

Original Article

Comparison of the Effect of Intra-Articular Injection of Autologous (Orthokine) Interleukin-1 Receptor Antagonist (IL-1Ra) and Hyaluronic Acid in Pain Control of Knee Osteoarthritis

Masoud Hashemi¹, Mehrdad Taheri^{2*}, Hossein Adlkhoo¹, Payman Dadkhah³, Mohammad Reza Abbasian⁴

¹ Anesthesiology Research Center, Akhtar Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran

² Anesthesiology Research Center, Imam Hossein Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran

³ Anesthesiology Research Center, Labbafinejad Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran

⁴ Department of Orthopedic surgery, Akhtar Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran

Received: 16 May 2019; Accepted: 16 August 2019

Abstract

Background: Due to the limitations of more common treatments of osteoarthritis, pharmaceutical research has been increasingly conducted during the past two decades with the aim of determining the rate of recovery of the disease' symptoms and making the process of disease progression slow. The purpose of this study was to compare the effect of intra-articular injection of autologous interleukin-1 Receptor Antagonist and hyaluronic acid in pain control of knee osteoarthritis.

Materials and Methods: A total of 60 patients with knee osteoarthritis were allocated to two groups of 30 in this randomized double-blind clinical trial. In the intervention group, injection of 2 milliliter of interleukin-1 receptor antagonist (Orthokine) into the knee joint was performed three times at intervals of one week (base time, seventh day and fourteenth day). In the control group, three injections of two milliliters of hyaluronic acid solution into the knee joint were performed at intervals of one week. Pain, symptoms, daily activities, sport-recreational performance, and knee-related quality of life were five outcomes investigated by completing two questionnaires, the knee injury and osteoarthritis outcome score (KOOS), Western Ontario, and McMaster Universities Arthritis Index (WOMAC) by the patients on two occasions before the start of treatment and six months after the last injection. Repeated measure and t-test were statistical tests used in this study.

Results: The mean score of pain in the first month ($p=0.005$) and the sixth ($p=0.049$) in the intervention group was less than the control group. Based on the scores of the KOOS questionnaire, the mean score of symptoms ($p=0.006$), daily activities ($p=0.001$) and sport-recreational performance ($p=0.037$) in the Orthokine group were higher than the hyaluronic acid group after six months. Also, the results of the questionnaire WOMAC show that while before the start of treatment, the mean of physical performance in the Orthokine group is greater than the control group, there is no difference in any of the indicators of pain, dryness of the joint, physical performance, and total score ($p=0.319$) in the sixth month.

Conclusion: According to the findings of this study, it seems that Orthokine has beneficial biological effects in patients with knee osteoarthritis. Intra-articular injection of Orthokine is a low invasive, safe and effective method, which can be considered as an appropriate choice in patients with chronic knee pain.

Keywords: Intraarticular injections, Intra-articular injection, IL-1Ra, Interleukin 1 receptor, Hyaluronic acid, Pain management

*Corresponding Author: Mehrdad Taheri, Anesthesiology Research Center, Imam Hossein Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran. Email: m.taheri@sbmu.ac.ir

Please cite this article as: Hashemi M, Taheri M, Adkhoo H, Dadkhah P, Abbasian MR. Comparison of the Effect of Intra-Articular Injection of Autologous (Orthokine) Interleukin-1 Receptor Antagonist (IL-1Ra) and Hyaluronic Acid in Pain Control of Knee Osteoarthritis. *Novel Biomed.* 2019;7(4):210-7.

Introduction

Osteoarthritis (OA) is degenerative joint disease with a slow progressive process that 10% of the world's population over the age of 60 years is involved with this disease according to the world health organization (WHO) estimation, and the increased incidence of this disease will be expected due to the increasing elderly in many parts of the world¹. Not only is osteoarthritis today one of the most common causes of clinical referrals of the elderly, but also makes the younger people involve after trauma or popular heavy sports²⁻⁴. Destruction of articular cartilage, remodeling of subchondral bones and synovitis are the most important causes of OA⁵ which consequently lead to the pain, reduced the range of joint movement, inflammation and swelling of the joint, involvement of total structure of the joint, cartilage, bone, ligament and muscle and ultimately lead to the patients' disability⁶⁻⁸. Modifying daily activities, physical treatment, consuming anti-inflammatory drugs, cryotherapy, hyperthermia, injection into the knee joint and arthroplasty are some of the treatment methods for OA control, which improve knee pain^{7,9}. Although low invasive treatments such as injection of steroid into the knee, lignocaine, hyaluronic acid¹⁰, non-steroidal anti-inflammatory and cyclooxygenase-2 inhibitors drugs^{11,12} have been reported to be effective in reducing pain and knee joint function. They do not prevent the progress of the disease and will have important consequences such as upper gastrointestinal bleeding and cardiovascular ischemia in the long-term consumption^{13,14}. Due to the limitations of more common treatments, pharmaceutical research has been increasingly designed and implemented during the past two decades with the aim of determining the rate of recovery of the disease' symptoms. Making the process of disease progression slow as well as preventing the need to arthroplasty using disease modifying osteoarthritis drugs (DMOADs) assuming the effect of intervention in the pathway of pro-inflammatory cytokines such as $1-\beta$ (IL-1 β)¹⁵.

Autologous conditioned serum (ACS) is one of the new methods of no-cell therapy which was developed in the mid-1990s. In this method, a significant amount of anti-inflammatory cytokines is produced including interleukin-1 receptor antagonist (IL-1Ra), IL-4, IL-10 and regenerative growth factors including TGF- β using the patient's venous blood process^{16,17}. Orthokine (IL-1Ra) is the product of the patient' complete blood, which is incubated with glass grains covered with CrSO₄ and improves pain and inflammation by blocking the destructive proteins which leads to the maintenance of the joint health¹⁸. Considering that a few human and animal studies have been conducted on the effectiveness of Orthokine in reducing the symptoms and reducing the progression rate of OA and no study has been conducted in Iran to compare the effectiveness of this treatment compared with other treatments. So far, the purpose of this study was to compare the effect of intra-articular injection of autologous interleukin-1 receptor antagonist and hyaluronic acid in pain control of knee osteoarthritis.

Methods

Study Design: This study was a randomized double-blind clinical trial to compare the effect of intra-articular injection of autologous interleukin-1 receptor antagonist and hyaluronic acid in pain control of 60 patients with knee osteoarthritis at the time of treatment, three and six months after treatment. The sampling method was sequential therefore; all eligible people referred respectively, and were selected to complete the desired sample size. For randomization of patients into two groups, we used balanced block method and then informed consent has been obtained from the patients.

Patients: Patients with knee osteoarthritis referring to pain clinic of Shohadaye Tajrish and Akhtar hospitals in Tehran were entered the study. The study included the patients who were 40-year old and older that they had knee osteoarthritis pain for more than three months. Radiological findings confirmed knee osteoarthritis based on the American College of Rheumatology (ACR) criteria, and were satisfied to participate in the study. Exclusion criteria were

included patients with a history of knee surgery, lower limb contracture and deformity, neuromuscular disease of the lower limb, acute lumbar pathology, injection of steroid drugs during the last two months, the history of inflammatory rheumatoid arthritis, infection, diabetes, pregnancy, lactation, BMI>35, anticoagulant drugs consumption, allergic to any of drugs, patients candidate for knee surgery, varus or valgus knee more than five degrees which had been confirmed by the three joint view graph, radicular pain in knee, post-traumatic arthritis, the history of intra-articular injection or ozone therapy during the past 12 months, systemic or psychiatric disease, severe osteoarthritis with grade over III, intra-articular injection of hyaluronic acid during the past 12 months, Hepatitis, AIDS, cytomegalovirus, syphilis, osteomyelitis, drug and alcohol abuse, and the lack of consent to participate in the project.

Drug administration: Patients were randomized into two groups receiving 2 ml of IL-1RA (intervention group) and receiving 2 ml of hyaluronic acid (control group). To prepare IL-1RA, in the intervention group, 10 ml of venous blood was taken from the patient using the orthokine syringe (manufacturing country: Germany, manufacturing company: Orthokine), which contains glass grains covered with CrSO₄. The syringe was slowly rotated and immediately stored at 37°C and transferred to a single laboratory in a special incubator within 24 hours to ensure complete mixing and maximum grain and blood contact. After making sure the tests are negative for HIV, and hepatitis A, B and C, the products of Orthokine (IL-1RA) were prepared by a single laboratory and were returned to the hospital in 2 ml vials at -20°C within 14-21 days. Protocol of diet therapy was included 3 injections at intervals of one week that were performed on the first day, 7 and 14 days after the first injection. In the control group, 2 ml of hyaluronic acid was injected into the knee joint that three injections were also performed in these patients at intervals of one week. To do the procedure, the patient was placed in supine position and the landmark of the injection site was determined with flexion of the knee of 30-45 degrees on the lateral side of the knee. The injection site was disinfected with Povidone iodine solution and two milliliters of 2% Lidocaine solution were injected

using a needle No. 27 for numbness of the skin and joint surface to enter the needle without pain. After aspiration and ensuring, the correct positioning of the needle by ultrasound guidance (Sono Site, PICO. probe Convex 3-7, Linear 5-12), IL-1RA / hyaluronic acid was injected into the joint using the same needle. During the study, patients did not consume steroid, antidepressant and sedation drugs and they could consume acetaminophen (Maximum 4 grams per day) in case of incidence of pain with a score of over 3 during the study.

Measurement and Tools: Pain, symptoms, daily activities, sport-recreational performance, and knee-related quality of life were five outcomes, which were investigated that its required information was obtained by completing two questionnaires Knee injury and osteoarthritis outcome score (KOOS) and WOMAC by the patient on two occasions before the start of treatment and six months after the last injection. The questionnaire KOOS uses a 4-point Likert scale (4-0) to respond and includes pain (the highest raw score of 36), symptoms (the highest raw score of 28), daily activities (the highest raw score of 68), sport-recreational performance (the highest raw score of 20) and quality. The questionnaire WOMAC uses a 5-point Likert scale (5-0) to respond and has three sections: assessment of pain (5 questions, the highest raw score of 36), dryness of the joint (2 questions, the highest raw score of 28), and physical performance (17 questions, the highest raw score of 68).

Ethical approval: The study protocol was approved by the Institutional Research Ethics Committee of Shahid Beheshti University of Medical Sciences, Tehran, Iran and was conducted according to the stated principles in the Declaration of Helsinki (Ethical code: IR.SBMU.RETECH.REC.1397.835; date: 2019-01-06)

Data analysis: Chi-squared (X²), t-test, paired t-test and repeated measurement ANOVA were used to compare patient characteristics which were treated by IL-1Ra or Hyaluronic Acid. Data were analyzed using SPSS 16 software, with a probability (P) value of less than 0.05 as statistically significant.

Results

Patient characteristics: The characteristics of the 60 patients enrolled were shown in Table 1. In short, the

Table 1: Baseline demographics of study subjects.

Characteristics	IL-1Ra	Hyaluronic Acid
Number of patients	30	30
Age (years) ^a	56.8±8.6	57.9±8.2
Male/female	14/16	9/21
Weight(Kg)	81.6±9.7	81.6±7.9
Height(cm)	164.8±6.3	162.2±6.7
BMI	31.3±2.4	30.4±3.5
Grade of osteoarthritis		
II	11(36.7%)	14(46.7%)
III	19(63.3%)	16(53.3%)
Job		
employee	12(40%)	15(50%)
housewife	13(43.3%)	13(43.3%)
worker	5(15.7%)	2(6.6%)

^aMean (S.D.).

mean age in two intervention and control groups was 56 and 57 years, and 36.7% of the patients receiving Orthokine and 46.7% of the patients receiving hyaluronic acid group were with Grade II osteoarthritis. In addition, 63.3% and 53.3% in two groups were with Grade III.

Outcome measurement: As shown in Table 2, the mean score of pain in the first and sixth months in the intervention group is lower than in the control group. However, the analysis of repeated measure showed that there was no significant difference between the two groups in terms of the changes in the amount of pain before the start of treatment, one month, three months and six months after treatment based on VAS score (p=0.319) (Figure 1). Comparison of KOOS scores before the start of treatment and six months after treatment in two groups based on the score of pain, symptoms, daily activities, sport-recreational activities and quality of life is shown in Table 3. Accordingly, the mean score of symptoms, daily activities, and sport-recreational activities in the control group before the start of treatment were significantly higher than the

Table 2: Comparison of VAS Score in over time.

Time of treatment	HA	Orthokine	P-Value
Baseline	5.6±1.0	5±8.5	0.262
1months	3.3±0.5	3±7.5	0.005
3 months	2.7±0.6	2±9.5	0.16
6 months	2.9±0.6	2±5.6	0.049

intervention group; while, this relationship was reversed after six months and the mean scores of these three indicators in the IL-1Ra group was higher than the hyaluronic acid group. In addition, the results of the questionnaire WOMAC showed that while before the start of treatment, the mean of physical performance in the group Orthokine was greater than the control group, there was no difference in any of the indicators of pain, dryness of the joint, physical performance, and total score in the sixth month (p=0.319).

Discussion

In the present study, there was a significant decrease in the amount of pain six months after treatment in the Orthokine group based on VAS score. However, these changes did not show significant differences between the two groups at different times. In addition, KOOS pain score was higher in the Orthokine group six months after treatment and there was a further decrease in the amount of pain and there was a further decrease in the amount of pain, but there was no significant difference compared to the hyaluronic acid group. Based on the criteria KOOS, our findings showed that there was a significant improvement in the scores of symptoms, daily activities, and sport-recreational activities in the Orthokine group. In addition, the scores of pain, dryness of the joint, physical activity, and total scores of WOMAC were higher in the Orthokine group and the patients had greater improvement compared to the hyaluronic acid, but comparison of the two groups did not show statistically significant difference. The treatment of osteoarthritis surgery has good results, but it is not the best option for the treatment of osteoarthritis in many cases due to the dangers of surgery and the patients' unwillingness to do so¹⁹. Therefore, over the past decades, many physicians have taken the use of drug therapies, which can change the process of disease progression and probably lead to delay or prevent surgical interventions, into consideration. The findings of our study, which are consistent with the results of similar studies, showed that improvement of symptoms in the group receiving Orthokine is more than the control group²⁰⁻²³. In a double blind clinical trial in comparing Orthokine with hyaluronic acid and normal saline for the first time in 2009, Baltzer et al,²⁴

Table 3: Comparison of scores in two group at baseline and six months after treatment.

Questioner	Time of treatment	Type of score	HA	Orthokine	P-Value
KOOS	Baseline	Pain score	46.9±10.4	40.1±8.9	0.317
		Sign score	46.9±10.4	41.9±2.5	0.035
		Daly activities score	52.9±8.9	37.9±8.1	0.001
		Sport and recreation score	29.8±13.4	22.7±3.5	0.002
		Quality of life	27.1±0.0	23.1±4.1	0.337
	6 months	Pain score	70.8±9.4	71.0±8.8	0.957
		Sign score	63.8±1.0	71.7±14.3	0.006
		Daly activities score	59.9±1.4	72.6±9.2	0.001
		Sport and recreation score	46.7±7.9	52.5±13	0.037
		Quality of life	44.1±8.6	46.1±9.4	0.483
WOMAC	Baseline	Pain score	43.5±16.0	46.1±0.5	0.548
		Dryness of the joint	41.4±14.9	45.9±6.5	0.199
		Physical function	22.8±9.4	45.1±2.2	0.001
		Total score	107±7.6	136.3±8.8	0.005
	6 months	Pain score	68.1±10.9	69.1±7.8	0.339
		Dryness of the joint score	55.4±10.1	58.8±2.6	0.253
		Physical function score	71.5±10.7	71.3±3.2	0.836
		Total score	194.2±9.3	199.2±2.5	0.391

showed that reduction of symptoms and improvement of quality of life in patients receiving

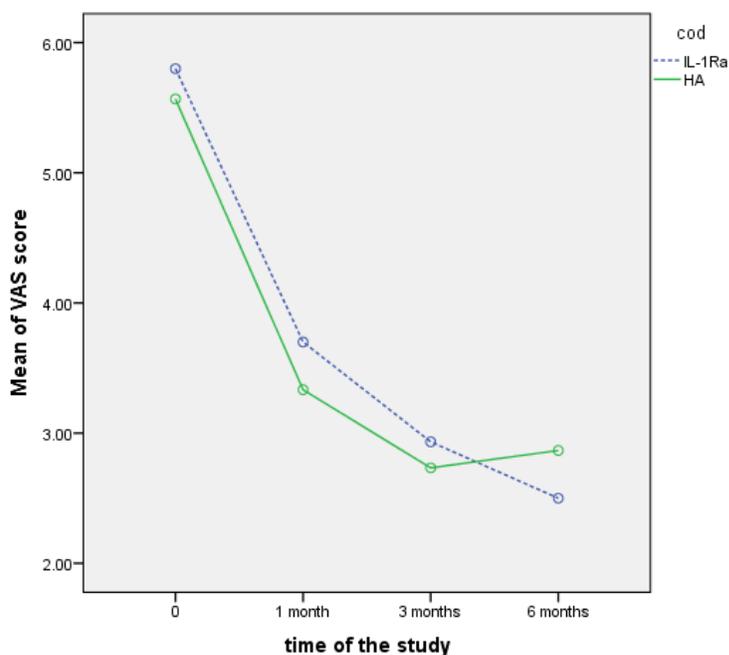


Figure 1. Mean of VAS scores based on time in two groups.

Orthokine were significantly higher than the hyaluronic acid group after 104 weeks of follow-up. This is while the researchers of this study did not observe any difference between the two groups of hyaluronic acid and normal saline. Similar findings observed in the study by Astolfi et al, in 2014²⁵. In a quasi-experimental study was done on 118 patients with knee osteoarthritis with chronic pain and candidate for the surgery, the researchers showed that regarding the patients who had been treated with ACS, the knee joint was replaced only in one person after 24 months and there was more than 60% to 80% improvement in pain in other patients. In addition, the duration of analgesia and the ACS therapeutic effects were long lasting and the patients tolerated this treatment well¹⁷. Few studies have also shown that there was no significant difference in improvement of clinical symptoms of knee osteoarthritis in the two groups of Orthokine and normal saline²⁶. While, it was shown in animal studies from the 1980s intra-articular injection of IL-1RA into the animal's knee leads to reduce the process of osteoarthritis progression and the level of IL-1RA is increased from a few weeks to several months after treatment²⁷⁻²⁹. In the first human studies, intra-articular injection of IL-1RA with a half-life of approximately 4 hours after injection in patients with painful knee did not lead to the clinical improvement of symptoms of osteoarthritis³⁰. Differences in the inclusion criteria of the study, tools used to measure, duration of patients' follow-up, long effect of placebo, and behavioral change of patients can be mentioned as the most important reasons for the difference in the results. Auw Yang et al,²⁰ showed that the KOOS score was significantly less than the score obtained in the study by Paradowski et al,³¹ with the same age group of patients. Also in studies by Waddell et al,³² and Altman et al,³³ that Orthokine was compared to hyaluronic acid, the number of patients treated with Orthokine without need for surgery was high. Furthermore, the perception of patients with osteoarthritis of their health status and symptoms of osteoarthritis will be changed with the passage of time and significantly affect the evaluation of the effect of therapeutic interventions³⁴. The severity of osteoarthritis plays an important role in response to

the treatment of Orthokine. The results of the study by Frizziero A et al,³⁵ showed that ACS containing anti-inflammatory endogenous cytokines includes IL-1RA and several growth factors which reduces the pain and increases the function and motion of knee in mild to moderate knee osteoarthritis. According to their findings, the use of ACS in the treatment of osteoarthritis is effective and safe and can increase the tissue regeneration and reduce the degenerative mechanisms. Also, the findings of another study that patients were followed-up more than seven years showed that the use of Orthokine in patients with end stages of knee osteoarthritis did not lead to delay in the surgical treatment and more than 40% of the patients receiving Orthokine received surgical treatment³⁶.

Conclusion

According to the findings of this study, it seems that Orthokine has beneficial biological effects in patients with knee osteoarthritis. Intra-articular injection of Orthokine is a low invasive, safe and effective method, which can be considered as an appropriate choice in patients with chronic knee pain. Thus, it is recommended to the pain specialists, orthopedic surgeons and rheumatologists to consider using therapeutic protocol of Orthokine according to the diet therapy of our study. It is suggested to conduct this study with larger sample size and longer follow up time and compared to the other drugs and placebo in future as well as to use other tools to evaluate the results of treatment and improvement of knee joint performance in addition to the KOOS and WOMAC tools.

Acknowledgment

This study is the result of a research project approved and registered by number 14176 through Anesthesiology Research Center. The researchers hereby express warm appreciation to Research and Technology Deputy as well as Anesthesiology Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

References

1. Woolf AD, Pfleger B. Burden of major musculoskeletal conditions. *Bulletin of the World Health Organization*. 2003;81:646-

- 56.
2. Buckwalter JA, Martin JA. Sports and osteoarthritis. Current opinion in rheumatology. 2004;16(5):634-9.
 3. Kim HJ, Lee YH, Kim CK. Biomarkers of muscle and cartilage damage and inflammation during a 200 km run. European journal of applied physiology. 2007;99(4):443-7.
 4. Lequesne MG, Dang N, Lane NE. Sport practice and osteoarthritis of the limbs. Osteoarthritis and cartilage. 1997;5(2):75-86.
 5. Martel-Pelletier J, Boileau C, Pelletier JP, Roughley PJ. Cartilage in normal and osteoarthritis conditions. Best practice & research Clinical rheumatology. 2008;22(2):351-84.
 6. Lane NE, Brandt K, Hawker G, Peeva E, Schreyer E, Tsuji W, et al. OARSI-FDA initiative: defining the disease state of osteoarthritis. Osteoarthritis and cartilage. 2011;19(5):478-82.
 7. Neustadt DH. Intra-articular injections for osteoarthritis of the knee. Cleveland Clinic journal of medicine. 2006;73(10):897-8, 901-4, 6-11.
 8. Reeuwijk KG, de Rooij M, van Dijk GM, Veenhof C, Steultjens MP, Dekker J. Osteoarthritis of the hip or knee: which coexisting disorders are disabling? Clinical rheumatology. 2010;29(7):739-47.
 9. Gregori D, Giacobelli G, Minto C, Barbetta B, Gualtieri F, Azzolina D, et al. Association of Pharmacological Treatments With Long-term Pain Control in Patients With Knee Osteoarthritis: A Systematic Review and Meta-analysis. Pharmacological Treatments for Pain Control in Knee Osteoarthritis. JAMA. 2018;320(24):2564-79.
 10. Review: evidence exists for 33 different treatment options for osteoarthritis of the knee. Evidence Based Medicine. 2004;9(3):81-.
 11. Cannon GW, Caldwell JR, Holt P, McLean B, Seidenberg B, Bolognese J, et al. Rofecoxib, a specific inhibitor of cyclooxygenase 2, with clinical efficacy comparable with that of diclofenac sodium: results of a one-year, randomized, clinical trial in patients with osteoarthritis of the knee and hip. Rofecoxib Phase III Protocol 035 Study Group. Arthritis and rheumatism. 2000;43(5):978-87.
 12. da Costa BR, Reichenbach S, Keller N, Nartey L, Wandel S, Juni P, et al. Effectiveness of non-steroidal anti-inflammatory drugs for the treatment of pain in knee and hip osteoarthritis: a network meta-analysis. Lancet (London, England). 2017;390(10090):e21-e33.
 13. Bresalier RS, Sandler RS, Quan H, Bolognese JA, Oxenius B, Horgan K, et al. Cardiovascular Events Associated with Rofecoxib in a Colorectal Adenoma Chemoprevention Trial. New England Journal of Medicine. 2005;352(11):1092-102.
 14. McGettigan P, Henry D. Cardiovascular risk and inhibition of cyclooxygenase: a systematic review of the observational studies of selective and nonselective inhibitors of cyclooxygenase 2. Jama. 2006;296(13):1633-44.
 15. Abramson SB, Amin A. Blocking the effects of IL-1 in rheumatoid arthritis protects bone and cartilage. Rheumatology (Oxford, England). 2002;41(9):972-80.
 16. Meijer H, Reinecke J, Becker C, Tholen G, Wehling P. The production of anti-inflammatory cytokines in whole blood by physico-chemical induction. Inflammation research : official journal of the European Histamine Research Society [et al]. 2003;52(10):404-7.
 17. Baselga Garcia-Escudero J, Miguel Hernandez Trillos P. Treatment of Osteoarthritis of the Knee with a Combination of Autologous Conditioned Serum and Physiotherapy: A Two-Year Observational Study. PloS one. 2015;10(12):e0145551.
 18. Wehling P, Moser C, Frisbie D, McIlwraith CW, Kawcak CE, Krauspe R, et al. Autologous conditioned serum in the treatment of orthopedic diseases: the orthokine therapy. BioDrugs : clinical immunotherapeutics, biopharmaceuticals and gene therapy. 2007;21(5):323-32.
 19. Watterson JR, JM. E. Viscosupplementation: therapeutic mechanisms and clinical potential in osteoarthritis of the knee. J Am Acad Orthop Surg 2000;8:277-84.
 20. Auw Yang KG, Raijmakers NJ, van Arkel ER, Caron JJ, Rijk PC, Willems WJ, et al. Autologous interleukin-1 receptor antagonist improves function and symptoms in osteoarthritis when compared to placebo in a prospective randomized controlled trial. Osteoarthritis and cartilage. 2008;16(4):498-505.
 21. Altman RD, Akermark C, Beaulieu AD, Schnitzer T. Efficacy and safety of a single intra-articular injection of non-animal stabilized hyaluronic acid (NASHA) in patients with osteoarthritis of the knee. Osteoarthritis and cartilage. 2004;12(8):642-9.
 22. Hughes R, Carr A. A randomized, double-blind, placebo-controlled trial of glucosamine sulphate as an analgesic in osteoarthritis of the knee. Rheumatology (Oxford, England). 2002;41(3):279-84.
 23. Pavelka K, Gatterova J, Olejarova M, Machacek S, Giacobelli G, Rovati LC. Glucosamine sulfate use and delay of progression of knee osteoarthritis: a 3-year, randomized, placebo-controlled, double-blind study. Archives of internal medicine. 2002;162(18):2113-23.
 24. Baltzer AW, Moser C, Jansen SA, Krauspe R. Autologous conditioned serum (Orthokine) is an effective treatment for knee osteoarthritis. Osteoarthritis and cartilage. 2009;17(2):152-60.
 25. Astolfi M, McGuire K, Kaminski TW. The effectiveness of autologous conditioned serum in the treatment of knee osteoarthritis. Journal of sport rehabilitation. 2014;23(4):365-9.
 26. Rutgers M, Saris DB, Dhert WJ, Creemers LB. Cytokine profile of autologous conditioned serum for treatment of osteoarthritis, in vitro effects on cartilage metabolism and intra-articular levels after injection. Arthritis research & therapy. 2010;12(3):R114.
 27. Frisbee DD, Ghivizzani SC, Robbins PD, al. e. Treatment of experimental equine osteoarthritis by in vivo delivery of the equine interleukin-1 receptor antagonist gene. Gene Ther. 2002;9 12-20.
 28. Fernandes J, Tardiff G, Martel-Pelletier J, al. e. In vivo transfer of interleukin-1 receptor antagonist gene in osteoarthritis rabbit knee joints,. Am J Pathol. 1999;154 1159-69.
 29. Zhang X, Mao Z, C. Y. Suppression of early experimental osteoarthritis by gene transfer of interleukin-1 receptor antagonist and interleukin-10. J Orthop Re. 2004;22 742-50.
 30. Chevalier X, Giraudeau B, Conrozier T, al. e. Safety study of intra-articular injection of interleukin 1 receptor antagonist in patients with painful knee osteoarthritis: a multicenter study. J Rheumatol. 2005;32 131. 23-27
 31. Paradowski PT, Bergman S, Sundén-Lundius A, Lohmander LS, EM. R. Knee complaints vary with age and gender in the adult population. Population-based reference data for the Knee injury and Osteoarthritis Outcome Score (KOOS). BMC Musculoskelet Disord.

2006;2(38).

32. Waddell DD, DWC. B. Total knee replacement delayed with Hylan G-F 20 use in patients with grade IV osteoarthritis. *J Manag Care Pharm.* 2007;13:113-21.

33. Altman R, Lim S, Steen RG, Dasa V. Hyaluronic Acid Injections Are Associated with Delay of Total Knee Replacement Surgery in Patients with Knee Osteoarthritis: Evidence from a Large U.S. Health Claims Database. *PLoS one.* 2015;10(12):e0145776.

34. Razmjou H, Yee A, Ford M, Finkelstein JA. Response shift in

outcome assessment in patients undergoing total knee arthroplasty. *The Journal of bone and joint surgery American volume.* 2006;88(12):2590-5.

35. Frizziero A, Giannotti E, Oliva F, Masiero S, N. M. Autologous conditioned serum for the treatment of osteoarthritis and other possible applications in musculoskeletal disorders. *Br Med Bull.* 2013;105:169-84.

Zarringam D, Bekkers JEJ, DBF. S. Long-term Effect of Injection Treatment for Osteoarthritis in the Knee by Orthokin Autologous Conditioned Serum. *Cartilage.* 2018 9(2):140-5.