr Aristide Serfioti" Military Emergency Hospital of Galați, Emergency Department;
- "Health with Ozone" Integrative Medicine Clinic of Galați.

We selected the patients co-opted into the study from those who requested consultation for back and knee pain of non-traumatic cause and who were investigated at presentation or in the last 6 months (by MRI examination) to establish the diagnosis and exclude situations requiring surgical intervention. Most of the patients in the study had chronic damage, but the unbearable pain led them to go to the emergency department. The fact that classical drug therapy was no longer adequate for some of them, they turned to a complementary medicine service. In both situations, the treatment was coordinated by the same doctor, specializing in medical-surgical emergencies, a graduate of the university course on ozone therapy in medical practice. The choice of therapy took into account the patient's clinical condition and comorbidities and benefited from the patient's informed consent

Conditions chosen in the study:

a. Knee osteoarthritis (gonarthrosis).

b. Lumbar pain, produced by herniation/protrusion of the disc of degenerative cause.

c. Cervical disc disease and cervical-brachial neuralgia (of spinal cause).

Goals:

- to compare the results of ozone treatment, compared to classical, drug treatment, according to stepwise pain therapy protocols,
- to demonstrate the efficacy, safety and tolerability of ozone in the treatment of pain in degenerative rheumatic diseases
- the PRP effects associated with ozone treatment and drug treatment.

Selection criteria:

- ✓ pathology frequency,
- ✓ prevalence of pain and functional disorders in the clinical picture,
- ✓ grade I, II and III arthroses,
- ✓ spinal pain due to degenerative causes (disc disease, herniated discs, spondylarthrosis),
- ✓ patient's informed consent.

Exclusion criteria:

- ✓ patients with neoplastic conditions and those undergoing treatment for mental disorders,
- ✓ those who had received corticosteroids one month before the start of the study,
- ✓ patients with residual pain after surgery,
- ✓ children,
- ✓ pregnant or breast-feeding patients,
- ✓ people who have changed the form of therapy during treatment,
- ✓ stage IV arthrosis,
- ✓ morbid obesity (BMI ≥ 40),
- ✓ presence of autoimmune conditions,
- ✓ presence of (known) allergies to any of the drugs used in stepwise pain therapy.

Recommendations:

Patients were informed about all forms of therapy that were suitable for them, and the choice of treatment option was up to them. The following recommendations were made:

- combination of active and passive movements (medical gymnastics),
- weight reduction in cases of obesity or overweight,
- it was forbidden to change the form of therapy during treatment; those who changed the form of treatment were excluded from the study,
- compliance with the treatment protocol (doses, frequency of administration).

The study batch comprised 256 patients, 134 females and 122 males, divided into five study groups.

Patient investigation:

Before the choice of treatment, each patient underwent a clinical examination, to which paraclinical investigations were added as appropriate:

- X-ray of both knees (front and profile),
- X-ray of the lumbar or cervical spine (front, profile)
- magnetic resonance imaging / CT scan of the lumbar / cervical spine/ knee,
- vascular Doppler ultrasound, lower limbs.

Depending on the situation, patients underwent interdisciplinary consultations: neurosurgical, neurological and orthopaedical.

122 men and 134 women participated in the study. (Figure 2**).**

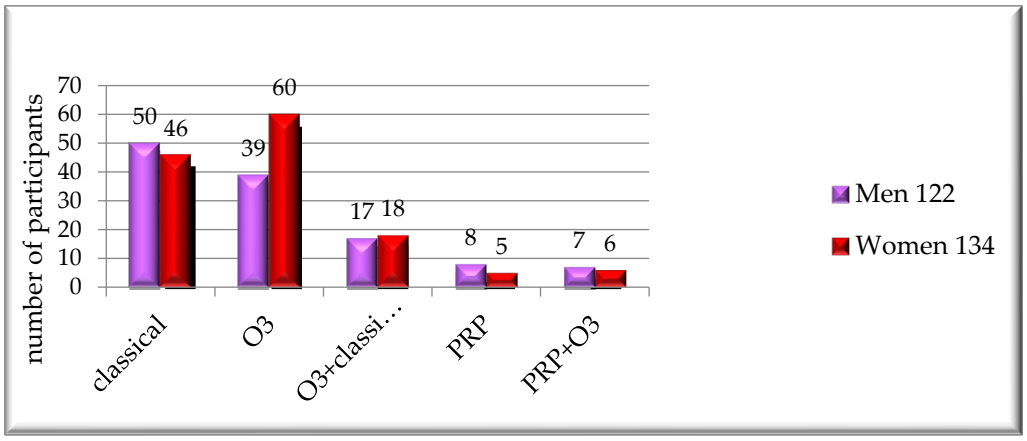


Figure 2. Structure of the group of patients participating in the study

The number of male patients was approximately equal to that of females, in all protocols used, except for the ozone protocol, where the number of women was 1/3 higher.

In this study we used five working protocols (figure 3):

Protocol 1 (P1) - – it included patients who followed classical treatment, according to the pain therapy protocol in stages [48] and who had no contraindications to analgesic or anti-inflammatory therapy:

- spinal conditions – 65;
- knee osteoarthritis – 31.

Protocol 2 (P2) - patients who underwent oxygen-ozone therapy (OOT), following their desire to benefit from an alternative therapy or because they had contraindications to usual analgesics and anti-inflammatory drugs due to comorbidities; this group also included patients whose response to classical medication was null:

- spinal conditions – 66;
- knee osteoarthritis – 33.

Protocol 3 (P3) - patients who responded only to the combination of anti-inflammatory drugs with opioids and gabapentin; in them, the classic treatment (analgesics and non-steroidal anti-inflammatory drugs) was combined with OOT to see if this combination could avoid polypharmacy, replacing steroidal anti-inflammatory drugs, opioids and gabapentin:

- spinal conditions – 16;
- knee osteoarthritis – 19.

Protocol 4 (P4) – included patients with osteoarthritis of the knee, with a VAS score ≤5, who were offered treatment with platelet-rich plasma (PRP) but who refused the combination with ozone–13;

Protocol 5 (P5) - patients with osteoarthritis, with recurrent exacerbations and VAS score ≥ 6, who underwent treatment with platelet-rich plasma (PRP) combined with OOT-13.

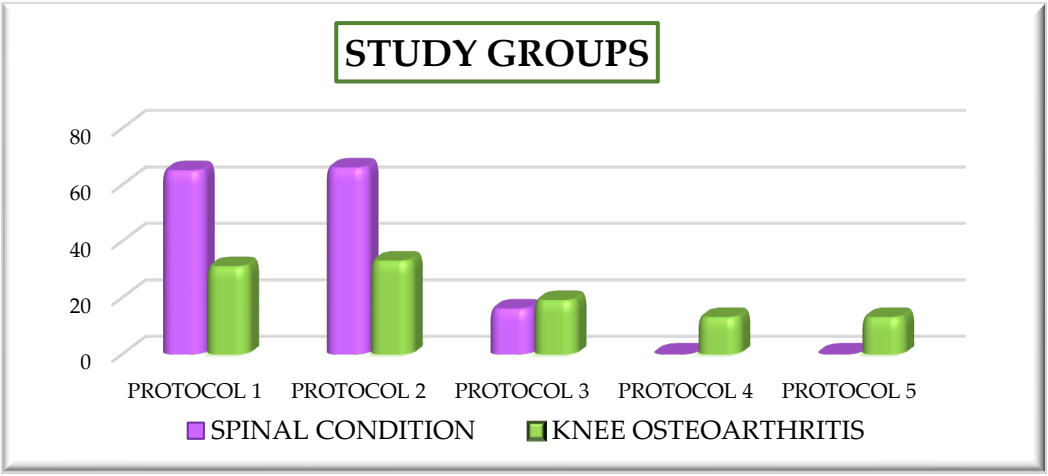


Figure 3. Working protocols: Protocol 1- classical treatment; Protocol 2 – ozone therapy; Protocol 3 – classical treatment and ozone therapy; Protocol 4- PRP; Protocol 5 – PRP and ozone therapy

The study monitored pain's evolution and its recurrence along three months from the onset.

Protocol 1

Depending on the degree of pain, drug treatment was administered in steps [48]:

- Analgesic + non-steroidal anti-inflammatory drug (NSAID) + muscle relaxant = 30.
- Analgesic + NSAID + muscle relaxant + opioid= 27.
- Analgesic + steroidal anti-inflammatory drug (SAID) + muscle relaxant + opioid = 19.
- Analgesic + NSAID + muscle relaxant + opioid + gabapentin = 20.

We recommended the treatment or seven days, and it was: analgesic (Paracetamol 3x 500 mg/day), non-steroidal anti-inflammatory (Ibuprofen 3x 400 mg/day), steroidal anti-inflammatory (Dexamethasone vials 8 mg, 1/day iv), muscle relaxant (Chlorzoxazone 3 x 250 mg/day), opioid (Tramadol 100 mg/day), Gabapentin (3 x 300 mg/day)

Protocol 2

In our study we performed 1322 O_3 sessions (mean: 11.7 / patient, mean concentration 8.83 μg/ml, Figure 4), using a protocol of 12 sessions, administered as follows:

- 2 sessions/week (Monday and Thursday), for 5 weeks = 10 sessions.
- 11th session one week after the 10th administration.
- 12th session 2 weeks after the 11th administration.

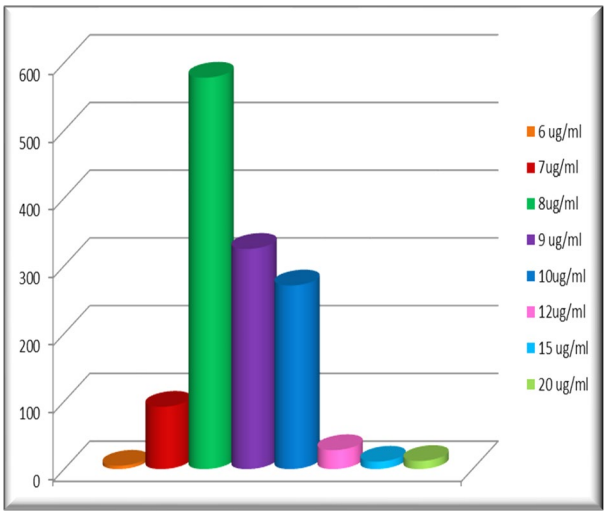
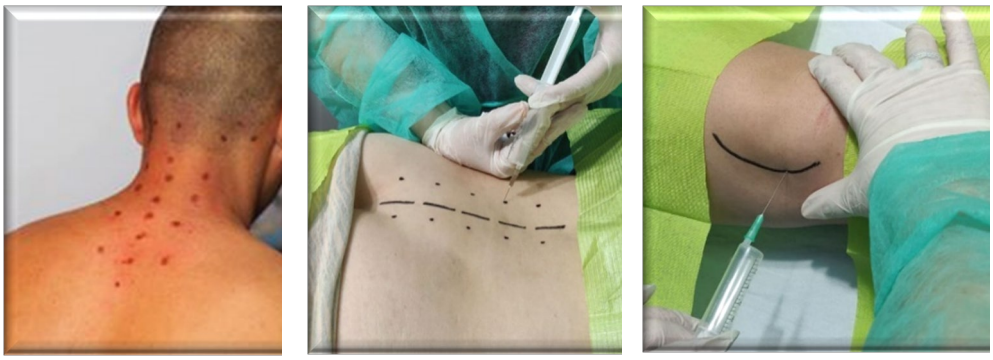


Figure 4. Average O_3 concentration, relative to the number of treatments

The most commonly used concentrations are 8-10 µg/ml, with the lowest concentration controlling pain. Concentrations of 8 µg/ml are usually used initially and increased or decreased by 1-2 µg/ml depending on the evolution of pain according to the VAS.

The O2-O3 mixture was obtained from pure medicinal oxygen using an Ozonosan generator. After being loaded into an O3-proof syringe, the gas mixture was administered via a 30G (0.30 x 13 mm) needle for subcutaneous or a 23G (0.60 x 32 mm) needle for intramuscular and intra-articular injections. We used the sterile compresses with chlorhexidine 4% to disinfect the skin.

For cervical spinal disorders, O_3 was administered subcutaneously along three parallel lines, two from the level of the mastoid to the angle of the scapula (5-6 points) and the third along the cervical spine, from C1 to T1-T2, along the path of the spinous processes (Figure 5 (a)). When the impairment was at the lumbar spine level, the injection was performed subcutaneously along the path of the spinous processes and on either side, 1 cm from the lumbar spinous processes. In persistent pain, we administered O_3 intramuscularly on both sides of the affected disc (Figure 5 (b)). In osteoarthritis of the knee, we administered O_3 via retro patellar infiltrations along the spinous apophyses (Figure 5 (c)).



(a) **(b)** **(c)**

Figure 5. (**a**) The cervical points where ozone is injected; (**b**) lumbar administration of ozone; (**c**) retropatellar administration of ozone.

Protocol 3 - Classical treatment [48] and OOT

Nonsteroidal anti-inflammatory (7 days, then as needed, without exceeding the daily dose) + analgesic (7 days, then as needed, without exceeding the daily dose)+ muscle relaxant (14 days) + one OOT session (20 cm^3 O_3, 8 – 10 μg / cm^3 / week, total 3 sessions).

Protocol 4 - We administered the PRP in six sessions (Figure 6), one session every seven days: three plasma and three gel sessions.

To obtain PRP, blood was collected in a special 9 ml test tube by venipuncture from a vein in the elbow pocket. After applying the tourniquet and disinfecting the skin with sterile gauze soaked in 4% chlorhexidine, I centrifuged the tube at 3500 rpm for 5 minutes. Centrifugation resulted in 5 ml of PRP, which was administered intra-articularly, retro patellar.

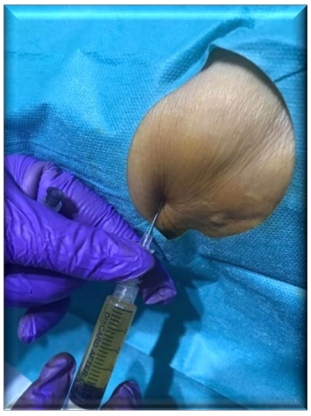


Figure 6. PRP administration in the knee joint was performed retropatellarly

In weeks 1, 3, and 5, I used one tube each, and in weeks 2, 4, and 6, we used two tubes each. One was kept in the thermostat for 5 minutes at 95°C, and then the gelled contents were mixed with the plasma from the other tube to get the plasmogel we injected.

We assessed the patient's knee mobility by going up and down stairs and by taking goniometric measurements of the amplitude of flexion and extension movements.

Protocol 5: We applied it to knee osteoarthritis, using O_3 (20μg/ml) and PRP, administered intra-articularly, to see if the presence of O_3 brings additional benefits compared to P4.

- session 1, 5: O_3 + PRP;
- session 2, 4, 6, 8: ozone;
- session 3, 7: O_3 + plasmogel.

The plasma preparation method was identical to the previous one. Treatment was done once every 7 days, with both plasma and O_3 administered intra-articularly. We monitored the pain according to VAS, and the number of sick leave days during treatment.

Results and discussion

We used two alternative therapies (OOT and PRP) to analyze their effects on knee osteoarthritis pain and vertebral back pain compared to drug therapy. Both ozone therapy and platelet-rich plasma therapy interact with natural healing processes. OOT, through moderate and controlled oxidative stress, activates body-specific repair processes, increases the supply of tissue oxygen, decreasing inflammation and, implicitly, the pain caused by it [5,36,49–51].

PRP, through the multitude of growth factors in activated platelets, is used in regenerative therapy because it favours the repair of degraded tissues [46,52].

We used IBM SPSS Statistics 23 for statistical analysis, since the data used in the study were nominal type (gender, background, treatment protocol) or numerical (O_3 concentration, pain scale, number of sick leave days). To see if there is a relationship between nominal type variables, we calculated nominal level correlation coefficients j, C, and V and their associated probabilities, considering a significance threshold a = 0.05. We calculated Spearman's coefficient and the corresponding probability, considering the same significance threshold to determine the correlations between numerical variables. We applied the t-test for independent samples and applied the Mann-Whitney test. The t-test can be used for small samples if the groups' distribution is normal and their variance does not differ significantly. We checked the equality of the variant with Levene's test, and then, taking into account its result, the two-way t-test was calculated. We used the Mann-Whitney test to test the difference between two independent groups, for which the dependent variable can be expressed in ordinal (rank) values. We did an ANOVA analysis to see if there were differences between groups defined by age groups or treatment protocols. Certain requirements condition the use of the ANOVA test: the values in each group must be independent and normally distributed, and the distribution of the values of the dependent variable within each defined group must be equal.

A limitation of the statistical analysis may be due to the number of subjects that form the research samples.

We analyzed various correlations between the type of injury (spinal/knee) and:

- patient's sex (we classified according to the XX or XY chromosome, unrelated to the patient's sexual orientation; we did not find patients with sex chromosome pair abnormalities);
- pain;
- age;
- the protocol followed.

We also analyzed:

- the evolution of pain, depending on the O_3 concentration used;
- the evolution of pain according to gender in correlation with the dose of O_3 used.

Furthermore, we questioned the existence of **correlations between the treatment protocol** used **and the affected region** (spine/knee), as seen in Table 1 and Table 2.

Table 1. The composition of the groups according to the affected region

Count

		Protocol					Total
		1	2	3	4	5	
Demage	spine	65	66	16	0	0	147
	knee	31	33	19	13	13	109
Total		96	99	35	13	13	256

Table 2. Symmetric Measures

		Value	Approximate Significance
Nominal by Nominal	Phi	0,418	0,000
	Cramer's V	0,418	0,000
	Contingency Coefficient	0,385	0,000
N of Valid Cases		256	

3. Results

The φ, C and V tests show that the treatment protocol correlates with the lesion's site. The correlation is of medium level (φ = V = 0.418, C = 0.385 and $p < 0.01 < \alpha = 0.05$). We observe that P1 and P2 were applied more to patients with spinal damage, P3 more to those with knee damage, and P4 and P5 were applied only to those with knee damage. We have not applied PRP in spinal conditions.

We studied whether there are ***correlations between age and the affected region***, spine or knee (Table3, 4):

According to correlation tests for nominal data, there is a strong relationship between the impairment location and age groups (φ = V = 0.565, C = 0.492, $p < 0.01 < \alpha = 0.05$). We observe that people up to 50 years of age suffer more from spinal damage (85% of patients aged 20-29, 96.42% of patients aged 30-39, 93.87% of patients aged 40-49), and patients over 50 years of age suffer more from knee damage (60% of patients aged 50-59, 68.91% of patients over 60 years old). So, consistent with the specialized literature, patients under 50 are more likely to suffer from spinal injuries [53], being of active age and those over 50 from knee injuries. According to existing studies, the percentage of knee deterioration increases progressively with age for several reasons [54,55].

Table 3. Age-affected region correlation

Crosstabulation, age and illness				
Age class		Spinal cord injury	Knee injury	Total
Age	20-29	17	3	20
	30-39	27	1	28
	40-49	46	3	49
	50-69	34	51	85
	Over 60	23	51	74
Total		147	109	256

Table 4. Correlation coefficients

Symmetric Measures			
		Value	Approximate Significance
Nominal by Nominal	Phi	0,565	0,000
	Cramer's V	0,565	0,000
	Contingency Coefficient	0,492	0,000
N of Valid Cases		256	

Pain monitoring was performed using the visual analog scale (VAS), from 0 (no pain) to 10 (most severe pain). In this sense, pain could be evaluated as:

- mild – score 1 – 3
- moderate – score 4 – 6
- severe – score 7 – 10

We noted outcomes in the Treatment Record Sheet: initially, every 14 days, one month, two months, and three months from the onset.

It was easy to use the VAS scale, both for us and especially for the patients, who found it easy to assess the pain level. It would have been interesting to analyze the Lequesne index (Samuel & Kanimozhi, 2019) for the assessment of knee pain and mobility in the context of therapy effectiveness, or the WOMAC score (Hmamouchi et al., 2012; Samuel & Kanimozhi, 2019), but patients responded more difficult to questions, communication with them during the 1, 2, 3-month evaluation being by phone.

Regardless of the protocol addressed, pain decreased linearly from the onset to 3 months afterward (Figure 7).

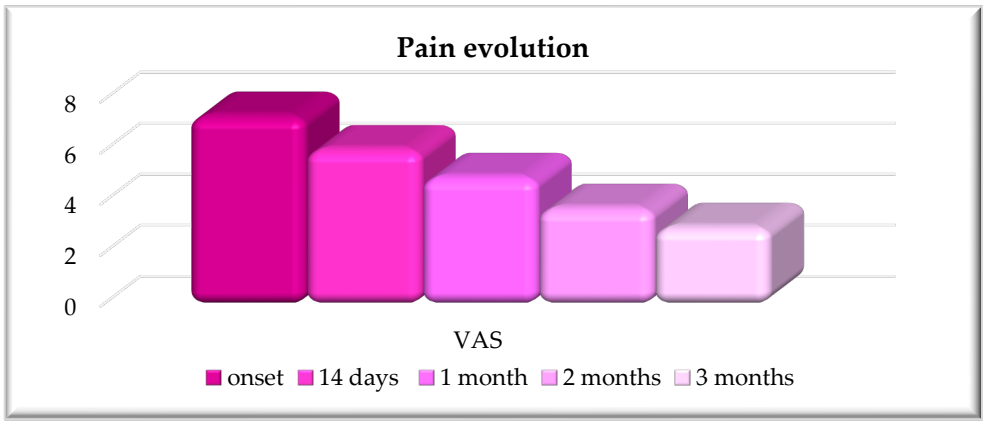


Figure 7. Pain evolution

During the study, the pain improved, regardless of the applied protocol (Figure 8). Although the average pain score was comparable in patients included in P1 – P3, the decrease in pain in patients in group P2 (which received only ozone treatment) was more significant than in those in P1 (classical treatment, medication) and those in P3 (classical treatment + O_3) who received fewer sessions with O3. This fact would be due to the property of OOT to modulate the response to pain [36], by combating allodynia [32]. Also, if we compare the pain score between P1 and P3 (both using classical therapy), we find that at 14 days, the VAS value decreases approximately equally. At measurements at one, two, and three months, pain decreased remarkably in patients following P3 (O_3 and drug treatment).

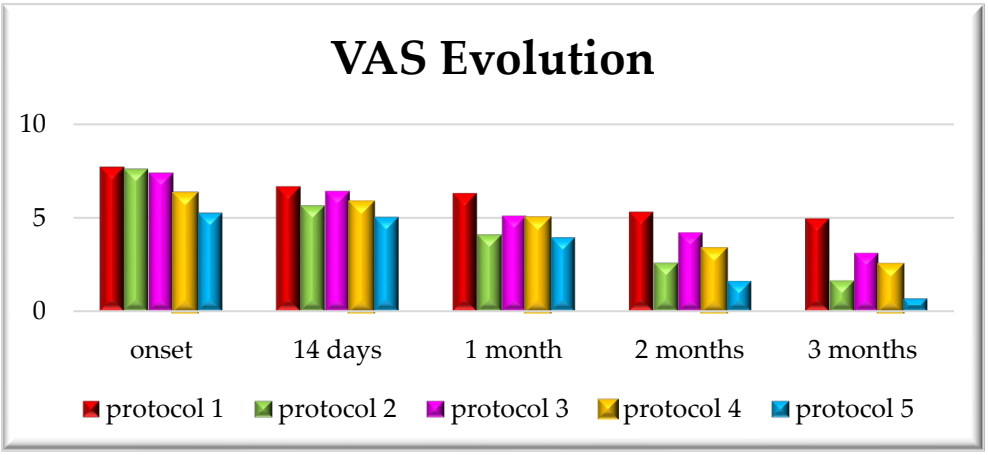


Figure 8. The evolution of pain during the study, by analyzing the VAS.

Initially, the patients included in P1-P3 had similar values on the VAS scale. At 14 days, the VAS scale at P1 and P3 has equal values; at P2, the pain level decreases, and at P4 and P5, the pain remains approximately at the same level. At 1, 2, and 3 months, the VAS scale decreased considerably in patients who received treatment with O_3.

We note that all non-drug therapy patients experienced significant and linear pain relief.

We see here that there is a negative correlation between O_3 concentration and pain progression, which could have two explanations:

- in the case of severe and persistent pain, deep intramuscular ozone was administered at concentrations of 20 µg/ml, resulting in rapid pain relief; however, we reduced the number of profound administrations;

- in knee osteoarthritis (protocols 2, 3 and 5), O_3 was administered intra-articularly, using a concentration of 20 μg/ml, which also resulted in rapid pain relief.

Spearman's test (Table 5) shows there is a negative correlation between O_3 administration and pain scale at 14 days ($p = -0.130$, $p = 0.038 < \alpha = 0.05$), pain scale at one month ($p = -0.302$, $p < 0.01 < \alpha = 0.05$), pain scale at two months ($p = -0.380$, $p < 0.01 < \alpha = 0.05$) pain scale at three months ($p = -0.482$, $p < 0.01 < \alpha = 0.05$). The higher the number of O_3 sessions, the lower the pain. Also, in protocols where O_3 was used, pain decreased over time.

Table 5. Correlation between O_3 administration and pain scale

			Initial pain scale	Pain scale at 14 days	Pain scale at 1 month	Pain scale at 2 months	Pain scale at 3 months
Spearman's rho	O_3 concentration	Correlation Coefficient	-0,075	-0,130*	-0,302**.	-0,380**.	-0,482**.
		Sig. (2-tailed)	0,234	0,038	0,000	0,000	0,000
		N	256	256	256	256	256

Analyzing the differences in pain scale (Table 6, 7), the "t" test (Table 8) and the Mann-Whitney test (Table 9) were used between the two groups (female and male), both showing that men had a higher pain intensity permanently.

Table 6. Analyse differences of pain scale between male and female

	Gender	N	Mean	Std. Deviation	Std. Error Mean
Initial pain scale	male	122	7,49	1,410	0,128
	female	134	7,38	1,325	0,114
Pain scale at 14 days	male	122	6,23	1,547	0,140
	female	134	6,02	1,643	0,142
Pain scale at 1 month	male	122	5,32	1,824	0,165
	female	134	4,78	2,068	0,179
Pain scale at 2 months	male	122	4,27	2,041	0,185
	female	134	3,41	2,292	0,198
Pain scale at 3 months	male	122	3,47	2,062	0,187
	female	134	2,72	2,318	0,200

Table 7. Comparison between the pain scale of men and women

Ranks				
	gender	N	Mean Rank	Sum of Ranks
Initial pain scale	male	122	131,66	16063,00
	female	134	125,62	16833,00
	Total	256		
Pain scale at 14 days	male	122	133,93	16339,00
	female	134	123,56	16557,00
	Total	256		
Pain scale at 1 month	male	122	138,55	16903,00
	female	134	119,35	15993,00
	Total	256		
Pain scale at 2 months	male	122	143,84	17548,00
	female	134	114,54	15348,00
	Total	256		
Pain scale at 3 months	male	122	142,45	17379,50
	female	134	115,79	15516,50
	Total	256		

Using the t-test for two independent samples (Table 8), we tested whether significant differences existed between women and men on the pain scale. Levene's test confirmed equality of variances in all cases because $p > \alpha = 0.05$ ($p = 0.28$ - initially, $p = 0.422$ at 14 days, $p = 0.105$ at one month, $p = 0.136$ at 2 months and $p = 0.228$ at three months). There are no significant differences between women and men for the initial pain scale ($p = 0.516 > \alpha = 0.05$, $t = 0.650$, difference between means 0.111) and the pain scale at 14 days ($p = 0.301 > \alpha = 0.05$, $t = 1.036$, difference between means 0.207). Significant differences appear for the pain scale at one month ($p = 0.029 < \alpha = 0.05$, $t = 2.191$, difference between means 0.536), at two months ($p = 0.002 < \alpha = 0.05$, $t = 3.159$, difference between means 0.860) and at three months ($p = 0.007 < \alpha = 0.05$, $t = 2.700$, difference between means 0.743). In all cases, higher values occur in men.

Table 8. „t-test" for two independent samples

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
Initial pain scale	Equal variances assumed	1,339	0,248	0,650	254	0,516	0,111	0,171	-0,225	0,448
	Equal variances not assumed			0,649	247,985	0,517	0,111	0,171	-0,226	0,449
Pain scale at 14 days	Equal variances assumed	0,647	0,422	1,036	254	0,301	0,207	0,200	-0,187	0,601
	Equal variances not assumed			1,039	253,705	0,300	0,207	0,199	-0,186	0,600
Pain scale at 1 month	Equal variances assumed	2,647	0,105	2,191	254	0,029	0,536	0,245	0,054	1,018
	Equal variances not assumed			2,204	253,752	0,028	0,536	0,243	0,057	1,015
Pain scale at 2 months	Equal variances assumed	2,231	0,136	3,159	254	0,002	0,860	0,272	0,324	1,396
	Equal variances not assumed			3,176	253,882	0,002	0,860	0,271	0,327	1,393
Pain scale at 3 month	Equal variances assumed	1,463	0,228	2,700	254	0,007	0,743	0,275	0,201	1,285
	Equal variances not assumed			2,715	253,866	0,007	0,743	0,274	0,204	1,282

The Mann-Whitney test (Table 9) indicates similar results to the independent samples t-test for differences in pain scale between women and men. There were no significant differences between women and men in the initial pain scale ($p = 0.502 > \alpha = 0.05$, $Z = -0.671$) and the pain scale at 14 days ($p = 0.2550 > \alpha = 0.05$, $Z = -1.139$). There are significant differences for the pain scale at one month ($p = 0.036 < \alpha = 0.05$, $Z = -2.101$), at two months ($p = 0.001 < \alpha = 0.05$, $Z = -3.192$) and at three months ($p = 0.004 < \alpha = 0.05$, $Z = -2.907$), the explanation requiring further studies related to the hormonal

status, the response to opioids, the type of therapy applied, the psycho-social and cognitive component [56].

Table 9. The Mann-Whitney Test statistics (grouping variable: sex)

	Initial pain scale	Pain scale at 14 days	Pain scale at 1 month	Pain scale at 2 months	Pain scale at 3 months
Mann-Whitney U	7788,000	7512,000	6948,000	6303,000	6471,500
Wilcoxon W	16833,000	16557,000	15993,000	15348,000	15516,500
Z	-0,671	-1,139	-2,101	-3,192	-2,907
Asymp. Sig. (2-tailed)	0,502	0,255	0,036	0,001	0,004

We investigated the existence of correlations between the type of protocol used and the pain scale (Tables 10 and 11).

Table 10. Descriptive statistics

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Initial pain scale	1	96	7,70	1,017	0,104	7,49	7,90	5	10
	2	99	7,62	1,323	0,133	7,35	7,88	4	10
	3	35	7,40	1,376	0,233	6,93	7,87	5	10
	4	13	6,38	1,758	0,488	5,32	7,45	4	9
	5	13	5,23	1,166	0,323	4,53	5,94	3	7
	Total	256	7,43	1,365	0,085	7,27	7,60	3	10
Pain scale at 14 days	1	96	6,67	1,382	0,141	6,39	6,95	3	9
	2	99	5,67	1,660	0,167	5,34	6,00	2	9
	3	35	6,43	1,539	0,260	5,90	6,96	4	9
	4	13	5,85	1,625	0,451	4,86	6,83	3	8
	5	13	5,00	1,225	0,340	4,26	5,74	3	7
	Total	256	6,12	1,598	0,100	5,92	6,32	2	9
Pain scale at 1 month	1	96	6,11	1,710	0,175	5,77	6,46	0	9
	2	99	4,10	1,876	0,189	3,73	4,48	0	8
	3	35	5,17	1,445	0,244	4,68	5,67	2	8
	4	13	5,00	1,871	0,519	3,87	6,13	2	9
	5	13	3,92	1,801	0,500	2,83	5,01	0	7
	Total	256	5,04	1,970	0,123	4,80	5,28	0	9
Pain scale at 2 months	1	96	5,30	1,730	0,177	4,95	5,65	0	9
	2	99	2,59	1,927	0,194	2,20	2,97	0	7
	3	35	4,23	1,767	0,299	3,62	4,84	0	8
	4	13	3,38	1,805	0,500	2,29	4,48	1	7
	5	13	1,62	1,387	0,385	0,78	2,45	0	4
	Total	256	3,82	2,214	0,138	3,55	4,09	0	9
Pain scale at 3 months	1	96	4,90	1,756	0,179	4,54	5,25	0	9
	2	99	1,68	1,583	0,159	1,36	1,99	0	6
	3	35	3,11	1,676	0,283	2,54	3,69	0	6
	4	13	2,62	1,609	0,446	1,64	3,59	0	5
	5	13	0,69	0,947	0,263	0,12	1,26	0	3
	Total	256	3,08	2,227	0,139	2,80	3,35	0	9

Table 11. ANOVA Test

		Sum of Squares	df	Mean Square	F	Sig.
Initial pain scale	Between Groups	87,433	4	21,858	14,161	0,000
	Within Groups	387,438	251	1,544		
	Total	474,871	255			
Pain scale at 14 days	Between Groups	69,649	4	17,412	7,515	0,000
	Within Groups	581,597	251	2,317		
	Total	651,246	255			
Pain scale at 1 month	Between Groups	214,985	4	53,746	17,415	0,000
	Within Groups	774,624	251	3,086		
	Total	989,609	255			
Pain scale at 2 months	Between Groups	433,149	4	108,287	33,285	0,000
	Within Groups	816,585	251	3,253		
	Total	1249,734	255			
Pain scale at 3 months	Between Groups	588,434	4	147,108	54,621	0,000
	Within Groups	676,004	251	2,693		
	Total	1264,438	255			

According to the ANOVA test, there are significant differences in pain intensity according to the treatment protocol used ($F = 14.161$, $p < 0.001$ – initial pain scale, $F = 7.515$, $p < 0.001$ – pain scale at 14 days, $F = 17.415$, $p < 0.001$ – pain scale at one month, $F = 33.285$, $p < 0.001$ – pain scale at two months, $F = 54.621$, $p < 0.001$ – pain scale at three months). The highest mean values occur for P1, possibly because pain relief may require the use of several drug classes, while ozone treatment addresses several pathways simultaneously [5,57].

To show if there are differences in the O_3 concentration used by the two sexes (Table 12), we used two statistical methods: the t-test and Levene's test (Table 13).

Table 12. Statistical analysis of the concentration of O_3 used in the study, in male and female

	Gender	N	Mean	Std. Deviation	Std. Error Mean
O_3 concentration	male	122	7,37	6,963	0,630
	female	134	6,28	6,861	0,593

Table 13. Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
O3 conc.	Equal variances assumed	0,000	0,991	1,255	254	0,211	1,085	0,865	-0,618	2,788
	Equal variances not assumed			1,254	251,023	0,211	1,085	0,865	-0,619	2,789

We performed the independent samples t-test to determine whether there were significant differences in mean O_3 concentration comparing the female and male groups.

Levene's test confirms the equality of the variances of the two groups ($p = 0.991 > \alpha = 0.05$). The t-test results are as follows: $t = 1.255$, $df = 254$, $p = 0.211$, mean concentration for women = 6.28, mean concentration for men = 7.37, and the difference between means = 1.085.

The confidence interval for the difference between means is (-0.618, 2.788), indicating that we are 95% confident that the true difference in mean O_3 concentration between women and men falls within this range. Since $p = 0.211 > \alpha = 0.05$ and the confidence interval contains the zero value, we accept the null hypothesis that there are no significant differences in O_3 concentration between women and men.

Number of sick leave days (Figure 9)

By analyzing the number of sick leave days (Image 11), we wanted to see if the patient's recovery was achieved faster, depending on the applied protocol. In this sense, we analyzed patients of active age.

Of the patients aged 20 to 59 who received treatment according to P1 - P3, 159 had an employment contract.

Of them:

- 69 underwent P1 (classical treatment, without O_3), amounting to a total number of 456 sick leave days, with an average of 6.6 days/patient;
- 75 underwent P2 (O_3 only), amounting to a total number of 87 sick leave days, with an average of 1.16 days/patient;
- 15 underwent P3 (classical treatment + O_3), amounting to a total number of 76 sick leave days, with an average of 5.06 days/patient.

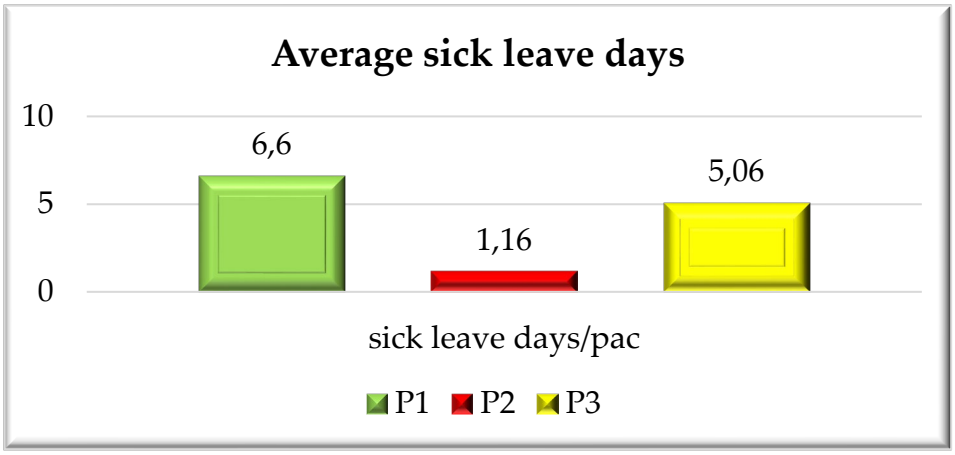


Figure 9. Average sick leave days in the P1-P3 groups

Patients who followed P2 required a few days of recovery and most quickly resumed their daily activities. Moreover, patients in P3 returned to work one day earlier than those in P1.

We investigated whether there are correlations between the affected region and the number of sick leave days (Table 14 and Table 15), P1 - P3.

Table 14. Descriptive statistics

	Injury	N	Mean	Std. Deviation	Std. Error Mean
days of medical leave	Spinal cord	147	2,54	5,450	0,450
P1	knee	109	0,76	2,902	0,278
days of medical leave	Spinal cord	147	0,47	1,737	0,143
P2	knee	107	0,17	0,916	0,089
days of medical leave	Spinal cord	147	0,20	1,070	0,088
P3	knee	109	0,43	1,931	0,185

Table 15. Independent Samples t-Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
days of medical leave P1	Equal variances assumed	35,641	0,000	3,092	254	0,002	1,776	0,574	0,645	2,907
	Equal variances not assumed	-	-	3,360	232,971	0,001	1,776	0,529	0,735	2,817
days of medical leave P2	Equal variances assumed	11,361	0,001	1,635	252	0,103	0,301	0,184	-0,062	0,664
	Equal variances not assumed	-	-	1,788	232,196	0,075	0,301	0,168	-0,031	0,633
days of medical leave P3	Equal variances assumed	6,281	0,013	-1,235	254	0,218	-0,234	0,189	-0,607	0,139
	Equal variances not assumed	-	-	-1,141	156,796	0,255	-0,234	0,205	-0,639	0,171

There are differences in the number of days of sick leave in the case of P1 patients between groups defined by the affected region (t = 3.360, p = 0.001, difference between means = 1.776), patients with spinal damage requiring more recovery days than those with knee osteoarthritis. There are no differences in sick leave days between groups by affected region for patients in P2 (t = 1.788, p = 0.074) and P3 (t = -1.141, p = 0.255). From the analyzed values, we can conclude that OOT has similar effects in the two types of conditions, while those with osteoarthritis respond better to drug treatment. However, further studies with more subjects are needed to consider these observations conclusive.

The beneficial effects of ozone treatment in musculoskeletal disorders are widely presented in the specialized literature and confirmed clinically and paraclinically. We recorded no adverse effects other than slight pain at the injection site, which disappeared in a few seconds, consistent with the reviewed literature [6,36,58].

4. Conclusions

This retrospective and statistical study was conducted in two different centers under the coordination of the same doctor. In this study, we analyzed five therapeutic plans for two conditions (osteoarthritis and degenerative spine pathology). For the statistical analysis, we used IBM SPSS Statistics 23. The obtained results show the advantages of using alternative therapies (ozone therapy and plasma enriched in platelets) and the importance of choosing personalized therapeutic protocols.

122 men and 134 women were involved in the study. The patient has chosen the treatment plan from the variants corresponding to him as recommended by the doctor. The groups of patients following P1 and P2 were homogeneous, as were those in P4 and P5, so the results were comparable. The five treatment options were: P1—drug treatment, according to the pain therapy protocol of the World Health Organization, valid since 1980; P2—ozone therapy; P3—drug treatment and ozone therapy; P4—platelet-rich plasma; and ozone therapy.

During the study, we administered 1322 sessions with an oxygen-ozone mixture, on average 11.7 sessions/patient, with an average O3 concentration of 8.83 μg/ml. In case of damage to the spine, we used O3 in the form of subcutaneous or deep intramuscular paravertebral infiltrations. In osteoarthritis of the knee, we administered O3 and PRP intra-articularly, separately or combined.

We analyzed various correlations between the type of injury (spine/knee) and the patient's sex (we classified according to the XX or XY chromosome), pain, age, and the protocol followed.

Applying the statistical analysis, we found several positive or negative correlations. There is a correlation between age and the affected region: patients under 50 are more likely to suffer from spinal injuries and those over 50 from knee injuries. According to specialized literature, the percentage of knee deterioration increases progressively with age for several reasons [54,55].

Protocols P1 and P2 were applied more to patients with spinal injuries, P3 more to those with knee injuries, and P4 and P5 were applied only to those with knee injuries. Regardless of the protocol applied, the immediate goals were the same: pain control and resumption of regular activity. Decreased pain led to increased activity, implying better joint mobility.

Pain, assessed with the visual analog scale (VAS), decreased over time. At 14 days, the decrease was comparable between the five groups. After 3 months, the groups that followed the protocol P2 and P5 had the lowest pain rate (a significant difference in the mean VAS score, compared to the initial moment and compared to the other groups).

There is a negative correlation between the concentration of O3 and the progression of pain, because concentrations of 20 μg/ml decrease inflammation by more than ten μg/ml, reducing pain. This aspect is consistent with the bibliography studied [5]. The persistence of low VAS at 1,2,3 months confirms the increase in quality of life [10,16]. The Spearman test shows a negative correlation between the administration of O3 and the pain scale at 14 days, one month, two months, and the pain scale at three months, with the pain decreasing with the increase in the number of sessions. The use of O3 treatment, compared to traditional drug therapy, is effective in pain management (two weeks, one month, two months, and three months after onset). In the protocols where we used O3, pain decreased more over time compared to the drug-only group. We note that in patients who followed P3, pain decreased, with OOT having an effect similar to opioids or corticosteroids. Studies in the specialized literature also state that OOT has an effect similar to cortisone [19,59] but without its side effects. The pain intensity was constantly higher in men than in women and protocol 1, compared to the others. We did not record significant differences in the concentration of O3 used in women compared to men.

There is a significant correlation between the administration of O3 and the number of sick leave days, regardless of the protocol in which O3 was used. Patients in Protocol 1 (drug therapy) required more recovery days than those who received ozone. During the study, most patients who received treatment with O2-O3 stated that they felt a decrease in pain and an increase in the quality of life, even after the first administration.

There were no correlations between:

- the genre of the patient and the affected region,
- type of affection and the environment of the patient (urban/ rural)
- O3 concentration and initial pain scale
- the concentration of O3 was higher in men than in women..

The results obtained are gratifying and generally consistent with the literature. The effect of OOT in the situations studied by us is in the same direction as the multiple studies that confirm the effects of ozone therapy on posture [60] and in musculoskeletal conditions [3,36,50,61].

Since the number of patients in the P4 and P5 groups was low, a larger number of participants is needed to conclude whether the combination of O3 with PRP leads to better results.

Considering the wide range of conditions in which OOT finds applicability as a complementary and sometimes alternative therapy, especially in musculoskeletal conditions, with a low number of contraindications, we consider that OOT is a safe therapy and can be used alone or associated with therapy medicinal or other type, the results obtained being superior to the classic ones. However, additional studies are needed to establish the association of the two alternative therapies on an increased number of subjects to establish the most faithful protocols of investigation and administration to combat pain and restore damaged tissues effectively.

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