

Efficacy of Intra-Articular Polynucleotides Associated With Hyaluronic Acid Versus Hyaluronic Acid Alone in the Treatment of Knee Osteoarthritis: A Randomized, Double-Blind, Controlled Clinical Trial

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Abstract

Objective: Pain and range of motion loss are the main clinical features of osteoarthritis (OA). Hyaluronic acid (HA) is one of the infiltrative therapies for OA treatment; however, its effectiveness is a matter of an ongoing debate in clinical practice. Polynucleotides (PNs), a DNA-derived macromolecule with natural origin and trophic activity, were found to favor cell growth and collagen production, in preclinical and clinical studies regarding cartilage regeneration. This study aimed at evaluating whether injection of PNs, in combination with HA [PNs associated with HA (PNHA)], can ameliorate pain and function of knees affected by OA, more than HA alone. **Design:** A randomized, double-blind, controlled clinical trial. **Patients:** The study enrolled 100 patients, then randomized to receive PNHA or HA alone (3 weekly knee I.A. injections). **Interventions and Main Outcome Measures:** Pain reduction, decrease of proinflammatory synovial fluid (SF) factors, and improvement in knee function were evaluated by Knee Society Score and WOMAC scores, after 2, 6, and 12 months and by biochemical and immunoenzymatic analyses of SF at the end of the treatment. **Results:** Knee Society Score total score and pain item significantly ameliorated in both groups, showing better results in PNHA- than in the HA-treated group. A significant reduction in the WOMAC score was observed over time for both groups. No significant adverse events were reported in either group. **Conclusions:** These findings suggest that I.A. injection of PNs, in combination with HA, is more effective in improving knee function and pain, in a joint affected by OA, compared with HA alone.

Key Words: osteoarthritis, hyaluronic acid, polynucleotides, pain, knee function

(*Clin J Sport Med* 2020;30:1–7)

INTRODUCTION

Osteoarthritis (OA) is characterized by degeneration of the entire joint tissues and by an inflammatory microenvironment. It is the primary cause of disability^{1,2} in older adults with a decrement in lifestyle, daily life quality, and work hours. Clinically, it is characterized by joint pain and stiffness and loss of range of motion, with a prevalence that increases with age.³ Overall, 10% of patients older than 55 years has symptomatic radiographic knee.

Current therapeutic strategies consist mainly of changes in lifestyle, physiotherapy, and intake of analgesics, opioids, nonsteroidal anti-inflammatory drugs (NSAIDs), corticoids, and COX-2 inhibitors.⁴ Recently, the injection of platelet-rich plasma (PRP) into the knees affected by OA was also proven to be an efficacious treatment, as found in some of the

randomized controlled trials (RCTs).⁵ The superiority of PRP in comparison to saline, hyaluronic acid (HA), or corticosteroid treatments in knee OA was also demonstrated in some RCTs.⁶ However, given the complexity of the disease, patients with severe symptomatic OA and who do not respond to the above-mentioned therapies are usually treated with total joint replacement (TJR).⁷

To avoid surgery, clinicians are oriented toward the use of infiltrative therapies and, among them, injection of HA is the most used. Hyaluronic acid is a natural component of soft connective tissue with the ability to restore the viscoelastic properties of the synovial fluid (SF) and joint lubrication. It also has antiapoptotic, anti-inflammatory, antiangiogenic, and antifibrotic properties.⁸ Exogenous HA injection aims at restoring the amount of HA lost in OA (from 6×10^6 to $0.5\text{--}3 \times 10^6$ Dalton).^{9–12}

Although preclinical and clinical studies have shown the effectiveness of HA in treating OA,¹³ its efficacy is still being discussed in clinical practice.¹⁴

Recently, there has been more demand to develop bioactive substances with regenerative function, which would act on the whole altered intra-articular microenvironment and that could restore cartilage physiological conditions.

Polynucleotides (PNs) are a mixture of purines, pyrimidines, deoxyribonucleotides, and deoxyribonucleosides with trophic activity. They are not synthetically produced, but they

Submitted for publication July 12, 2017; accepted December 4, 2017.

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The authors report no conflicts of interest.

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<http://dx.doi.org/10.1097/JSM.0000000000000569>

TABLE 1. Demographic Data of the Enrolled Patients

	Overall (N = 98)	Study Group: PNHA (N = 49)	Control Group: HA (N = 49)
Age, yrs	50-75 (63.8 ± 5.8)	63.4 ± 6.5	64.2 ± 5.1
Kellen–Lawrence grade	2 ± 0.7	1.9 ± 0.6	2.1 ± 0.7
Sex, male/female, n	46/54	24/26	22/28
Body mass index, kg/m ²	28.1 ± 3.5	28.1 ± 3.4	28.1 ± 3.7
Weight, kg	80.0 ± 11.6	80.2 ± 10.2	79.8 ± 13
Height, cm	168.5 ± 9.2	168.9 ± 9.5	168.1 ± 9.0

are of natural origin, being derived from the trout sperm or human placenta. They link water and have viscoelastic property but also induce cell growth, collagen (COLL) production, migration of several cell types, and can reduce inflammation.^{15–17} In preclinical and clinical studies, PNs have shown positive results in musculoskeletal tissue regeneration.¹⁸ Regarding cartilage, preclinical and clinical studies have shown a reduction in proteoglycan degradation and in metalloproteinase activity in normal chondrocytes or in those harvested from patients affected by rheumatoid arthritis. A reduction in arthritis signs and proinflammatory cytokines production was also observed in mice affected by arthritis.^{15,19} In addition, PNs lead to a reduction in knee OA symptoms, with an effect comparable with HA, but also with an earlier response compared with HA, in patients affected by OA.^{20,21}

To date, no study evaluated the effects of the combination of PNs and HA [PNs associated with HA (PNHA)] in cartilage, and only 1 clinical study used PNHA as a topical treatment for venous lower limb ulcers, showing higher wound healing and re-epithelialization than with the topical use of HA.²² A synergic effect of PNs and HA has also been observed in fibroblasts.²³

The hypothesis of the present study was that the original association of PNs and HA injections would reduce pain in patients affected by knee OA, more than HA alone, after 12 months from the beginning of treatment. Second, the overall knee functionality and the production of proinflammatory factors in SF were also evaluated. For this purpose, a randomized, double-blind, controlled study was performed in patients affected by knee OA, making a comparison between PNHA and HA treatment.

METHODS

Patient Selection and Study Design

A randomized, double-blind, controlled study was conducted in 100 patients affected by knee OA. This study was performed according to the guidelines of the Declaration of Helsinki and the general principles of the “ICH Harmonised Tripartite Guidelines for Good Clinical Practice” (ICH Topic E6, CPMP/ICH/135/95, June 1996). The study was conducted according to local regulations. Before the enrollment of the first patient, the study protocol, the informed consent, and any other document were submitted to the analysis of the local Ethical Committee and the Health Authorities, whose approval was obtained before the start of the study.

From September 2014 to July 2015, 100 patients were enrolled. The inclusion and exclusion criteria are summarized in Table 1 and Table 2, respectively.

Inclusion criteria required patients without pre-existing infiltrative therapies or patients with a single previous infiltration cycle, performed at least 6 months before enrollment. In this way, the sample could be considered homogeneous and not susceptible to bias. We have not distinguished between patients with pre-existing therapies and patients without previous treatment. Therefore, we believe that there is no imbalance between the 2 groups.

There was no restriction on the use of NSAIDs during the study period and, at each visit, the physician recorded the consumption of anti-inflammatory drugs on the Case Report Form.

The sample size calculation was performed considering the percentage change of WOMAC at 12 months as the primary end point, according to the following formula:

$$\Delta \text{Womac } \% = \frac{\text{Womac } 12 - \text{Womac } T0}{100 - \text{Womac } T0} \times 100.$$

The literature shows that the SD is 26.9% for patients treated with the treatment object of study and 39.1% for patients treated with HA. The assumption is that the population to be enrolled has an SD similar to that found in the literature and that the 2 treatments differ by at least 20% (minimal clinically significant difference) against a null hypothesis that the 2 techniques have an analogous Womac percentage variation. Assuming an alpha error of 0.05 and a power of at least 0.8 with a minimum clinically meaningful difference of 20%, and a drop-out of 10%, the minimum number of cases to be studied per group is 50, for a total of 100 cases.⁶

Patients were randomized into 2 groups: (1) the study group, treated with intra-articular injection of PNHA and (2) the control group, treated with intra-articular injection of HA.

The study was double blinded both for the staff, involved in the treatment, and the patients. A randomization method that gave the same chances to every patient to be assigned to 1 of the 2 treatment modalities was used: the numbering, shown on

TABLE 2. Exclusion Criteria

Abuse of alcohol or drugs
Pregnancy or breastfeeding
Patients with pre-existing infiltrative therapies or patients with a single previous HA infiltration cycle, performed less than 6 mo before enrollment
Systemic anticoagulants and steroids ongoing or suspended for less than 1 mo
Hypersensitivity to the therapeutic products, previous bone fractures, knee severe trauma, joint deformities, rheumatoid arthritis, articular inflammatory diseases, and previous surgery (such as meniscectomy and scope debridement)
Hematological diseases or local skin lesions in the site of treatment inoculation

the boxes containing the treatments corresponded to the randomization list, and the investigator received the randomization code in a sealed envelope for each patient. To create the randomization list, the Simple Interactive Statistical Analysis website was used. This uses a linear congruential generator, an algorithm for the generation of pseudo-random numbers based on Lehner formula (1948): $[\text{rnd} (i + 1) = (\text{rnd} (i) \times b + a) \text{ mod } \text{max}]$. In particular, the method used was MINSTD 31 bit.

Polynucleotides associated with HA and HA syringes were packaged in an identical manner and provided in separate boxes for each individual patient. There were identical sleeves placed over the syringes to blind both injector and patient. In addition, data collectors and outcome assessors were also blinded to the type of treatment administered.

Treatments

Polynucleotides associated with HA (Condrotide Plus; Mastelli srl, San Remo, Italy) is an innovative Class III, CE marked, medical device for the intra-articular treatment of degenerative chondral pathologies. This product is registered in the whole of Europe as a Class III medical device and the Notified Body is the Italian National Health Institute [Istituto Superiore di Sanitàh Inst]. Outside Europe, the regulatory status depends on each National Health Institute, but many countries accept the European regulatory documentation as a medical device.

This product is a gel composed of PNs (10 mg/mL) of controlled natural origin (fish sperm) and 10 mg/mL of an HA of biotechnological origin, with a total content of active ingredients of 40 mg in 2 mL.

Ialart (Mastelli srl, San Remo, Italy) is the low-molecular-weight HA (0.8×10^6 - 1.3×10^6 Daltons), a Class III, CE marked, medical device used as the control. The syringe contains a gel with a concentration of 20 mg/mL (40 mg/2 mL) and derived from bacterial fermentation.

An amount of 2 mL of PNHA or HA was intra-articularly injected by an 18 to 22 G needle, every week for a total of 3 infiltrations (T0, T1, and T2). The injections were performed by highly skilled medical personnel, under aseptic conditions, and following the standard technical rules for intra-articular administration. Before the first infiltration (T0) and at the end of the treatment (T2), the excess of SF was removed and an aliquot (nearly 6 mL) was sent to the laboratory for SF analyses. The collection of SF was performed under sterile conditions (Figure 1).

Follow-Up

At T0 and after 2 (T3), 6 (T4), and 12 (T5) months from the beginning of the treatments, the evaluations of clinical

function and pain were performed with the WOMAC score²⁴ and Knee Society Score (KSS score).²⁵ At T0 and T2, biochemical and immunoenzymatic analyses of SF were performed. More precisely, fresh samples of SF were collected and immediately evaluated (Guideline on SF analysis RIMeL/IJLaM 2008; 4). Synovial fluid was aspirated only if present before the injection, without using joint lavage and only in a small group of patients (Figure 1).

Briefly, for the biochemical assays, after color and clarity observation, the mucin clot assay was performed to assess viscosity by adding 300 μ L of SF to 3 mL of 5% acetic acid and observing the formation of a clot. Cell count was performed by counting white cells (1:20 in Turk solution) using a Burkner chamber. Samples were then centrifuged at 2000g for 10 minutes to remove cells and debris, and the aliquots were stored at -80°C for further immunoenzymatic measure (ELISA kits) of the following parameters: matrix metalloproteinase-1 (MMP1) (Boster, Pleasanton, California), MMP13 (Cloud-Clone Corp, Texas), tissue inhibitor of MMP1 (TIMP1) (Boster), proinflammatory cytokines interleukin 1 β (IL1 β) (Boster), IL6 (Boster), tumor necrosis factor- α (TNF- α) (Boster), chemokine (IL-8) (Boster), and prostaglandin E₂ (PGE₂) (Arbor Assays, Ann Arbor, Michigan).

Statistical Analysis

Statistical analysis of clinical data was performed using IBM SPSS Statistics 21 software. Data were expressed in terms of mean with ranges or as boxplots. The analyses on the primary end points (WOMAC, KSSpain, KSStot) were only 3. We used the general linear model for repeated measures (GLM repeated measures) corrected for age, body mass index (BMI), and Kellen-Lawrence (KL) grade to assess the influence of treatments on the follow-up curve.

Only if the follow-up curves were significantly influenced by treatment (GLM repeated measures $P < 0.05$), the Sidak test was also used as a post hoc pairwise analysis to find out when the curve started to diverge.

For SF analyses, statistical evaluation of the data was performed with the use of the software package SPSS/PC + Statistics TM 23 (SPSS Inc, Chicago, Illinois). Data were the result of 3 replicates, and they are reported as mean $\pm$ SD at a significance level of $P < 0.05$. The normal distribution of data and the homogeneity of variance were verified. A paired samples Student t test was applied for comparison between experimental times within groups and Student t test between groups.

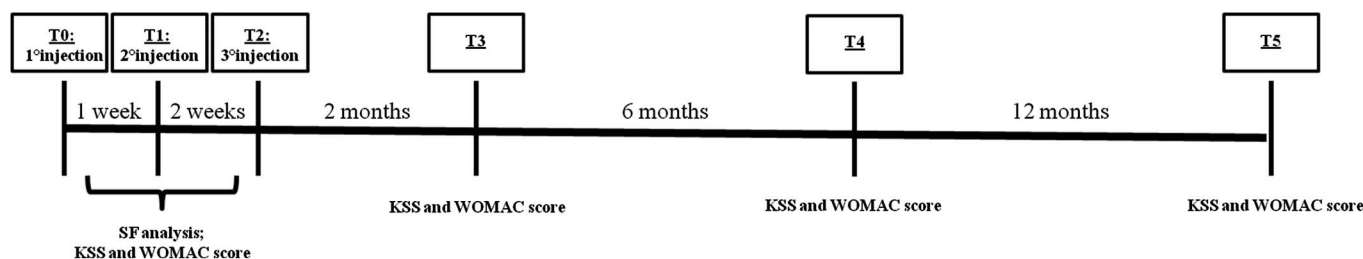


Figure 1. Experimental design: schedule of the 3 injections and follow-up.

Finally, correlations between the results of SF markers, both among them and between them, and KSS or WOMAC scores at T0 and at the end of treatment (T2 for SF and T3 for KSS and WOMAC scores) were also investigated by the Pearson test.

RESULTS

As observed in Figure 2, 98 of 100 screened patients were enrolled in the study, 2 patients were excluded because they did not meet the inclusion criteria. Forty-nine patients were randomly assigned to the study group and the other 49 to the control group. A total of 90/98 patients completed the study at T5 (46 in the study group and 44 in the control group): 3 patients dropped out from the study group and 5 from the control group for personal reasons.

The 2 groups were homogeneous for age ($p = 0.54$), Kellgren–Lawrence grade ($p = 0.13$), sex ($p = 0.84$), BMI ($p = 1$), weight ($p = 0.86$), and height ($p = 0.67$) (Table 1). No complication related to the infiltrations was observed for both treatments and for the entire duration of the follow-up. The biochemical evaluation showed that SFs of all the patients were normal for color, clarity, and density (mucin clot test). The color was yellow or, more frequently, light yellow, and all samples were transparent or translucent. All samples showed a well-defined clot. In few cases, total white cell count was higher than physiological values (<200 cells/mm³), but within the range for noninflammatory state (<2000 cells/mm³).

Knee Society Score and WOMAC Scores

The KSS score was assessed in its entirety, but particular attention was paid to the “pain” item.

The KSS total score showed significantly better results in the study group compared with controls at each follow-up time ($*p < 0.05$; at T3, T4, and T5 $p = 0.009$) (Figure 3A).

Regarding the KSS “pain” item, results were significantly better in the group treated with PNHA than in the group treated with HA at T3 and at T5 ($*p < 0.05$) (Figure 3B). At T4, the 2 groups did not statistically differ ($p = 0.08$), even if the study group showed better results. Both groups significantly improved the KSS total score over time, and there was a reduction in KSS “pain” item between T0 and the other experimental times (T3, T4, and T5) ($^{***}p < 0.0005$). Only the

study group showed a significantly lower pain at T5 in comparison to T3 in the KSS “pain” item ($^{\circ}p < 0.05$).

No significant differences were observed for the WOMAC score between groups during the follow-up (Data not shown). Regarding the “Pain” item of the WOMAC score, no significant differences were observed between the 2 groups at each follow-up times, even if a better trend was observed in the study group in comparison to the control one (at T3, $p = 0.46$, at T4, $p = 0.14$ and at T5, $p = 0.46$). However, a significant decrease was observed between T0–T3, T0–T4 ($^{***}p < 0.0005$), and T0–T5 ($^{***}p < 0.0005$) in the study group. In the control group, a significant reduction of pain was observed between T0–T3 and T0–T4 ($^{\circ}p < 0.05$), whereas no significant differences were observed between T0 and T5 ($p = 0.657$) (Figure 4).

DISCUSSION

Pain is the main clinical drawback of a joint affected by OA, and TJR is sometimes inevitable because pain is usually not resolved with current treatments.^{26,27}

The results of the present randomized, controlled, double-blind clinical study have demonstrated a reduction in pain in patients affected by knee OA 12 months after the first injection of HA, and even more after the combined treatment with PNHA. The same positive results were also observed in terms of physical improvement.

Because surgery has some disadvantages, especially in older subjects due to the presence of associated comorbidities, the aim of current pharmacological OA treatments is to reduce pain and improve function of the joints.²⁸

It is already known that viscosupplementation of SF with intra-articular injection of HA is able to favor the native rheological and protective characteristics of SF, ameliorating pain symptoms and joint function.^{29,30}

Clinical trials, in patients affected by knee OA showed good tolerability and efficacy in reducing joint pain with PN injection.^{31–33} Two studies, with 3 or 5 injections of PNs or HA, observed an equal reduction in pain and an improvement in knee function, after 6 and 4 months,^{20,21} with an earlier effect on physical parameters with PNs.²⁰ One clinical study observed an improvement in function and pain in patients affected by OA or by chondropathy of grade III to IV, 2 months after an injection of PNs.³⁴

Based on these promising results, the present clinical study combined the use of PNs and HA in the same formulation (PNHA) for the treatment of patients affected by knee OA, and this combination represents the novelty of the study. It had already been observed that PNHA induced better results than PNs and HA alone in human fibroblasts’ growth and extracellular matrix (ECM) production.²³ In this way, the trophic effect due to PNs is combined with the viscoelastic property of HA, a useful feature for intra-articular treatments.

In the present clinical study, although HA showed a significant effect in knee function, PNHA improved clinical aspects more than HA, up to 12 months. Similarly, both groups showed a significant improvement in pain over time: PNHA reduced pain at all experimental times (after 2, 4, and 12 months), whereas HA only after 2 and 4 months and not after 12 months. Some commercially available HA-based products, Food and Drug Administration (FDA) approved and currently used in OA treatment, are characterized by low-molecular weight (from 0.5 to 3×10^6 Da).¹¹ Their characteristics are similar to HA used in the present clinical study. However, the statistically inferior

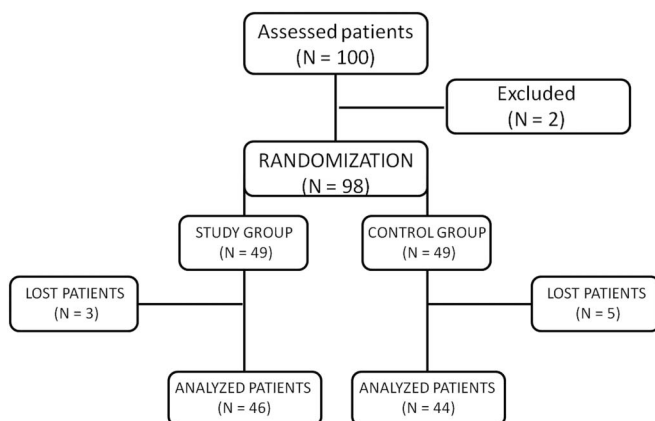


Figure 2. Study flowchart.

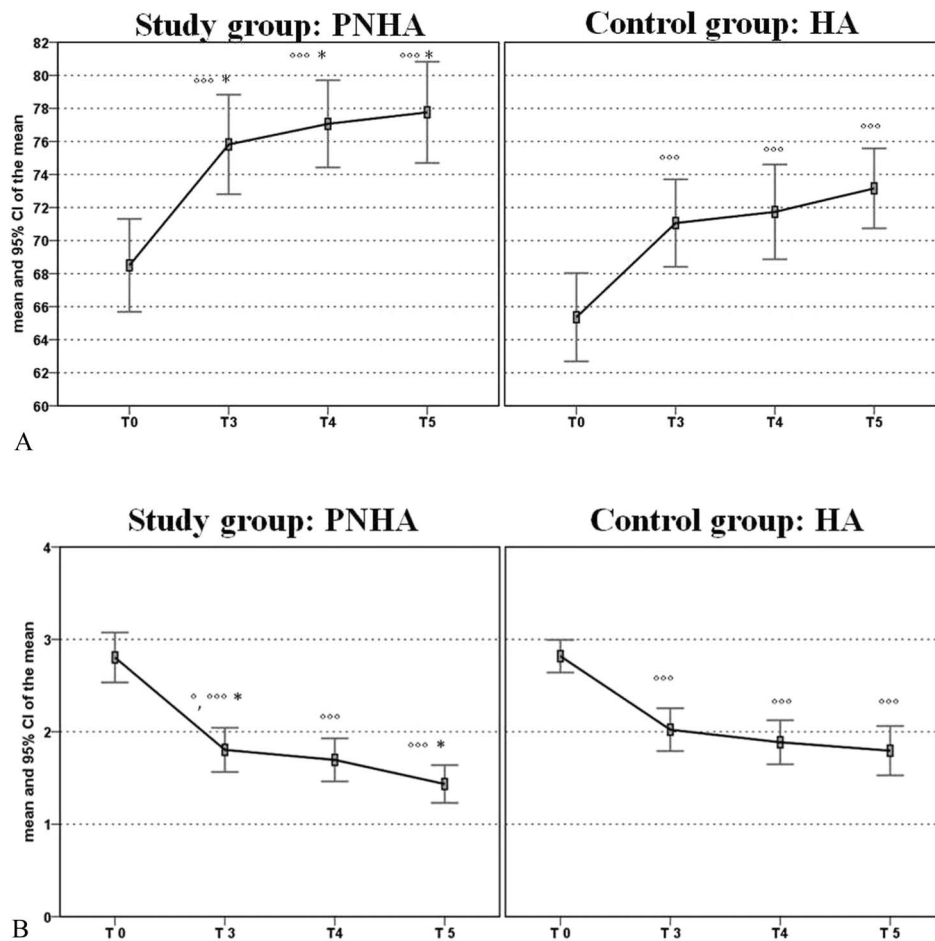


Figure 3. Knee Society Score. A, KSS total score; (B) KSS pain item. * $p < 0.05$ between experimental groups; $^{\circ\circ\circ}p < 0.0005$; $^{\circ}p < 0.05$ between experimental times.

results obtained with HA compared with PNHA are probably due to the use of low-molecular-weight HA, which can be rapidly degraded. Indeed, HA with low-molecular weight has a structure similar to natural molecules, but requires a greater number of infiltrations to achieve a reasonable effectiveness due to its low elastoviscosity.³⁵ Inflammation is often related to joint pain and, in turn, induces further degeneration of the joint

structures. The association between pain and SF inflammation is yet to be confirmed by several studies and, for this reason, the evaluation of inflammatory biomarkers in SF of joints affected by OA is important.^{36,37} Several different proinflammatory factors are identified in the SF of knees affected by OA and, among them, IL1- β , TNF- α , and IL-6 are the most associated with OA severity and are mostly produced

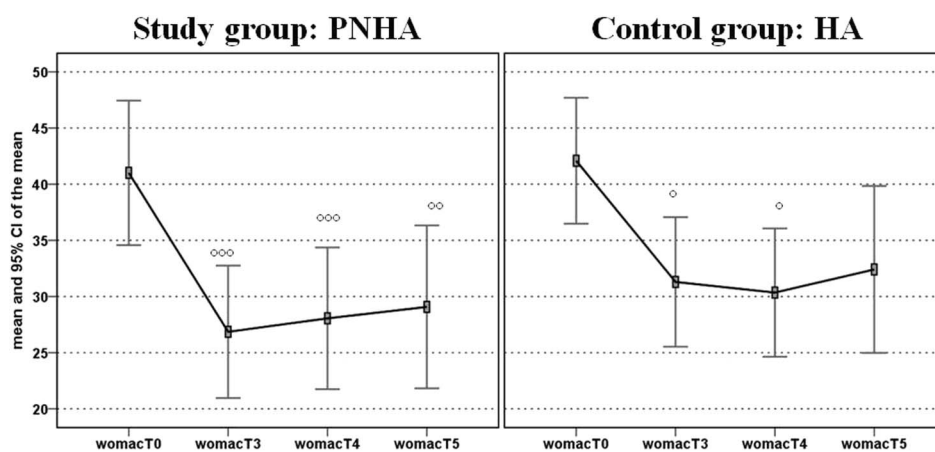


Figure 4. "Pain" item of the WOMAC score. $^{\circ\circ\circ}p < 0.0005$; $^{\circ\circ}p < 0.005$; $^{\circ}p < 0.05$ between experimental times.

in the earlier phases of OA.^{36,37} IL1- β , together with TNF- α , upregulates other proinflammatory cytokines and chemokines, such as IL-6, thus amplifying inflammation.³⁸ The proinflammatory IL-8 induces the production of MMP13, chondrocyte hypertrophy, and leukocyte activation in the SF of patients with OA. Even if its relationship with OA severity is still unclear, it is increased in SF of patients affected by OA.³⁹ MMP13 is responsible for the COLL II degradation, inducing heavy damages of the cartilage structure and reducing cartilage ability to respond to mechanical loads.⁴⁰ Metalloproteinase activity is inhibited by TIMPs and, among them, TIMP-1 reduces several MMPs including MMP1 and MMP13. Therefore, the balance between MMPs and TIMPs (ie, ratio between MMPs and TIMPs) is important to be evaluated in OA.⁴¹ Finally, PGE₂ is responsible for cartilage and bone alterations observed in OA and reduces ECM cartilage synthesis.⁴⁰

In the present study, the aforementioned SF markers were evaluated but, because SF was not available for all patients at both T0 and T2 experimental times, the statistical comparison did not yield significant results. This is also due to the wide SD found in all markers. Moreover, because of the restrictive exclusion criteria (such as hypersensitivity to the therapeutic products, previous bone fractures, severe knee trauma, joint deformities, rheumatoid arthritis, articular inflammatory diseases, and previous surgery, such as meniscectomy and scope debridement), only a small part of patients could be analyzed for SF markers. However, the calculated percentage of difference between final and basal values for each patient is an index of SF factors changes between the end and the beginning of treatment. By observing this percentage, it can be concluded that, up to 2 months, PNHA treatment produced a slight reduction of MMP1, MMP13, IL-6, TNF- α , and PGE₂ in SF, whereas treatment with HA induced a reduction of only IL-6, IL-8, and PGE₂ (data not shown).

For both treatments, an inverse correlation between total KSS score and IL6 was found. The reduction trend, observed in MMP1 and MMP13 after PNHA treatment between T0 and T2, was not clinically relevant because no correlation was found between these markers and the clinical parameters analyzed in this study. Probably, 12 months after the treatment and with a larger number of SF samples, clinically relevant differences would be detectable, but this is an interesting aspect to be considered in future works. Two months of treatment is not a sufficient period to observe a clinically relevant MMP1 and MMP13 reduction.

In addition, the maximum quantity of SF aspirated from these patients was 5 mL, so we can conclude that there was not enough effusion to compromise the efficacy of the injected treatments.

To conclude, this is the first clinical study that used a combination of PNs and HA in a single formulation for the treatment of knee OA, through clinical evaluations of pain, function, and SF biomarkers during the 1-year follow-up. This study has underlined the superiority of PN and HA combined treatment in comparison to HA alone, the most widely used treatment for a joint affected by OA, in reducing pain and improving overall knee function as measured by the KSS score.

ACKNOWLEDGMENT

The authors thank Mrs. Elettra Pignotti for her help in the interpretation of data and statistical support.

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