Efficacy of single high-molecular-weight versus triple low-molecular-weight hyaluronic acid intra-articular injection among knee osteoarthritis patients

**CURRENT STATUS:** UNDER REVIEW

**BMC Musculoskeletal Disorders**  
**BMC Series**

Adel Ebrahimpour  
Shaheed Beheshti University of Medical Sciences  
✉️ a.ebrahimpour@sbmu.ac.ir **Corresponding Author**

Mohammad Hasan Bahrami  
Shaheed Beheshti University of Medical Sciences

Seyed Ahmad Raeissadat  
Shaheed Beheshti University of Medical Sciences

Mohsen Cheraghi  
Shaheed Beheshti University of Medical Sciences

Shahram Rahimi-Dehgolan  
Shaheed Beheshti University of Medical Sciences

**DOI:**

10.21203/rs.2.16399/v1

**SUBJECT AREAS**

*Orthopedics*

**KEYWORDS**

*Intra-articular Injections, Cross-linked, Hyaluronic Acid, Knee Osteoarthritis*
Abstract

Purpose To compare intra-articular (IA) knee injections of a cross-linked high-molecular-weight hyaluronic acid (HMW-HA) with a linear low-molecular weight HA (LMW-HA) in terms of pain and functional improvement among knee osteoarthritis (OA) patients.

Methods In this single-blinded RCT, the patients were randomly divided into two groups for HA injections. The first group received an HMW-HA (Arthromac) injection, while the other received three weekly LMW-HA (Hyalgan) injections. Pain and function were assessed using the outcome measures including WOMAC, Lequesne and VAS indices, once prior to injection, as well as 2 and 6 months after injections.

Results A total of 90 patients were included. There was no significant difference in baseline characteristics including age and sex between the two groups. Our analysis showed that total WOMAC, Lequesne and VAS mean scores remarkably improved at both follow-up time-points compared to the baseline measurements (p<0.001). There was no significant superiority between the two therapeutic protocols according to our outcome measures at any time-point of follow-up. The only except was about the improvement in WOMAC stiffness subscale that was significantly higher in LMW-HA group compared to HMW-HA (p=0.021). Moreover, no significant difference was observed in minor complications and injection-induced pain scores between the two groups.

Conclusion This study proved that a single HMW-HA injection is as effective as multiple injections of LMW-HA counterparts in periods of 2 and 6 months follow-up.

Background

Osteoarthritis (OA) has been known to be the most common articular disease. Knee OA is responsible for a large proportion of the total burden of OA. The prevalence of knee OA after 1950s became twice from 1900s to 1950s. Even after removing the interaction of
obesity and aging, the prevalence of OA was found to be doubled, possibly related to physical inactivity and western diet.\textsuperscript{2} By examining the DALY among selected conditions throughout the world, knee and hip OA was determined to be at the 11\textsuperscript{th} rank of global disability;\textsuperscript{3} and its social cost lies between 0.25\% and 0.50\% of a country’s gross domestic product.\textsuperscript{4}

Treatments of knee OA can be performed using several approaches, although none of them can be considered a disease-modifying therapy.\textsuperscript{5} Oral and topical medications including nonsteroidal anti-inflammatory drugs (NSAIDs), acetaminophen, duloxetine, opioids, topical NSAIDs and capsaicin are effective in reducing symptoms.\textsuperscript{6} Intra-articular injection, as one of the most effective therapies, can be carried out using corticosteroid,\textsuperscript{7} viscosupplementation (hyaluronic acid),\textsuperscript{8} ozone,\textsuperscript{9} plasma rich in growth factor (PRGF),\textsuperscript{10} and Platelet-rich plasma (PRP).\textsuperscript{11} Physical agent modalities including high-power laser and biofeedback have also been investigated regarding knee OA.\textsuperscript{12,13} Many of international scientific associations have recommended or endorsed Intra-articular hyaluronic acid (IA-HA) injections as part of knee OA treatment.\textsuperscript{14} There are a wide-variety of randomized clinical trials (RCTs) and systematic reviews with meta analyses concerning hyaluronic acid efficacy in knee OA;\textsuperscript{15,16} most of which reported beneficial effects. HA has been compared with ozone,\textsuperscript{17} PRP,\textsuperscript{18} PRGF,\textsuperscript{19} and corticosteroid.\textsuperscript{20}

Hyaluronic acid, which plays a role in traumatic energy dissipation and lubrication, as well as shock absorption, is a natural constituent of normal cartilage and synovial fluid. It also acts as a protective coating of the surface of articular cartilage.\textsuperscript{21} IA-HA is capable of decreasing nerve impulses related to OA pain. By benefiting from exogenous hyaluronic acid, endogenous proteoglycan and hyaluronic acid production are improved, while
decreasing the synthesis and activity level of matrix metalloproteinases (MMPs) and pro-inflammatory mediators; besides, it can activate immune system.²² HA binds to CD44 on chondrocytes and reduces IL-1β action that decrease activity of MMP-1, 2, 3, 9 and 13.²³ HA also binds to hyaluronan mediated motility (RHAMM) receptor and could be helpful for chondroprotection.²⁴ Synovium nitric-oxide production is also inhibited through reduction of IL-1β preventing apoptosis of chondrocytes.²⁵ Aggrecan, an important proteoglycan can be degradaded in OA knees, while IA-HA is capable to reduce this degradation process.²⁶ IA-HA treatment can inhibit many inflammatory pathways through Toll-Like Receptors reducing TNF-a, IL-1β, IL-6, IL-17, MMP-13 and Nf-kB.²⁷,²⁸ IA-HA also affects the sub-chondral bone and its abnormal metabolism in OA by lowering the activity of MMPs, mainly MMP-13 and IL-6.²⁹ The concentration and molecular weight of IA-HA in OA knee joints are lower than normal, thus decreasing the amount of viscoelasticity in synovial fluid.³⁰ Many formulations of HAs can be found in the market with a wide range of differences. This results in a more difficult understanding of the proper effects of this treatment. Differences exist in concentration, molecular weight, source of HA (biological fermentation-derived HA or avian-derived HA), dosage (number of injections and intervals), expected duration of effects, cross linkage and added formulations.³¹ Based on HA molecular weight, these products are classified in three groups (high ≥3000 kDa, moderate 1500-3000 kDa and low ≤1500 kDa). Many studies claim that high-molecular-weight intra-articular hyaluronic acids (HMW IA-HA) have better chondro-protective, anti-inflammatory, proteoglycan production, rheologic, analgesic and mechanical properties.³² They suggest that HMW IA-HA and those biological fermentation-derived HAs probably provide better efficacy and safety.³³ Our utilized HMW-HA (Arthromac, NOVATEX
Bioengineering SA Switzerland) is one of these cross-linked products which is indicated for single intra-articular injection in knee OA patients.\textsuperscript{34} In a few studies, the efficacy of single cross-linked HMW-HA has been investigated, though there exists a discrepancy between them.\textsuperscript{35–38}

The aim of current trial was to compare the efficacy and safety of the single cross-linked HMW injection versus triple injection of low-molecular-weight IA-HA among knee OA patients in terms of function and pain improvement during a six-month period. Also, we aimed to determine the best IA-HA injection-protocol based on HA characteristics including molecular weight, cross-linkage and number of injections.

Methods

Participants

In this RCT according to ACR criteria,\textsuperscript{39} 90 patients aged between 45–75 years suffering from knee OA symptoms lasting for at least three months were included. The diagnosis was made based on a famous radiological classification, i.e. Kellgren and Lawrence score (KLS).\textsuperscript{40} Only subjects with KLS grade of II-III were eligible. The other exclusion criteria were as the followings: breastfeeding or pregnancy, vascular collagen and immunodeficiency disorders, diabetes mellitus, a history of malignancy, body mass index (BMI) >32 kg/m\textsuperscript{2}, mal-alignment as genu varum or valgum greater than 20°, any knee trauma or intra-articular injection during the last 6 months, prior hypersensitivity reaction to avian products or egg protein.

This study protocol was also registered in Iranian database of RCTs (IRCT; www.irct.ir) with the trial registration number IRCT20130523013442N24 and registration date 2018-07-13. Besides, the Ethics Committee of Shahid Beheshti University of Medical Sciences was in charge of approving this study (No: IR.SBMU.MSP.REC.1396.899). A written
informed consent was obtained; moreover, a physiatrist described the methodology, probable advantages and disadvantages of HA injections for every participant.

**Interventions**

The patients were randomly divided into two groups of 44 and 46 subjects using a computer software for random number generation. In the first group, a HMW-HA called Arthromac® (NOVATEX Bioengineering SA Switzerland) was administered as a single intra-articular knee injection for 44 participants. HMW-HA solution was provided in a 3 mL prefilled syringe (60 mg of sodium hyaluronate). In the second group, 46 subjects received a low-molecular weight HA (molecular weight 500–730 kDa) (Hyalgan, Fidia Pharmaceutici S. P.A Italy) as three weekly sessions of IA injection. LMW-HA was provided in a 2 mL prefilled syringe (20 mg of sodium hyaluronate). All injection were performed by an expert physiatrist who had 15 years of experience in musculoskeletal injections. A sealed aluminum envelope was employed to hide the material and blind the latter physician, at least during the first visit of participants. However, since the injections in LMW-HA group should be repeated, the actual blinding was not achieved. Rather, the assessor physicians who were three senior residents remained unaware to patients’ group till the end.

Our patients did not receive any anti-inflammatory or analgesic agents since 2 weeks before the first injection known as washout period. Prior to injection, routine skin cleansing with the aid of povidone-iodine was performed. Twenty-two gauge (22G) needles through lateral midpatellar approach for knee intra-articular injections were used to administer HMW-HA and LMW-HA in a sterile manner. Upon completion of injections, the participants were requested to flex and extend their knees 10 times. Next, the patients of both groups rested briefly, after which they were given a written protocol of exercises and recommendations to be performed at home. A period of 24–48 hours rest along with 20-minute cold therapy 3 times a day and restricted weight-bearing over knee joints were
The exercise therapy protocol comprised of isometric strengthening workouts that gradually progressed to closed-chain isotonic exercises. Hamstring stretching and muscles strengthening (quadriceps femoris, hip adductor groups, gluteus medius and maximus) were executed three times a day, each time lasting 15 seconds and repeating 5 times. All patients were followed-up for 8 and 24 weeks after the therapy using visual analog scale (VAS), Lequesne index, and Western Ontario and McMaster Universities Arthritis Index (WOMAC) questionnaire. VAS, WOMAC, and Lequesne indices were employed to investigate the patients’ function and pain at three time-points; once at the baseline and two other times at the 2nd and 6th month after the injections. Moreover, minor adverse events such as the injection-induced pain was assessed in both groups. For all indices, lower scores indicate a better condition.

**Statistical analysis**

Final data before and after the treatment were imported and analyzed in SPSS v.22. Normality of the data was evaluated using Shapiro–Wilk’s test. Qualitative variables were expressed as frequency and percent. Chi square test was applied to analyze the differences of these qualitative parameters between the two groups. Also, the paired t-test and independent t-test were used to compare mean values within and between the two groups, respectively. To evaluate the relationship between quantitative variables, correlation coefficients of Pearson was employed. Statistical significance value was set at P < 0.05.

**Results**

Among 158 patients with knee OA as candidates for IA-HA injection, 68 patients were excluded from the study. Ninety subjects who met our criteria were randomized to the HMW-HA (44 patients) and LMW-HA (46 patients) groups and received IA-HA in a manner
demonstrated in Figure–1. Eleven participants discontinued the study; therefore the final number of subjects for the analysis was 39 in the HMW-HA group and 40 in the LMW-HA group. There was no significant difference in baseline characteristics between the two groups [Table–1]. The majority of participants were female in both groups (71.8% in HMW-HA and 75% in LMW-HA group).

Changes in WOMAC, Lequesne index and VAS mean values in each group have been demonstrated in Table–2. Findings showed that all outcome measuring tools statistically improved at 2 months and 6 months of follow-up, compared to the baseline level (p < 0.001). However, there was no definite superiority between the two groups in any of the outcome measures, neither at the short term follow-up, nor at the long term [Table–3]. In fact, there was no significant difference between the LMW-HA and the HMW-HA groups based on three subscales of Lequesne index. Our analysis revealed a similar pattern in VAS and WOMAC subscales. There was no superiority between two groups with one exception in WOMAC stiffness subscale at 2 months follow-up. When comparing WOMAC stiffness improvement, LMW-HA was statistically superior to HMW-HA at the 2nd month follow-up (P = 0.021). Furthermore, success rates [defined as ≥30% decrease from baseline scores in WOMAC, Lequesne and VAS] have been presented in Table–4.

Eventually, the frequency of minor complications and injection-induced pain have also been showed in Table–5. Joint stiffness and swelling occurred in 8 (20.5%) patients in the HMW-HA group versus 5 (12.5%) subjects of the LMW-HA group (P = 0.378). The mean value of injection-induced pain was 2.64 and 1.9 in HMW-HA and LMW-HA groups, respectively (P = 0.286). Fortunately, no systemic adverse event or major complication such as septic arthritis was reported in the present RCT.

Discussion
Our study proved that clinical improvement with a single cross-linked HMW-HA injection could be relatively equal to that of triple injection of a linear LMW-HA, within the periods of two and six months follow-up. Moreover, a comparison between the two groups indicates that there exists no statistically significant superiority. An exception was the improvement of WOMAC stiffness subscale which was significantly higher in LMW-HA group in 2 months.

Altman\textsuperscript{33} review which included 68 randomized trials proved that HMW-HA efficacy was superior to LMW-HAs. Conversely in our study, there was no difference in efficacy between these two types of HAs. In the study conducted by Zhang et al.\textsuperscript{44} the therapeutic effectiveness of single injection of a cross-linked HMW-HA (Durolane) was compared to five injections of a LMW-HA (Artz), showing that during a period of 26 weeks, Durolane was non-inferior to Artz in terms of pain, physical activity and knee-stiffness.\textsuperscript{45} Our study revealed a similar result within a period of same length.

Similarly, Diracoglu et al\textsuperscript{35} evaluated the efficacy of two HA types with different molecular weights and number of injections. The first group received a single cross-linked moderate-molecular-weight HA (Monovisc), while the other one underwent three consecutive weekly injections of a linear LMW-HA (Adant). In both groups, WOMAC scores and VAS-pain showed statistically significant improvements compared to the baseline level, without any remarkable superiority between the two groups. However in both groups, WOMAC stiffness showed no significant improvement. Meanwhile, VAS improvement for the group receiving Adant was remarkably higher than the Monovisc group. The latter study proved that a single cross-linked HA can be as effective as a triple linear LMW-HA, exactly similar to our study. It should be pointed out that the HA used in our trial was much heavier than the one used by Diracoglu. Unlike the mentioned results, WOMAC stiffness in our investigation
was associated with a statistically significant improvement. This change was even more evident in the group receiving LMW-HA compared to HMW-HA group.

Another study by Estades-Rubio et al,37 evaluated a single dose of NASHA (Durolane) versus a five-time GO ON® injection. Mobility and WOMAC were assessed during six months. A statistically significant change was observed for both groups compared to their baseline level. In addition, a remarkable superiority was observed in WOMAC scores of the group receiving NASHA compared to the GO ON® group, although no difference was detected in mobility values. From the economic point of view, the total price of using a single injection of Durolane was lower than that of multiple injections of GO ON®. In comparison, the results of our RCT showed some dissimilarities since the improvement in the NASHA as a cross-linked HMW-HA is statistically more significant than GO ON®. However, this finding proves that a single cross-linked HMW-HA can be as effective as or even better than multiple linear LMW-HA injections.

In the meta-analysis conducted by Concoff et al,36 the efficacy of multiple HA injections versus a single dose of HA was studied. The pooled data showed that a single HA injection was not significantly more effective than IA-Saline in a period of six months. Another systematic review and meta-analysis by Zhao et al,46 was carried out to compare the results of Hylan G-F 20 and LMW-HA in knee OA patients. The final results indicate a similarity between the Hylan G-F 20 and LMW-HA groups in terms of their pain-relief effect. However, Hylan G-F 20 was more effective in pain improvement from 2–3 months. It should be pointed out that in the present meta-analysis, Hylan G-F 20 injections were administered more than once; however, this number was less than the number of LMW-HA injections in most trials. These findings, similar to our study, showed the effectiveness of HMW-HA with lower number of injections compared to LMW-HA with multiple injections.
In our study, the rate of minor complications and injection-induced pain was not statistically different between two HA products. In Bannuru’s meta-analysis, none of the HA products were significantly different from each other with regard to incidence of adverse events and were relatively equal to IA placebo. Altman et al. concluded that there is no significant difference in the occurrence of effusion across molecular weight subgroups. Different brand names of HA exist in the market, claiming to be effective by a single injection. So far, few studies have been conducted to compare a single HA injection with multiple HA injection. Although most single-injection HAs are of HMW and cross-linked type, some differences can be observed in their structure. To examine the exact effects of such viscosupplements, further well-designed investigations should be performed. Evidently, single injections possess the advantage of lower cost, patients’ comfort and lower risk of complications owing to the lower number of injections. An advantage of this research is employing various outcome measures for evaluating patients’ symptoms including WOMAC subscales, Lequesne and VAS indices. The washout period was considered in this study; besides, only three patients did not complete our intervention. The fact that the physician was not blind in this study, is an important limitation. Due to ethical issues, it was not possible to do second and third sham injections in HMW-HA group. Rather, all assessors in this study were completely blinded. It would be better to enroll a higher number of patients in the future studies. Although our follow-up was for 6 months, yet longer follow-up time can be suggested. As the last limitation to be mentioned, no economic analysis was conducted in our trial.

Conclusions

Both HMW IA-HA and LMW IA-HA caused significant function improvement and pain relief; however, there was no significant difference between HMW IA-HA versus three weekly
LMW IA-HA in terms of pain relief and function improvement in knee osteoarthritis patients in six months of follow-ups. This study proved that a single HMW-HA injection is as effective as multiple injections of LMW-HA counterparts in periods of 2 and 6 months. Further research into the subject probably sheds lights on choosing the more suited protocol of HA injections.

Abbreviations

HMW-HA: high-molecular-weight hyaluronic acid; LMW-HA: low-molecular-weight hyaluronic acid; OA: osteoarthritis; NSAIDs: nonsteroidal anti-inflammatory drugs; PRGF: plasma rich in growth factor; PRP: Platelet-rich plasma; IA-HA: intra-articular hyaluronic acid; RCTs: randomized clinical trials; MMPs: matrix metalloproteinases; KLS: Kellgren and Lawrence score; BMI: body mass index; VAS: visual analog scale; WOMAC: Western Ontario and McMaster Universities Arthritis Index.

Declarations

Ethics approval and consent to participate

This study protocol was also registered in Iranian database of RCTs (IRCT; www.irct.ir) with ID number of 31564. Besides, the Ethics Committee of Shahid Beheshti University of Medical Sciences was in charge of approving this study (No: IR.SBMU.MSP.REC.1396.899). A written informed consent was obtained; moreover, a physiatrist described the methodology, probable advantages and disadvantages of HA injections for every participant.

Consent for publication

Not Applicable

Availability of data and material

The authors do not intend to share substantial data of this study; but they are ready to share the de-identified file of substantial data in excel format and all other study-related
documents, at any specific time for any period, on the demand of editorial board via the corresponding author’s email.

**Competing interests**
The authors declare that they have no financial or non-financial competing interests. *Funding*
This RCT had no external funding source.

**Authors’ contributions**
SAR and MC designed the clinical trial. Data analysis was done by SAR, SRD and MC. MC prepared the first draft of the article. AE and MHB took care of its revisions. All the authors contributed to interpretation of the results and preparation of the article. All authors approved the final version of the article to be published.

**Acknowledgements**
This article has been extracted from the thesis written by Dr. Mohsen Cheraghi in School of Medicine, Shahid Beheshti University of Medical Sciences. The authors would like to appreciate the help of Mrs Mehrnaz Mehrabi in Shahid Modarres Clinical Research Development Center.

**CONSORT guidelines**
Our study adheres to CONSORT guidelines and a completed CONSORT checklist is added in an additional file.

**References**
1. Vos, T., et al., Years lived with disability (YLDs) for 1160 sequelae of 289 diseases and injuries 1990–2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet, 2012. 380(9859): p. 2163–96.
2. Wallace, I. J., et al., Knee osteoarthritis has doubled in prevalence since the mid-20th
century. Proc Natl Acad Sci U S A, 2017. 114(35): p. 9332–9336.

3. Cross, M., et al., The global burden of hip and knee osteoarthritis: estimates from the global burden of disease 2010 study. Annals of the rheumatic diseases, 2014: p. annrheumdis-2013-204763.

4. Puig-Junoy, J. and A. R. Zamora. Socio-economic costs of osteoarthritis: a systematic review of cost-of-illness studies. in Seminars in arthritis and rheumatism. 2015. Elsevier.

5. Bannuru, R. R., et al. Relative efficacy of hyaluronic acid in comparison with NSAIDs for knee osteoarthritis: a systematic review and meta-analysis. in Seminars in arthritis and rheumatism. 2014. Elsevier.

6. McAlindon, T. E., et al., OARSI guidelines for the non-surgical management of knee osteoarthritis. Osteoarthritis and cartilage, 2014. 22(3): p. 363–388.

7. da Costa, B. R., R. Hari, and P. Juni, Intra-articular Corticosteroids for Osteoarthritis of the Knee. Jama, 2016. 316(24): p. 2671-2672.

8. He, W. W., et al., Efficacy and safety of intraarticular hyaluronic acid and corticosteroid for knee osteoarthritis: A meta-analysis. Int J Surg, 2017. 39: p. 95–103.

9. Raeissadat, S. A., et al., An investigation into the efficacy of intra-articular ozone (O2-O3) injection in patients with knee osteoarthritis: a systematic review and meta-analysis. Journal of pain research, 2018. 11: p. 2537.

10. Sánchez, M., et al., A randomized clinical trial evaluating plasma rich in growth factors (PRGF-Endoret) versus hyaluronic acid in the short-term treatment of symptomatic knee osteoarthritis. Arthroscopy: The Journal of Arthroscopic & Related Surgery, 2012. 28(8): p. 1070-1078.

11. Ayhan, E., H. Kesmezacar, and I. Akgun, Intraarticular injections (corticosteroid,
hyaluronic acid, platelet rich plasma) for the knee osteoarthritis. World Journal of Orthopedics, 2014. 5(3): p. 351-361.

12. Nouri, F., et al., Efficacy of High-Power Laser in Alleviating Pain and Improving Function of Patients With Patellofemoral Pain Syndrome: A Single-Blind Randomized Controlled Trial. 2018, 2018. 10(1): p. 7.

13. Raeissadat, S. A., et al., The efficacy of electromyographic biofeedback on pain, function, and maximal thickness of vastus medialis oblique muscle in patients with knee osteoarthritis: a randomized clinical trial. Journal of pain research, 2018. 11: p. 2781.

14. Cooper, C., et al., Use of intraarticular hyaluronic acid in the management of knee osteoarthritis in clinical practice. Arthritis care & research, 2017. 69(9): p. 1287-1296.

15. Bellamy, N., et al., Viscosupplementation for the treatment of osteoarthritis of the knee. The Cochrane Library, 2006.

16. Chen-Ti, W., et al., THERAPEUTIC EFFECTS OF HYALURONIC ACID ON OSTEOARTHRITIS OF THE KNEE: A META-ANALYSIS OF RANDOMIZED CONTROLLED TRIALS. Journal of Bone and Joint Surgery, 2004. 86(3): p. 538.

17. Raeissadat, S. A., et al., Intra-articular ozone or hyaluronic acid injection: which one is superior in patients with knee osteoarthritis? A 6-month randomized clinical trial. Journal of pain research, 2018. 11: p. 111.

18. Raeissadat, S. A., et al., Knee osteoarthritis injection choices: platelet-rich plasma (PRP) versus hyaluronic acid (a one-year randomized clinical trial). Clinical Medicine Insights: Arthritis and Musculoskeletal Disorders, 2015. 8: p. CMAMD. S17894.

19. Raeissadat, S. A., et al., Efficacy of Intra-articular Injection of a Newly Developed Plasma Rich in Growth Factor (PRGF) Versus Hyaluronic Acid on Pain and Function of
Patients with Knee Osteoarthritis: A Single-Blinded Randomized Clinical Trial. Clinical MEDicine Insights: Arthritis and Musculoskeletal Disorders, 2017. 10: p. 1179544117733452.

20. Stahl, S., et al., Comparison of intraarticular injection of depot corticosteroid and hyaluronic acid for treatment of degenerative trapeziometacarpal joints. JCR: Journal of Clinical Rheumatology, 2005. 11(6): p. 299-302.

21. Tascioglu, F. and C. Öner, Efficacy of intra-articular sodium hyaluronate in the treatment of knee osteoarthritis. Clinical rheumatology, 2003. 22(2): p. 112-117.

22. Moreland, L. W., Intra-articular hyaluronan (hyaluronic acid) and hylans for the treatment of osteoarthritis: mechanisms of action. Arthritis Res Ther, 2003. 5(2): p. 54.

23. Julovi, S. M., et al., Inhibition of interleukin-1β-stimulated production of matrix metalloproteinases by hyaluronan via CD44 in human articular cartilage. Arthritis & Rheumatism: Official Journal of the American College of Rheumatology, 2004. 50(2): p. 516-525.

24. Karna, E., et al., Protective effect of hyaluronic acid on interleukin-1-induced deregulation of β 1-integrin and insulin-like growth factor-I receptor signaling and collagen biosynthesis in cultured human chondrocytes. Molecular and cellular biochemistry, 2008. 308(1-2): p. 57-64.

25. Peng, H., et al., Hyaluronic acid inhibits nitric oxide-induced apoptosis and dedifferentiation of articular chondrocytes in vitro. Inflammation research, 2010. 59(7): p. 519-530.

26. Yatabe, T., et al., Hyaluronan inhibits expression of ADAMTS4 (aggrecanase-1) in human osteoarthritic chondrocytes. Annals of the rheumatic diseases, 2009. 68(6): p. 1051-1058.
27. Campo, G. M., et al., Inhibition of hyaluronan synthesis reduced inflammatory response in mouse synovial fibroblasts subjected to collagen-induced arthritis. Archives of biochemistry and biophysics, 2012. 518(1): p. 42–52.

28. Campo, G. M., et al., Hyaluronan reduces inflammation in experimental arthritis by modulating TLR-2 and TLR-4 cartilage expression. Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease, 2011. 1812(9): p. 1170-1181.

29. Hiraoka, N., et al., Intra-articular injection of hyaluronan restores the aberrant expression of matrix metalloproteinase-13 in osteoarthritic subchondral bone. Journal of Orthopaedic Research, 2011. 29(3): p. 354–360.

30. Dahl, L., et al., Concentration and molecular weight of sodium hyaluronate in synovial fluid from patients with rheumatoid arthritis and other arthropathies. Annals of the rheumatic diseases, 1985. 44(12): p. 817–822.

31. Migliore, A., et al., Differences among branded hyaluronic acids in italy, part 1: data from in vitro and animal studies and instructions for use. Clinical Medicine Insights: Arthritis and Musculoskeletal Disorders, 2016. 9: p. CMAMD. S38857.

32. Altman, R. D., et al., The mechanism of action for hyaluronic acid treatment in the osteoarthritic knee: a systematic review. BMC musculoskeletal disorders, 2015. 16(1): p. 321.

33. D Altman, R., et al., Product Differences in Intra-articular Hyaluronic Acids for Osteoarthritis of the Knee. Vol. 44. 2015.

34. ORGEV, A. L. What is ARTHROMAC. 2019 [cited 2019; Available from: http://www.arthromac.com/en/osteoarthritis-treatment.html.

35. Diracoglu, D., et al., Single versus multiple dose hyaluronic acid: Comparison of the results. J Back Musculoskelet Rehabil, 2016. 29(4): p. 881-886.

36. Concoff, A., et al., The efficacy of multiple versus single hyaluronic acid injections: a
systematic review and meta-analysis. BMC musculoskeletal disorders, 2017. 18(1): p. 542.

37. Estades-Rubio, F. J., et al., Knee viscosupplementation: cost-effectiveness analysis between stabilized hyaluronic acid in a single injection versus five injections of standard hyaluronic acid. International journal of molecular sciences, 2017. 18(3): p. 658.

38. Zhang, H., et al., Comparison of two hyaluronic acid formulations for safety and efficacy (CHASE) study in knee osteoarthritis: a multicenter, randomized, double-blind, 26-week non-inferiority trial comparing Durolane to Artz. Arthritis research & therapy, 2015. 17(1): p. 51.

39. Altman, R., et al., Development of criteria for the classification and reporting of osteoarthritis: classification of osteoarthritis of the knee. Arthritis & Rheumatism: Official Journal of the American College of Rheumatology, 1986. 29(8): p. 1039–1049.

40. Kellgren, J. and J. Lawrence, Radiological assessment of osteo-arthritis. Annals of the rheumatic diseases, 1957. 16(4): p. 494.

41. Bellamy, N., et al., Validation study of WOMAC: a health status instrument for measuring clinically important patient relevant outcomes to antirheumatic drug therapy in patients with osteoarthritis of the hip or knee. The Journal of rheumatology, 1988. 15(12): p. 1833–1840.

42. Carlsson, A.M., Assessment of chronic pain. I. Aspects of the reliability and validity of the visual analogue scale. Pain, 1983. 16(1): p. 87–101.

43. Faucher, M., et al., Assessment of the test-retest reliability and construct validity of a modified Lequesne index in knee osteoarthritis. Joint Bone Spine, 2003. 70(6): p. 520-525.

44. Zhang, H., et al., Comparison of two hyaluronic acid formulations for safety and
efficacy (CHASE) study in knee osteoarthritis: a multicenter, randomized, double-blind, 26-week non-inferiority trial comparing Durolane to Artz. Arthritis Res Ther, 2015. 17: p. 51.

45. Lindqvist, U., et al., Elimination of stabilised hyaluronan from the knee joint in healthy men. Clinical pharmacokinetics, 2002. 41(8): p. 603-613.

46. Zhao, H., et al., Hylan GF 20 versus low molecular weight hyaluronic acids for knee osteoarthritis: a meta-analysis. BioDrugs, 2016. 30(5): p. 387-396.

47. Bannuru, R. R., et al., Comparative safety profile of hyaluronic acid products for knee osteoarthritis: a systematic review and network meta-analysis. Osteoarthritis Cartilage, 2016. 24(12): p. 2022-2041.

Tables

Table 1

| Variable                          | HMW-HA* (N=39) | LMW-HA* (N=40) | P value |
|-----------------------------------|----------------|----------------|---------|
| Age [year] Median (Range)         | 56(41-66)      | 59.5(45-70)    | 0.305   |
| Weight [kg] Median (Range)        | 74(59-98)      | 75(57-89)      | 0.879   |
| Height [cm] Mean (SD)             | 1.66(0.09)     | 1.65(0.07)     | 0.521   |
| BMI [kg/m2] Median (Range)        | 27.05(23-34)   | 27.45(22-32)   | 0.462   |
| Female : Male (%)                 | 71.8% : 28.2%  | 75% : 25%      | 0.747   |
| Right : Left (Number)             | 21 : 18        | 16 : 18        | 0.21    |
| KLS Grade II : III (Number)       | 20 : 19        | 24 : 16        | 0.43    |

Table 2
|                | WOMAC |       |       | Lequesne |       |       |
|----------------|-------|-------|-------|----------|-------|-------|
|                | pain  | stiffness | Function | Total | pain | walk | ADL |
| HMW-HA [Mean]  |       |       |       |          |       |       |
| Before         | 9     | 3     | 30    | 42      | 5    | 2    | 5.5 |
| 2 months       | 5     | 2     | 19    | 25      | 4    | 1    | 4.5 |
| 6 months       | 5     | 1     | 17    | 22      | 3    | 1    | 4   |
| *P value       | <0.001| <0.001| <0.001| <0.001  | <0.001| <0.001| <0.001|
| LMW-HA [Mean]  |       |       |       |          |       |       |
| Before         | 9     | 3     | 30    | 44      | 5.5  | 1    | 5.5 |
| 2 months       | 5     | 1     | 18    | 25      | 3    | 1    | 4   |
| 6 months       | 5     | 1     | 17    | 24.5    | 3.5  | 1    | 4.5 |
| *P value       | <0.001| <0.001| <0.001| <0.001  | <0.001| <0.001| <0.001|

* P values refer to changes over time within each group, based on the Friedman test

# P values refer to changes over time within each treatment group, based on Repeated Measures

Table 3
|                | HMW-HA | LMW-HA | P value |
|----------------|--------|--------|---------|
| **Before [Mean]** |        |        |         |
| WOMAC          | 42     | 44.00  | 0.713a  |
| Lequesne       | 12.42  | 12.50  | 0.866b  |
| VAS            | 8      | 8.00   | 0.276a  |
| **2 months [Mean]** |       |        |         |
| WOMAC          | 26.03  | 25.00  | 0.59b   |
| Lequesne       | 9.6    | 8.93   | 0.202b  |
| VAS            | 2      | 2.00   | 0.788a  |
| **6 months [Mean]** |      |        |         |
| WOMAC          | 24.08  | 26.55  | 0.247b  |
| Lequesne       | 8.97   | 9.73   | 0.126b  |
| VAS            | 3      | 4.00   | 0.411a  |

a P values refer to comparison between the two groups, based on the Mann-Whitney test

b P values refer to comparison between the two groups, based on the paired T-test

Table 4
WOMAC (points)

|               | pain       | stiffness  | Function   | Total      | Pain       |
|---------------|------------|------------|------------|------------|------------|
|               |            |            |            |            |            |
| HMW-HA        |            |            |            |            |            |
| Before        | 9.74 (0.22)| 2.72 (0.19)| 29.92 (1.04)| 42.38 (1.24)| 5.08 (0.16)|
| 2 months      | 5.00 (0.27)| 2.65 (0.17)| 19.46 (1.12)| 26.03 (1.4) | 3.95 (0.19)|
| 6 months      | 5.05 (0.28)| 1.41 (0.19)| 17.62 (1.18)| 24.08 (1.55)| 3.49 (0.22)|
| Mean Difference\(^a\) (SD) | -4.7 (0.32) | -1.3 (0.26) | -12.30 (0.9) | -18.30 (1.23) | -1.6 (0.23) |
| Change\(^b\) (%) from baseline [SD] | 47.77 [2.96] | 58.16 [5.56] | 41.82 [3.17] | 43.76 [3.06] | 37.38 [3.63] |
| Success Rate\(^c\) (Number) [%] | 31 [79.5] | 30 [83.3] | 28 [71.8] | 25 [64.1] | 24 [61.5] |
| \(^*\)P value | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |

|               |            |            |            |            |            |
| LMW-HA        |            |            |            |            |            |
| Before        | 9.28 (0.26)| 2.65 (0.19)| 29.88 (1.24)| 41.33 (1.65V) | 5.53 (0.15) |
| 2 months      | 4.83 (0.23)| 1.05 (0.16)| 18.40 (1.01)| 25.00 (1.33) | 3.50 (0.17) |
| 6 months      | 5.30 (0.24)| 1.00 (0.14)| 19.00 (1.05)| 26.55 (1.43) | 3.75 (0.19) |
| Mean Difference\(^a\) (SD) | -3.97 (0.25) | -1.65 (0.14) | -10.87 (1.07) | -14.77 (1.05) | -1.77 (0.19) |
| Change\(^b\) (%) from baseline [SD] | 42.56 [2.26] | 63.20 [4.72] | 36.55 [2.52] | 35.62 [2.33] | 32.62 [2.98] |
| Success Rate\(^c\) (Number) [%] | 34 [85] | 34 [87.2] | 27 [67.5] | 29 [72.5] | 21 [52.5] |
| \(^{**}\)P value | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |

\(^*\)P values refer to changes over time within each group, based on the Repeated Measures; \(^{**}\)p value between groups, based on the Repeated Measures; \(^a\) (6\(^{th}\) month-Baseline); \(^b\) ([6\(^{th}\) month-Basline]/Baseline) *100; \(^c\) for each participant 30% change was considered as the success.

Table 5
|                          | HMW-HA     | LMW-HA     | p-value |
|--------------------------|------------|------------|---------|
| Post injection pain      | 2.64 (2.265) | 1.90 (1.392) | 0.286   |
| Mean (SD)                |            |            |         |
| Stiffness and heaviness  | 4 (10.26%) | 3 (7.5%)   | 0.712   |
| Number (Frequency %)     |            |            |         |
| Swelling                 | 4 (10.26%) | 2 (5%)     | 0.432   |
| Number (Frequency %)     |            |            |         |
| Total                    | 8 (20.51%) | 5 (12.5%)  | 0.378   |
| Number (Frequency %)     |            |            |         |

**Figures**

**Figure 1**

Flowchart of study population.

**Supplementary Files**
This is a list of supplementary files associated with the primary manuscript. Click to download.

CONSORT 2010 Checklist.doc