applied sciences

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Rehabilitation of Patients with Moderate Knee Osteoarthritis Using Hyaluronic Acid Viscosupplementation and Physiotherapy

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Abstract: Knee osteoarthritis (KOA) is one of the most common public health problems which cannot be cured and ultimately leads to disability. Current management is largely limited to the treatment of the symptoms. To avoid the late stages of KOA that lead to knee replacement, the key point is to control and reduce destructive processes using efficient pharmacological products combined with physiotherapy (PT). Herein, we perform a monocentric observational study to compare the effect of combining a multi-modal physiotherapy regime and intra-articular (IA) injection with hyaluronic acid (HA) on the non-surgical treatment of KOA. Patients with mild KOA were randomly assigned to two groups to receive an HA injection with PT or an HA injection only. The assessment tools for pain, clinical disease severity, and disability were the total score on the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) (scores range from 0 to 96, with higher scores indicating worse pain, function, and stiffness), knee range of motion (ROM), pain on the visual analog scale (VAS), and muscle strength testing (MST). All tests were evaluated every 3 months up to 1 year from the baseline. The study enrolled 52 patients with ages between 47 and 61 years who were divided into two groups. Thirty-seven (n = 37) patients were randomized in the pilot group (PG) and received IA injections with the viscoelastic hyaluronic acid product (HA) combined with a multi-modal PT regime. The PT program included 10 sessions of transcutaneous electrical nerve stimulation, low-level laser therapy, ultrasound, physical exercise, and cryotherapy. Fifteen patients (n = 15) from the control group (CG) received the IA HA injections only. All patients were confirmed with mild KOA of Kellgren–Lawrence grade 2 on radiographs at the beginning of the treatment. The baseline characteristics, including the severity of pain and level of disability, were similar in the two groups. At baseline, the mean (±SD) WOMAC scores reported were 64.6 ± 4.08 in the CG and 64.5 ± 2.99 in the PG. Notably, at only 3 months into the study, the mean scores were significantly improved to 56.7 ± 5 in the CG and 48.27 ± 2.13 in the PG (mean between-group difference = 16.19 points; 95% confidence interval), finding favor for the combination of HA injections and physiotherapy. At the study's endpoint (12 months), the scores were improved in both groups, with the mean between-group difference remaining significant (7.08 points, 95% confidence interval). A decrease in pain, as evaluated by the VAS scale, was reported for both groups, with the PG reporting a better VAS score that decreased from 5.7 to 2 when compared to the CG, which decreased from 5.7 to 3. The

Citation: Onu, I.; Matei, D.; Sardaru, D.-P.; Cascaval, D.; Onu, A.; Gherghel, R.; Serban, I.L.; Mocanu, G.D.; Iordan, D.A.; Murariu, G.; et al. Rehabilitation of Patients with Moderate Knee Osteoarthritis Using Hyaluronic Acid Viscosupplementation and Physiotherapy. *Appl. Sci.* **2022**, *12*, 3165. https://doi.org/10.3390/app12063165

Academic Editors: Stefano Masiero and Daniele Coraci

Received: 2 February 2022
Accepted: 16 March 2022
Published: 20 March 2022

physical assessment parameters (ROM and MST) followed the same trend, with a rapid improvement in the ROM in the PG, changing from 98° to 115° in the first 3 months, and a slower and more steady evolution in the CG group, changing from 100° to 112° in 9 months. Herein, we report on the combination therapy of an intra-articularly administered HA viscoelastic product and a multi-modal physiotherapy regime, which can play a key role in the non-surgical treatment of KOA, effectively controlling pain, stiffness, and the ROM value and improving patients' quality of life.

Keywords: knee osteoarthritis; hyaluronic acid; viscoelastic infiltration; physiotherapy; combination therapy

1. Introduction

Osteoarthritis (OA) is one of the most important public health problems and the most common joint disorder in the elderly. It is well recognized that OA leads to decreased quality of life due to pain and stiffness, including limited activity in daily life and work with a decreasing capacity of the affected individual for integration in the family and society, which often results in depression and anxiety [1]. Moreover, OA poses a huge financial burden on the healthcare systems around the globe, and although not fatal, it significantly reduces the quality of life of tens of millions globally.

OA is an inflammatory disease characterized by chronic and progressive cartilage degeneration, osteophyte formation, subchondral sclerosis, margin hypertrophy of the bone, and changes in the synovial membrane. There are several risk factors linked to the onset of OA, such as age, body weight, and metabolic and genetic factors. The occurrence and rate of OA across studies vary greatly, depending on the used definition, the population tested (primary versus tertiary care), and the dispersal of OA risk factors, such as age, sex, obesity, and geographical region [2]. Aging, inflammation, and oxidative stress appear to be major contributing factors to the development and progression of OA. The progressive loss and breakdown of articular cartilage induced by inflammation play an essential role in the pathogenesis of OA [3]. The main manifestation of OA is synovial inflammation, with loss of articular cartilage and degenerative changes in other tissues such as the synovium, menisci, ligaments, and subchondral bone.

Knee osteoarthritis (KOA) is a disease that eventually becomes disabling, and in the last stage, the solution is total knee arthroplasty. A multitude of factors leads to KOA's progression that are associated with the destruction of articular cartilage. Along with these cartilage changes, a reduction in the elastic and viscous properties of the synovial fluid occurs. The molecular weight and concentration of the naturally occurring hyaluronic acid decrease.

Both early diagnosis and conservative treatment methods are critical in the subsequent evolution of the disease. The treatment of patients depends on the clinical diagnosis associated with the imaging diagnosis based on radiographic images.

Hyaluronic acid (HA), also known as hyaluronan or hyaluronate, is widely distributed in many tissues and fluids in the human body but more abundantly in the articular cartilage and synovial fluid (SF) [4]. It is well established that in KOA, the viscoelastic properties of the SF are compromised, as both the concentration and the molecular weight decrease with time and aging. [5,6]. HA has many biological properties, such as articular cartilage lubrication and antioxidative or anti-nitrosative, analgesic, anti-inflammatory, chondroprotective, ECM degradation prevention, and cartilage repair effects, and is actively synthesized by B synoviocytes, fibroblasts, and chondrocytes. HA can enhance the synthesis of chondroitin sulphate and proteoglycans and reduce the production and activity of metalloproteinases (MMPs) [7]. HA was found to counteract the effect of IL-1 by inhibiting the IL-1-induced downregulation of type II collagen mRNA expression [8]. HA may be involved in various cellular interactions (cell differentiation, proliferation, development, and recognition) and

physiological functions [9]. A comprehensive review of the molecular mechanisms and therapeutic applications can be found elsewhere [10].

From the literature, it is known that the pathological changes of SF HA, with its decreased molecular weight and concentration, led to the concept of viscosupplementation (VS). VS is a safe, effective, and well-established treatment for OA that involves injecting a solution based on HA into the affected synovial joint. Optimal treatment with VS should delay the degeneration of the cartilage and even help to regenerate the structure of the articular cartilage [11]. VS with HA in KOA restores the properties of healthy SF, activates the physiological production of HA by synovial membrane cells, has a painkiller and anti-inflammatory effect, and inhibits cartilage degradation enzymes such as metalloproteinases (MMPs) [12–14]. Hsieh et al. measured the levels of MMP-2, MMP-9, urokinase-type plasminogen activator (u-PA), and plasminogen activator inhibitor (PAI-1) in a series of chondral, meniscal, and synovial cultures of early OA after treatment with or without three HA products with different molecular weights (MWs) and demonstrated that all of the HA products could decrease the secretion of MMP-2 and MMP-9. They suggested that HA with a high MW might be more effective in inhibiting MMP-2, MMP-9, u-PA, and PAI-1 expression [15]. HA can bind to receptors such as CD44 (the most widely distributed cell surface receptor recognized for HA binding), the receptor for hyaluronate-mediated motility (RHAMM), and Intercellular Adhesion Molecule 1 (ICAM-1) [16].

Moreover, VS could restore the rheological properties of the SF and promote the endogenous synthesis of a higher MW and possibly more functional HA, thereby improving mobility and articular function and decreasing pain [17]. The intra-articular (IA) administration of HA is reported to be more effective than oral administration because it avoids systemic exposure and potential adverse side effects.

HA, being a physiological component, is not expected to produce adverse reactions even after repeated administration [18]. In clinical trials, IA administration of HA is safe and well-tolerated in OA patients [18,19], with only minor side effects that might occur such as pain at the injection site, local joint pain, swelling, and local skin reactions.

However, HA treatment is contraindicated in individuals who are hypersensitive to HA products, women who are pregnant or nursing, pediatric patients, patients with bacteremia, or patients with infections in or around the target knee [20].

Physical therapy (PT) in KOA rehabilitation is an essential part of healthcare that provides services for the development, maintenance, and restoration of movement capacity and functional ability to the maximum possible level throughout life. PT is a form of musculoskeletal disorder treatment with the help of physical agents.

Transcutaneous electrical nerve stimulation (TENS) is a method of pain therapy used worldwide by physiotherapists and medical practitioners. TENS reduces pain in KOA by selectively stimulating the large-diameter, low-threshold, non-noxious afferents in dermatomes and increasing the excitability of the quadriceps motor neuron [21]. It has been shown that TENS activates the native opioid receptors targeting δ and μ receptors with high- and low-frequency current pulses [22]. The Philadelphia panel published a randomized control trial (RCT), and they concluded that TENS currents provided significant pain relief in KOA patients compared with a placebo [23]. The results found in the meta-analysis studies showed that TENS currents have a significant effect on the reduction of pro-inflammatory cytokines, especially IL-6 [24].

Low-level laser therapy (LLLT) is a form of non-invasive PT that applies low-power lasers or light-emitting diodes (LEDs) to the surface of the body. LLLT is used by physiotherapists to treat various musculoskeletal conditions, including KOA. LLLT has an analgesic and pain-relieving effect as well as a bio modulatory effect on microcirculation. LLLT has strong anti-inflammatory and analgesic effects, including tissue healing and improving lymphedema [25,26]. In a 2009 study, Béla Hegedűs et al. showed that LLLT improved knee flexion and decreased knee pain in KOA-affected joints over a 2-month period after the therapy session, using the WOMAC questionnaire or the Lequesne index to evaluate the output. The study was performed on 27 patients, and treatment was performed with

an LLLT twice a week over a period of 4 weeks with a GaAlAs laser diode at 50 mW power and at continuous wave with a 830-nm wavelength [27].

Ultrasound (US) therapy is a non-invasive and safe form of PT used for musculoskeletal conditions, including KOA. US not only relieves symptoms but also can provide potential cartilage reparation effects [28]. Multiple studies have shown that US promotes collagen formation, regulates inflammatory responses, and induces cartilage repair [29,30]. In a meta-analysis published by Zhang C et al. in 2016 comprising RCTs from 1991 to 2014, they demonstrated that US is a safe and valid type of PT in relieving OA-related pain and improving joints' ROM [31]. In PT, US is most often used in continuous mode and rarely in pulsed mode with a low duty cycle. Priscila Daniele de Oliveira Perrucini et al. showed that low-intensity pulsed US had a bio-stimulating effect on fibroblast cells in vitro. They proved that US with a duty-cycle of 10% and 0.2 W/cm^2 intensity presented superior bio-stimulation response in contrast to 0.5 W/cm^2 intensity and 20% duty-cycle. After 48 h from US therapy, the treatment affected the IL-6 cytokine production and genetic modulation, confirming its therapeutic properties related to the initial phases of tissue healing [32].

Cryotherapy is a non-pharmacological intervention that has been widely used in controlling inflammation, edema, and pain management [33]. Cryotherapy is a technique considered to be safe, inexpensive, and easy to administer for physiotherapists and patients. Cryotherapy on an animal model with induced KOA reduces synovial inflammation due to lower leukocyte migration and inflammatory cytokine concentration at the knee joint cavity [34]. Controlling pro-inflammatory cytokines is a key factor in the treatment of KOA and has been considered a pharmacological therapeutic approach. However, anti-cytokine drugs exhibit potential iatrogenic effects, and cryotherapy controls the inflammation and improves the clinical condition without side effects. Cryotherapy is rarely prescribed as an adjuvant treatment for KOA, and unfortunately, there are not enough studies to show the long-term benefits of this therapy [35]. In this study, we introduced cryotherapy at the end of the PT program to reduce the joint temperature in order to protect the application of HA-biopolymer.

Physical therapy exercises (PTEs) are used to limit the loss of joint function caused by KOA. PTE is used to manage the symptoms of KOA and optimize the quality of life. Regular exercise prevents joint degradation and maintains joint mobility and muscle tone, thus increasing joint stability and coordination in patients with KOA. Most clinical guidelines recommend PTE, patient education, and weight loss [36,37].

VS with HA products and PT are the most common means used worldwide to protect articular cartilage in the synovial joints, control the pain, and stimulate joint tissues. Despite the large body of literature in the field, currently, there are no standardized PT protocols that use combinations of physical agents and pharmacological agents to control the progression of KOA.

In this study, we sought to evaluate a treatment regime that would limit the progression of KOA by combining HA VS with a multi-modal PT approach that includes transcutaneous TENS, LLLT, cryotherapy, US, and PTE.

2. Materials and Methods

2.1. Trial Procedure

A monocentric observational study was performed from January 2020 to July 2021 in the orthopedics and physiotherapy departments of the Piatra Neamț Micromedica Clinic. The study was approved by the Ethical Committee for Scientific Research of the Micromedica Clinic in Piatra Neamț and was carried out in accordance with the Helsinki Declaration of Ethical Principles. All patients included in the study signed their informed consent. The study was performed on a group of 52 patients diagnosed with KOA in stage 2 of the "Kellgren and Lawrence system" (KL) based on radiological examination of the anterior-posterior view of an X-ray of the knee. KL classification is commonly used in epidemiological research studies of KOA to guide health professionals in their clinical

decision making, especially for managing patients with surgical indications [38]. The KL system uses five classes: KL 0 (normal), KL 1 (narrowing of the joint space), KL 2 (osteophytes and narrowing of the joint space), KL 3 (multiple osteophytes, well-defined joint narrowing, sclerosis, and possible bone deformity), and KL 4 (large osteophytes, marked joint narrowing, severe sclerosis, and defined bone deformity). The study report follows the Consolidated Standards of Reporting Trials (CONSORT) model using the CONSORT checklist (Figure 1) [39].

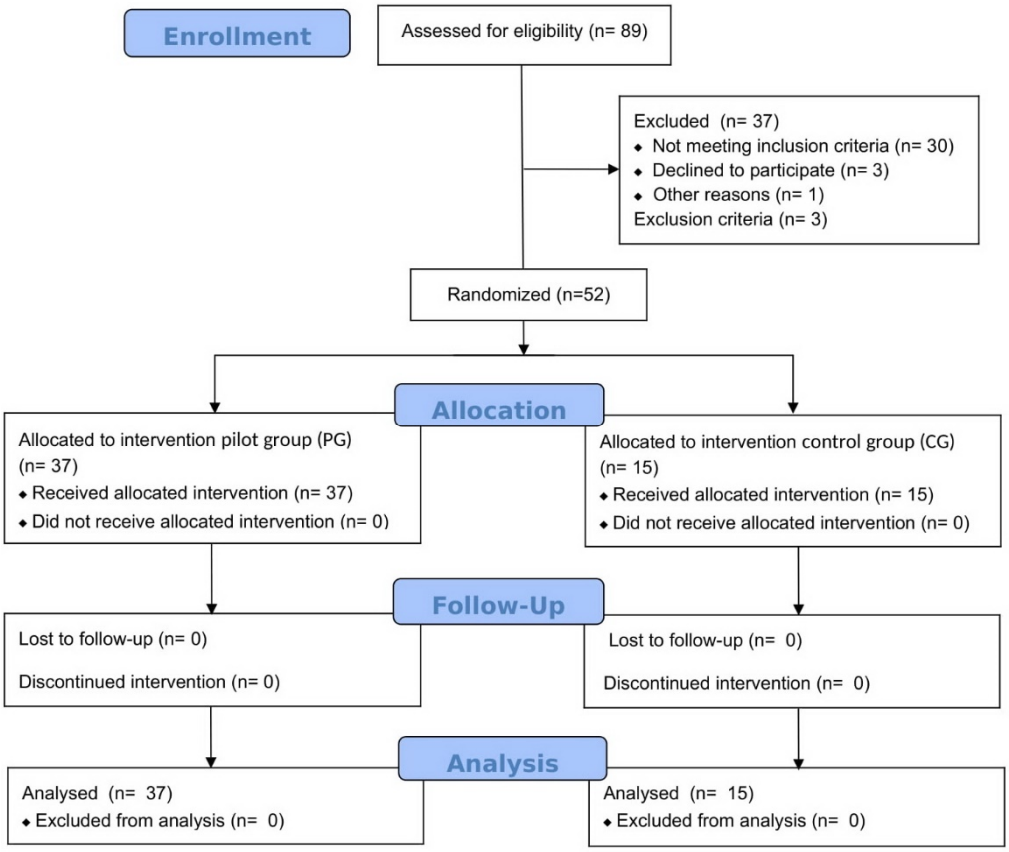


Figure 1. The CONSORT flow diagram of the progress through the phases of a randomized trial of two groups (enrolment, intervention, follow-up, and data analysis).

2.2. Patients and Demographics

The pilot group (PG) consisted of 37 patients who benefited from intra-articular (IA) VS with a Kombihylan® viscoelastic HA product and 10 consecutive sessions of PT. The control group (CG) consisted of 15 patients treated with Kombihylan® without PT sessions. The patients had an equal distribution by gender; 26 were men and 26 were women aged 47–61 years, weighing between 65 and 110 kg and with heights between 154 and 186 cm.

The PG included 16 women and 21 men, with 7 having bilateral KOA, 13 having left KOA, and 17 patients having right KOA. Of these, 22 patients entered the occupational risk group, in which they stood in a bipedal position for more than 4 h per day. The CG included 15 patients, of which 10 women and 6 men aged between 50 and 61 years, weighing between 65 and 110 kg and having heights between 154 and 186 cm. In this group, 3 patients suffered from bilateral KOA, 11 had left KOA, and 2 had right KOA, of which 14 of them were in the occupational risk group.

The CONSORT flow diagram of the progress through the phases of a parallel randomized trial of two groups (enrolment, intervention, follow-up, and data analysis) is presented in Figure 1.

2.3. HA Injections

The viscoelastic product with the commercial name Kombihylan® was purchased from Ropharm® (Romania). Kombihylan® is a biological matrix with a molecular weight of 3 MDa in the form of a viscoelastic solution containing HA which is obtained by bacterial fermentation of a *Streptococcus* strain. The product was administered IA by an orthopedic specialist.

2.4. Procedure Steps (PS)

PS1: Using appropriate sterile techniques, an aspiration from the knee was performed from the suprapatellar region with a needle and syringe to depressurize the joint capsule. About 2 mL of SF was aspirated from the knee joint to reduce post-procedural swelling, preventing the increase in IA pressure.

PS2: IA infiltration was performed with the viscoelastic product Kombihylan® (3 mL) in the suprapatellar region using the same needle.

PS3: After removing the needle, the patient was asked to walk for 5–10 min to "homogenize" the viscoelastic product.

PS4: After 72 h, each patient started the program of PT for 10 consecutive days.

2.5. Study Design

For the inclusion criteria, the eligible patients were those who did not have increased inflammatory markers above the accepted values (normal blood count, erythrocyte sedimentation rate, and C-reactive protein), patients diagnosed with KOA and confirmed radiologically with KL 2 stage without previous infiltration of other viscoelastic substances or glucocorticoids in the last 12 months, and patients without knee synovitis in the last 12 months. Patients with bilateral KL 2 stage KOA were also included but only if they had a single symptomatic knee.

The exclusion criteria included a known allergy or hypersensitivity to sodium hyaluronate, chondroitin sulphate, N-acetylglucosamine, or any of the ingredients of Kombihylan product and patients with signs of local inflammation or medium hydrarthrosis. Moreover, patients with preexisting infections or skin diseases at the injection site, known infection of the affected joint, patients diagnosed with inflammatory rheumatic diseases or systemic diseases, or known systemic bleeding disorders, as well as bleeding or a tendency to bleed were not included in the study. Patients with bilateral KOA who had different stages from KL 2 and who were symptomatic were excluded. Consumption of NSAID drugs or other food supplements was prohibited.

Backup medication for PG and CG, consisting of 1000 mg metamizole sodium with 500 mg of acetaminophen taken twice a day for up to 5 consecutive days, was taken into account in case of insufficient improvement. However, it was not necessary to administer the backup medication for PG and CG throughout the study.

The PT program in KOA was the same for all patients for the 10-day duration. The goal of the PT program was to reduce pain without using other medications (NSAIDs, painkillers, or local anesthetics) or other procedures. All procedures were performed in the cold to avoid modification on the previously administered viscous HA-based biopolymer.

2.6. Physiotherapy Treatment

PT1: electrotherapy-conventional TENS electroanalgesia for 30–40 min using two frequency channels at 100 Hz and 100 μs.

PT2: LLLT, 3-kHz frequency with a 904-nm GaAlAs probe, 108 mW of power at 5 Joules/point, and a maximum of 40 Joules/application.

PT3—US: 8 min of 0.2–0.3 W/cm^2 at 1 MHz with a 10% duty cycle.

PT4—PTE: over 40 min per session with moderate-intensity exercise that included the following: warm up for 5 min with a stationary bike, static quads with a hold for 7 s, knee extensions over a roll with a hold for 7 s, single-leg raises for 50 reps, step-ups for 50 reps, calf raises for three sets of 10–15, and wall squats with a hold for 5–10 s.

Neuro-proprioceptive facilitation (PNF) techniques were used in four movement patterns (MPs) repeated 2–3 times as a set: MP1 (flexion-abduction-internal), MP2 (extension-adduction-external), MP3 (flexion-adduction-external), and MP4 (extension-abduction-internal) rotations. The PNF techniques included PNF1 (contract-relax), PNF2 (hold-relax); PNF3 (reversal of antagonists), and PNF4 (repeated stretch) [37].

PT5: cryotherapy ice packs were applied at the end of the PT to cool down the affected knee (at least 15 min).

2.7. Assessment Tests and Outcomes

All results were analyzed from the perspective of multiple indicators: the visual analog scale (VAS), the WOMAC scale, muscle strength testing, and knee goniometry, namely the range of motion (ROM).

The VAS is a pain assessment tool used worldwide by clinicians and physiotherapists [40]. The VAS is scored on a scale from 0 to 10, with 0 representing the absence of pain and 10 representing extreme pain [41].

The Western Ontario and McMaster Universities Arthritis Index (WOMAC) is used for the evaluation of KOA. The WOMAC self-administered questionnaire consists of three subscales: pain, stiffness, and function. The maximum WOMAC score is 96 and represents an assessment made by the patient that refers to daily activities, functional mobility, walking, general health, and quality of life. A high WOMAC score correlates with a decrease in quality of life [42].

Muscle strength testing is a system consisting of manual examination techniques that assess the strength of each muscle or muscle group. The most commonly accepted method of evaluating muscle strength is the Oxford Scale (also known as the Medical Research Council Manual Muscle Testing Scale). This method involves testing key muscles from the upper and lower extremities against the examiner's resistance and grading the patient's strength on a 0–5 scale accordingly [43]. In this study, we chose to evaluate the quadriceps muscle, since it is the extensor of the knee that helps maintain a bipedal position and performs gate control. Moreover, it has been previously reported that inhibition of the arthrogenic muscle in KOA causes a decrease in quadriceps muscle strength through a loop phenomenon [44,45].

Knee flexion or knee range of motion (ROM) is the movement of the knee in the sagittal plane. The functional value of the knee is 90 degrees and is calculated from the extension being 0 degrees and then onward through 45, 90, 125, and 135 degrees. The wide angle of the knee represents more bending, so the leg approaches on the posterior side of the thigh [46,47].

Statistical Approach

Regarding the statistical methodology approach, the procedure is described in the literature by several authors [48]. Thus, if in the first phase a preliminary statistical description is made presenting the average values, the standard deviations, and the comparisons between the series of values, in the second phase, an evaluation of the correlation coefficients is made. This second step is necessary to identify the grouping of the factors that influences the expected result. Finally, the principal components analysis (PCA) method was applied, a procedure based on the use of correlation coefficient values. This last step is enough to highlight the dynamic grouping of the factors that influence the desired result.

3. Results

All patients were assessed by VAS, WOMAC, muscle strength testing, and knee goniometry at baseline (Table 1) and after 3, 6, 9, and 12 months with the same tests. Additionally, the within-group effect size was evaluated for each test as Ω = initial − final (12 months).

Table 1. Baseline characteristics of the patients *.

Characteristic	Total Cohort (n = 52)	Pilot Group (HA Injection + PT) (n = 37)	Control Group (HA Injection) (n = 15)
Age (years)	55.9 ± 4.1	55.8 ± 4.2	56.1 ± 4.0
Female gender—no. (%)	26 (13.5)	16 (5.9)	10 (1.6)
Body mass index	30.0 ± 7.8	29.0 ± 3.5	32.3 ± 13.3
Symptomatic knee—no. (%)			
Right knee	19 (9.9)	17 (6.3)	2 (0.3)
Left knee	24 (12.5)	13 (4.8)	11 (1.8)
Both knees	10 (5.2)	7 (2.6)	3 (0.5)
Baseline measures			
WOMAC total score	64.5 ± 3.3	64.5 ± 2.99	64.6 ± 4.1
VAS scale	5.7 ± 0.5	5.7 ± 0.5	5.6 ± 0.5
ROM (degrees)	98.4 ± 11.4	98.1 ± 12.5	99.1 ± 8.6
Muscle strength testing (Oxford scale)	3.8 ± 0.5	3.8 ± 0.5	3.8 ± 0.4

* Plus and minus values are means ± SD. Percentages may not total 100 because of rounding.

3.1. Patient Groups' Descriptive Statistical Results

For completeness of exposure, a comparison of the distribution by gender, age, symptomatic knee, and weight for the PG and CG is presented in Figures 2–5. These representations were made for ease and quality of exposure.

Figure 2 shows the distribution of the CG and PG by gender. It can be easily noted that the distributions were comparable, with the ratios of the components being approximately equal.

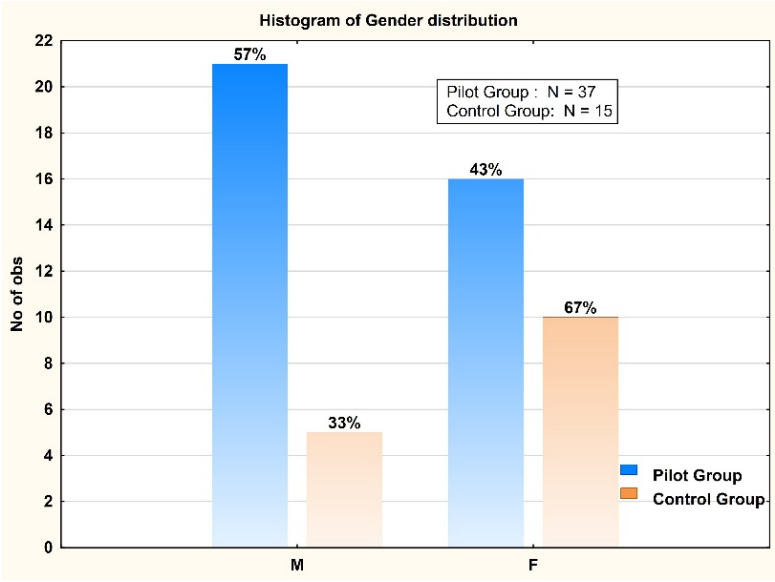


Figure 2. Histogram representation for the gender distribution of the control group (CG) and the pilot group (PG).

For the aim of easier exposure, a comparison of the distribution by age interval for the PG and CG is presented in Figure 3.

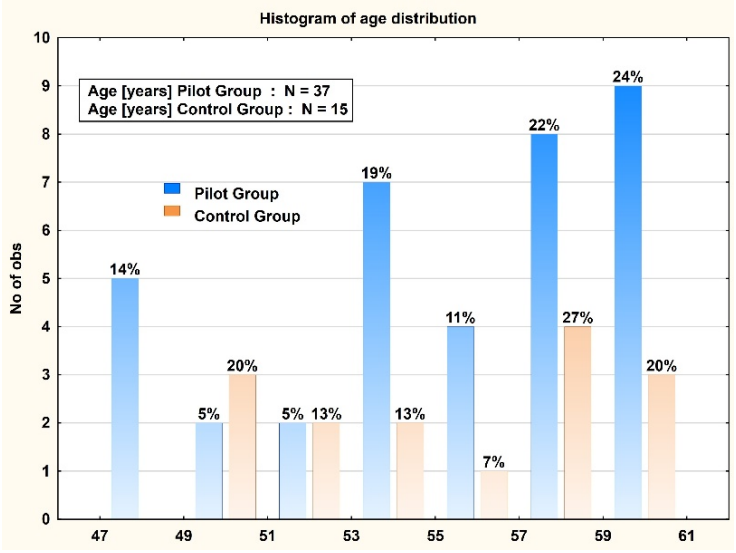


Figure 3. Histogram representation for the age interval distribution of the control group (CG) and the pilot group (PG).

A *t*-test for the independent samples (i.e., the variables were treated as independent samples) showed that there were no differences between the age mean values, as is shown below (Table 2).

Table 2. Characteristics of the *t*-test results.

C.G. vs. P.G.	Mean Group 1 CG	Mean Group 2 PG	*t*-Value	df	*p*	t Separ. Var. Est.	df	*p* 2-Sided	Std. Dev. Group 1	Std. Dev. Group 2
CG Age (years) vs. PG Age (years)	55.86667	55.83784	0.022647	50	0.982022	0.022828	26.4375	0.981959	4.103425	4.180069

Furthermore, a comparison of the distribution by the affected knees for the PG and CG is presented in Figure 4.

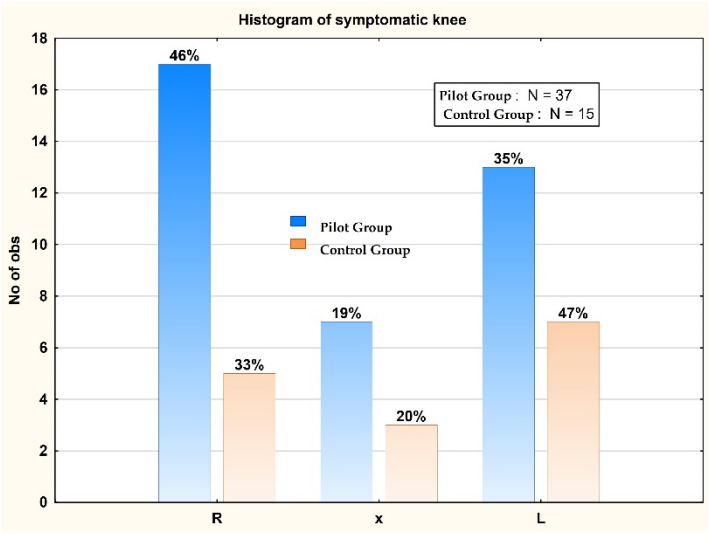


Figure 4. Histogram representation for the affected knee distribution of the control group (CG) and the pilot group (PG).

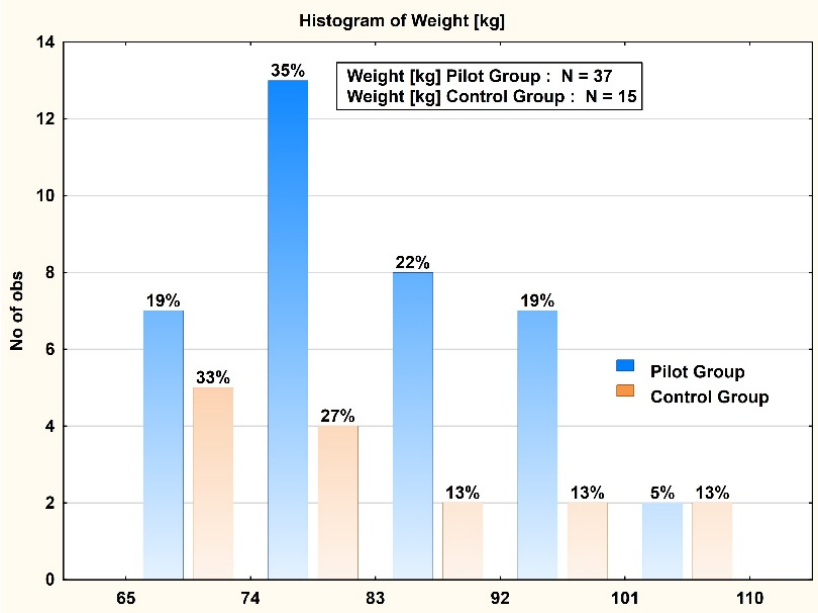


Figure 5. Histogram representation for the weight distribution of the control group (CG) and the pilot group (PG).

For completeness of the presentation, a Mann–Whitney U test (also called the Mann–Whitney–Wilcoxon test) is a nonparametric test of the null hypothesis. Using this procedure, we could show that there were no significant differences. As is known from the literature [48], the Mann–Whitney U test is significant at $p < 0.05000$, and the p value for our case was $p = 1.000$.

A *t*-test for the independent samples (i.e., the variables treated as independent samples) showed that there were no differences between the weight mean values, as is shown below (Table 3).

Table 3. Characteristics of the *t*-test results.

CG vs. PG	Mean Group 1	Mean Group 2	*t*-Value	df	*p*	t Separ. Var. Est.	df	*p* 2-Sided	Std. Dev. Group 1	Std. Dev. Group 2
CG Weight (kg) vs. PG Weight (kg)	83.4	83.89189	−0.13462	50	0.893455	−0.11851	20.50209	0.906819	14.54451	10.75429

3.2. Patient Results Description

As seen in Figure 6, at month 3 in the treatment program, the pain as assessed by the VAS scores decreased considerably from the baseline in both groups, with a larger and sharper effect observed in the PG from 5.730 ± 0.450 to 2.108 ± 0.614 ($p = 0.5$). A more moderate improvement was observed from months 3 to 6. Beyond 6 months, the VAS scores started to plateau in both groups but remained better in the PG patients receiving the combination treatment.

From a clinical perspective, the patients in the PG that underwent the combined approach (HA+ PT) no longer experienced noticeable pain or morning stiffness as early as 3 months into the treatment period. In contrast, while a pain decrease was evident in the CG patients, as observed by the improved VAS scores, intermittent morning stiffness was still experienced throughout the study period. The within-group effect size for the VAS score Ω_{VAS} between the two groups was statistically significant ($p < 0.0001$), with a Ω_{VA} of 3.757 ± 0.683 in the PG compared with the Ω_{VAS} of 2.750 ± 0.683 in the CG group.

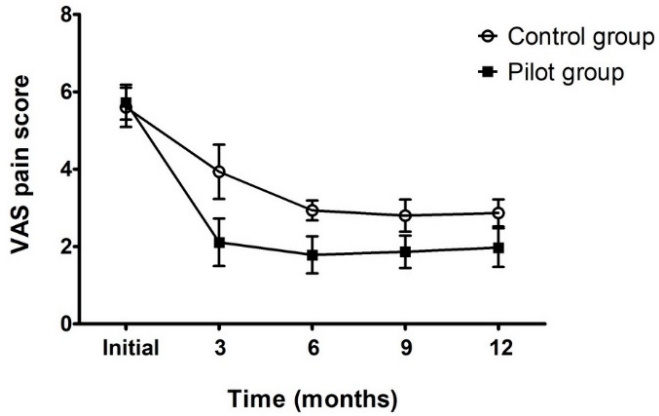


Figure 6. Evolution of pain over time in the control group (n = 15) and pilot group (n = 37), measured on a visual analog scale (VAS) at inclusion (initial point) and every 3 months up to 12 months.

At the baseline, the mean (± SD) WOMAC scores reported were 64.6 ± 4.08 in the CG and 64.5 ± 2.99 in the PG. Notably, at only 3 months into the study, the mean scores were significantly improved to 56.7 ± 5 in the CG and 48.27 ± 2.13 in the PG (mean between-group difference: 16.19 points; 95% confidence interval), finding favoring of the combination of VS with HA and PT. At the study's endpoint in 12 months, the scores were improved in both groups, with the mean between-group difference remaining significant (7.08 points; 95% confidence interval) (Figure 7). This translated into a significant improvement in pain and discomfort when the PT regimes were added to the pharmacological treatment, highlighting the importance of a combined, multimodal approach for the management of pain in KOA patients.

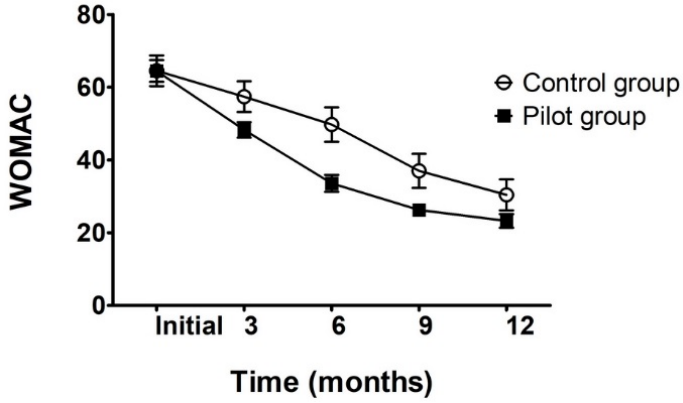


Figure 7. Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) total scores over the 12-month follow-up period. WOMAC total scores range from 0 to 96, with higher scores indicating worse pain, function, and stiffness. The values in parentheses are 95% confidence intervals (also indicated by the I bars). All 52 participants were included in the analysis in the CG (n = 15) and PG (n = 37).

From a clinical perspective, both groups of patients obtained considerably good results from the perspective of WOMAC classification. Both groups registered in the first stage (first 3 months) an improvement in functional mobility and walking, followed by significant improvement in the quality of life at the 12-month endpoint. These results validate the efficiency of the IA infiltration with Kombihylan® in patients with KOA, yet the PG recorded superior results from the first 3 months, thus separating the two groups of patients until the end of the study.

When calculating the within-group effect size, ΩWOMAC in the PG was 41.22 ± 3.65, and in the CG, it was 34.75 ± 3.36, with a significant statistical difference between groups as $p < 0.0001$.

Figure 8 depicts the muscle strength testing scale where, notably, the PG reported a fast and sharp increase from a value of 3.81 ± 0.52 at the baseline to 4.76 ± 0.44 at 3 months, which remained stable up to 1 year. A smaller size effect was registered in the CG, which did not receive the PT program. Thus, the CG remained around a value of 4, during the 12 months of screening (Figure 8). Muscle strengths values of four and five are clinically acceptable. From the patients' perspective, based on the feedback interviews, there were no noticeable differences between groups, since a value of four is considered good and five is normal. However, from the perspective of arthrogenic inhibition, for long-term maintenance, a value as close to 5 as possible means limiting this negative loop phenomenon that contributes to the acceleration of KOA.

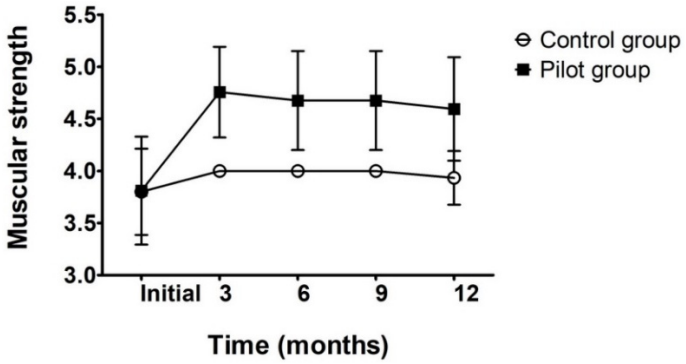


Figure 8. Evaluation of the strength of the quadriceps muscle over time in the control group (n = 15) and pilot group (n = 37), measured on a scale from 0 to 5 at inclusion (initial point) and every 3 months up to 12 months. ΩForce of 0.78 ± 0.58 for the PG was in contrast with the ΩForce of 0.19 ± 0.40 obtained for the CG with statistical significance at $p < 0.0001$.

While IA infiltration with Kombihylan® for KOA did not change the quadriceps muscle strength parameters for the 12-month study interval, the training through PTE toned the quadriceps and achieved a great overall within-group effect, with a value for muscle strength testing ΩForce of 0.78 ± 0.58 for the PG in contrast with the ΩForce of 0.19 ± 0.40 obtained for CG (statistical significance: $p < 0.0001$).

Similar results were obtained for the knee flexion evaluation test (ROM) as shown in Figure 9, where it is observed that the PG started from the average value of 98 degrees at the baseline, followed by a sudden increase to 115 degrees in the first 3 months and reaching a plateau between 6 and 12 months, as previously observed for all the other tests performed. In the case of the CG, the value at the baseline was 100 degrees of flexion, with a slow increase up to 112 degrees at month 9 followed by a significant decrease to 105 degrees at the endpoint (12 months).

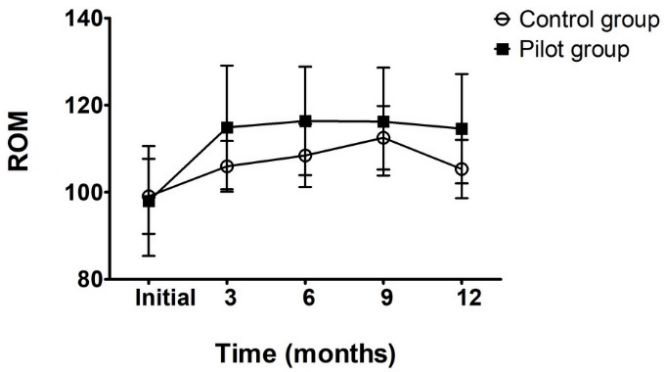


Figure 9. ROM evaluation of knee flexion in the PG (n = 37) and the CG (n = 15). Statistically, the within-group ROM effect size ΩROM was 16.62 ± 6.24 in the PG versus 8.75 ± 8.27 in CG, with $p < 0.0004$.

3.3. Descriptive Statistics

In this paragraph, we present the preliminary statistical analysis of the results obtained.

For the PG, the descriptive statistics of the results obtained after the successive tests are presented in Table 4. Herein, the correlation values for the PG's measured parameters are presented. A p value of 0.05 was considered significant. The significant correlations are marked in bold.

Table 4. Correlations between PG parameters of the patients.

Variable for PG	Initial Muscle Strength	Muscle Strength after 3 Months	Muscle Strength after 6 Months	Muscle Strength after 12 Months	Muscle Strength after 9 Months	Initial ROM Flexion Test	ROM Flexion Test after 3 Months	ROM Flexion Test after 6 Months	ROM Flexion Test after 9 Months	ROM Flexion Test after 12 Months
Initial muscle strength	1.0000	0.7757	0.4211	0.3404	0.4211	0.6834	0.6749	0.6431	0.6210	0.6497
	p = ---	p = 0.000	p = 0.009	p = 0.039	p = 0.009	p = 0.000	p = 0.000	p = 0.000	p = 0.000	p = 0.000
Muscle strength after 3 months	0.7757	1.0000	0.5492	0.4300	0.5492	0.7180	0.8032	0.7804	0.7269	0.7703
	p = 0.000	p = ---	p = 0.000	p = 0.008	p = 0.000	p = 0.000	p = 0.000	p = 0.000	p = 0.000	p = 0.000
Muscle strength after 6 months	0.4211	0.5492	1.0000	0.8390	1.0000	0.7923	0.8168	0.8283	0.8253	0.8169
	p = 0.009	p = 0.000	p = ---	p = 0.000	p = ---	p = 0.000	p = 0.000	p = 0.000	p = 0.000	p = 0.000
Muscle strength after 12 months	0.3404	0.4300	0.8390	1.0000	0.8390	0.7507	0.6987	0.6959	0.7132	0.6845
	p = 0.039	p = 0.008	p = 0.000	p = ---	p = 0.000	p = 0.000	p = 0.000	p = 0.000	p = 0.000	p = 0.000
Muscle strength after 9 months	0.4211	0.5492	1.0000	0.8390	1.0000	0.7923	0.8168	0.8283	0.8253	0.8169
	p = 0.009	p = 0.000	p = ---	p = 0.000	p = ---	p = 0.000	p = 0.000	p = 0.000	p = 0.000	p = 0.000
Initial ROM flexion test	0.6834	0.7180	0.7923	0.7507	0.7923	1.0000	0.9128	0.8981	0.8969	0.8769
	p = 0.000	p = 0.000	p = 0.000	p = 0.000	p = 0.000	p = ---	p = 0.000	p = 0.000	p = 0.000	p = 0.000
ROM flexion test after 3 months	0.6749	0.8032	0.8168	0.6987	0.8168	0.9128	1.0000	0.9858	0.9675	0.9730
	p = 0.000	p = 0.000	p = 0.000	p = 0.000	p = 0.000	p = 0.000	p = ---	p = 0.00	p = 0.00	p = 0.00
ROM flexion test after 6 months	0.6431	0.7804	0.8283	0.6959	0.8283	0.8981	0.9858	1.0000	0.9888	0.9813
	p = 0.000	p = 0.000	p = 0.000	p = 0.000	p = 0.000	p = 0.000	p = 0.00	p = ---	p = 0.00	p = 0.00
ROM flexion test after 9 months	0.6210	0.7269	0.8253	0.7132	0.8253	0.8969	0.9675	0.9888	1.0000	0.9775
	p = 0.000	p = 0.000	p = 0.000	p = 0.000	p = 0.000	p = 0.000	p = 0.00	p = 0.00	p = ---	p = 0.00
ROM flexion test after 12 months	0.6497	0.7703	0.8169	0.6845	0.8169	0.8769	0.9730	0.9813	0.9775	1.0000
	p = 0.000	p = 0.000	p = 0.000	p = 0.000	p = 0.000	p = 0.000	p = 0.00	p = 0.00	p = 0.00	p = ---

In all cases, there were strong correlations between the measured values of muscle strength and the values of the ROM test. Due to this, it turned out that the ROM flexion test was a fairly accurate tool for evaluating the evolution in the case of the parameters for measuring muscle strength. Notably, the correlation observed in the PG between the WOMAC test after 3 months and the initial WOMAC test (corr = 0.6155, p = 0.000) can also be highlighted.

For the CG, the correlation matrix showed a different representation (Table 5). The critical value for the p parameter was also considered to be p = 0.05. The significant correlations are marked in bold.

Table 5. Correlations between CG parameters of the patients.

Variable for CG	Initial WOMAC Test	WOMAC Test after 3 Months	WOMAC Test after 6 Months	WOMAC Test after 9 Months	WOMAC Test after 12 Months	Initial VAS Test	VAS Test after 3 Months	VAS Test after 6 Months	VAS Test after 9 Months	VAS Test after 12 Months	Initial Muscle Strength
CG initial WOMAC test	1	0.9405	0.8527	0.8501	0.8456	−0.0934	−0.2515	0.5589	0.3104	0.0032	−0.4657
	p = ---	p = 0.000	p = 0.000	p = 0.000	p = 0.000	p = 0.741	p = 0.366	p = 0.030	p = 0.260	p = 0.991	p = 0.080
CG WOMAC test after 3 months	0.9405	1	0.8475	0.796	0.7774	−0.0867	−0.2788	0.6159	0.2942	−0.1538	−0.4413
	p = 0.000	p = ---	p = 0.000	p = 0.000	p = 0.001	p = 0.759	p = 0.314	p = 0.015	p = 0.287	p = 0.584	p = 0.100
CG WOMAC test after 6 months	0.8527	0.8475	1	0.9181	0.9044	−0.0772	−0.1555	0.5677	0.3346	−0.1084	−0.4292
	p = 0.000	p = 0.000	p = ---	p = 0.000	p = 0.000	p = 0.784	p = 0.580	p = 0.027	p = 0.223	p = 0.700	p = 0.110
CG WOMAC test after 9 months	0.8501	0.796	0.9181	1	0.9405	−0.2109	−0.2605	0.4734	0.1476	0.0868	−0.4428
	p = 0.000	p = 0.000	p = 0.000	p = ---	p = 0.000	p = 0.451	p = 0.348	p = 0.075	p = 0.600	p = 0.758	p = 0.098
CG WOMAC test after 12 months	0.8456	0.7774	0.9044	0.9405	1	−0.1191	−0.2289	0.611	0.2513	0.2289	−0.4783
	p = 0.000	p = 0.001	p = 0.000	p = 0.000	p = ---	p = 0.672	p = 0.412	p = 0.016	p = 0.366	p = 0.412	p = 0.071
CG initial VAS test	−0.0934	−0.0867	−0.0772	−0.2109	−0.1191	1	0.7206	0.3273	0.6124	−0.3203	0.6124
	p = 0.741	p = 0.759	p = 0.784	p = 0.451	p = 0.672	p = ---	p = 0.002	p = 0.234	p = 0.015	p = 0.245	p = 0.015
CG VAS test after 3 months	−0.2515	−0.2788	−0.1555	−0.2605	−0.2289	0.7206	1	−0.0262	0.1961	−0.3269	0.6864
	p = 0.366	p = 0.314	p = 0.580	p = 0.348	p = 0.412	p = 0.002	p = ---	p = 0.926	p = 0.484	p = 0.234	p = 0.005
CG VAS test after 6 months	0.5589	0.6159	0.5677	0.4734	0.611	0.3273	−0.0262	1	0.5345	−0.1048	−0.1336
	p = 0.030	p = 0.015	p = 0.027	p = 0.075	p = 0.016	p = 0.234	p = 0.926	p = ---	p = 0.040	p = 0.710	p = 0.635
CG VAS test after 9 months	0.3104	0.2942	0.3346	0.1476	0.2513	0.6124	0.1961	0.5345	1	−0.1961	0.1667
	p = 0.260	p = 0.287	p = 0.223	p = 0.600	p = 0.366	p = 0.015	p = 0.484	p = 0.040	p = ---	p = 0.484	p = 0.553
CG VAS test after 12 months	0.0032	−0.1538	−0.1084	0.0868	0.2289	−0.3203	−0.3269	−0.1048	−0.1961	1	−0.1961
	p = 0.991	p = 0.584	p = 0.700	p = 0.758	p = 0.412	p = 0.245	p = 0.234	p = 0.710	p = 0.484	p = ---	p = 0.484
CG initial muscle strength	−0.4657	−0.4413	−0.4292	−0.4428	−0.4783	0.6124	0.6864	−0.1336	0.1667	−0.1961	1
	p = 0.080	p = 0.100	p = 0.110	p = 0.098	p = 0.071	p = 0.015	p = 0.005	p = 0.635	p = 0.553	p = 0.484	p = ---
CG muscle strength after 3 months	–	–	–	–	–	–	–	–	–	–	–
	p = ---	p = ---	p = ---	p = ---	p = ---	p = ---	p = ---	p = ---	p = ---	p = ---	p = —
CG muscle strength after 6 months	–	–	–	–	–	–	–	–	–	–	–
	p = ---	p = ---	p = ---	p = ---	p = ---	p = ---	p = ---	p = ---	p = ---	p = ---	p = —
CG muscle Strength after 9 months	–	–	–	–	–	–	–	–	–	–	–
	p = ---	p = ---	p = ---	p = ---	p = ---	p = ---	p = ---	p = ---	p = ---	p = ---	p = —
CG muscle strength after 12 months	−0.2271	−0.1703	−0.1322	−0.1775	−0.169	0.3273	0.3669	−0.0714	−0.1336	−0.1048	0.5345
	p = 0.416	p = 0.544	p = 0.639	p = 0.527	p = 0.547	p = 0.234	p = 0.179	p = 0.800	p = 0.635	p = 0.710	p = 0.040

3.4. Statistical Difference Analysis

Difference analysis for each group was performed by applying the *t*-test analysis. For the control group (CG), the *t*-test values for the measured muscle strength magnitudes are presented in the Table 6 below. It is easily observed that the *t*-tests gave insignificant values between successive measurement sets. This observation is in agreement with the graphical representation in Figure 9.

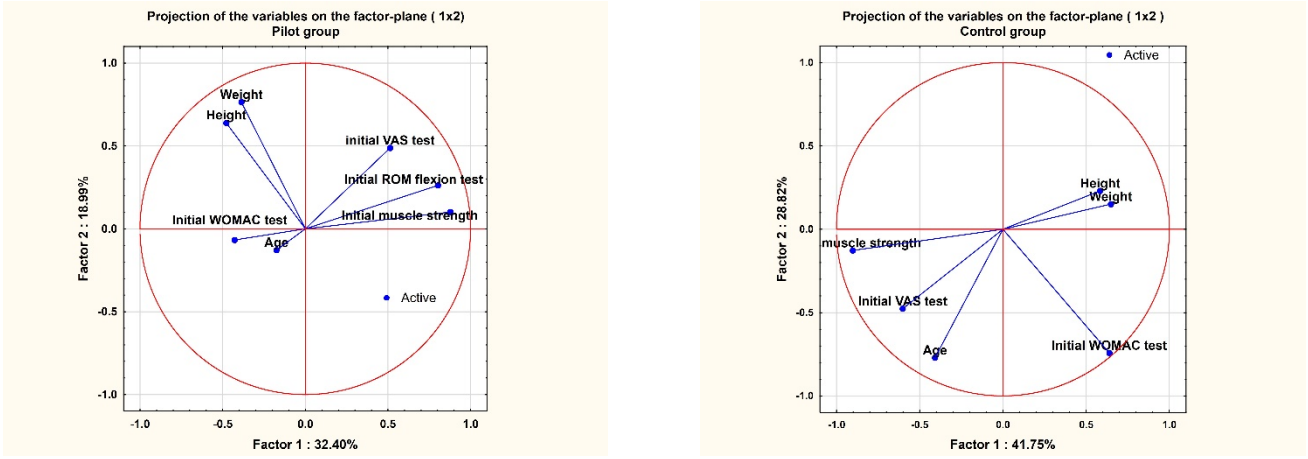
Table 6. *T*-test between CG muscle strength measurement sets of the patients.

CG Grouped by Measurement Set vs. CG Grouped by Measurement Set	*t*-Value	df	*p*	t Separ. Var. Est.	df	*p* 2-Sided	Std. Dev. Group 1	Std. Dev. Group 2
CG initial muscle strength vs. CG muscle strength after 3 months	−1.87083	28	0.071854	−1.87083	14	0.082418	0.414039	0
CG muscle strength after 3 months vs. CG muscle strength after 6 months		28	-				0	0
CG muscle strength after 6 months vs. CG muscle strength after 9 months		28	-				0	0
CG muscle strength after 9 months vs. CG muscle strength after 12 months	1	28	0.325875	1	14	0.334282	0	0.258199
CG initial muscle strength vs. CG muscle strength after 6 months	−1.87083	28	0.071854	−1.87083	14	0.082418	0.414039	0
CG initial muscle strength vs. CG muscle strength after 9 months	−1.87083	28	0.071854	−1.87083	14	0.082418	0.414039	0
CG muscle strength after 12 months vs. CG initial muscle strength	1.0583	28	0.298964	1.0583	23.45845	0.300695	0.258199	0.414039

For the PG, the *t*-test measured values for the muscle strength magnitudes are presented in Table 7. The *t*-tests gave significant values (marked in bold), and there were substantial differences between successive measurement sets. This observation is coherent with the graphical representation from Figure 10.

Table 7. *t*-test between CG muscle strength measurement sets of the patients.

PG Grouped by Measurement Set vs. PG Grouped by Measurement Set	*t*-Value	df	*p*	t Separ. Var. Est.	df	*p* 2-Sided	Std. Dev. Group 1	Std. Dev. Group 2
PG initial muscle strength vs. PG muscle strength after 3 months	−8.5027	72	0	−8.5027	69.8896	0	0.518429	0.43496
PG initial muscle strength vs. PG muscle strength after 6 months	−7.4849	72	0	−7.4849	71.44489	0	0.518429	0.47458
PG initial muscle strength vs. PG muscle strength after 9 months	−7.4849	72	0	−7.4849	71.44489	0	0.518429	0.47458
PG initial muscle strength vs. PG muscle strength after 12 months	−6.6337	72	0	−6.6337	71.88094	0	0.518429	0.49774
PG muscle strength after 3 months vs. PG muscle strength after 6 months	0.7661	72	0.446103	0.7661	71.45966	0.446122	0.434959	0.47458
PG muscle strength after 3 months vs. PG muscle strength after 9 months	0.7661	72	0.446103	0.7661	71.45966	0.446122	0.434959	0.47458
PG muscle strength after 3 months vs. PG muscle strength after 12 months	1.4922	72	0.140003	1.4922	70.72959	0.140081	0.434959	0.49774
PG muscle strength after 6 months vs. PG muscle strength after 9 months	0	72	1	0	72	1	0.474579	0.47458
PG muscle strength after 6 months vs. PG muscle strength after 12 months	0.7171	72	0.47561	0.7171	71.8371	0.475615	0.474579	0.49774
PG muscle strength after 9 months vs. PG initial muscle strength	7.4849	72	0	7.4849	71.44489	0	0.474579	0.51843
PG muscle strength after 9 months vs. PG muscle strength after 12 months	0.7171	72	0.47561	0.7171	71.8371	0.475615	0.474579	0.49774
PG muscle strength after 12 months vs. PG initial muscle strength	6.6337	72	0	6.6337	71.88094	0	0.497743	0.51843
PG muscle strength after 12 months vs. PG muscle strength after 9 months	−0.7171	72	0.47561	−0.7171	71.8371	0.475615	0.497743	0.47458



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

Figure 10. PCA analysis results for the (**a**) pilot group and (**b**) control group at baseline (initial).

3.5. PCA Analysis

We sought to perform a thorough data analysis that included all the elements in the database of the study. Of all the methods of statistical analysis, the method adopted by us was the principal component analysis (PCA) method. Figure 10 shows the results of the PCA method obtained at the initial time (Figure 10a) comparing the two groups of patients.

It was observed that at the initial moment, the configuration of the factors was somewhat similar (Figure 10a,b). Thus, at the baseline, factor 1 had important contributions from the VAS test, muscle strength, and ROM flexion for the PG (Table 8), and for the CG, factor 1 had important contributions from the same factors (Table 3b). Notable differences were observed in the level of contributions of factor 2. While for the PG the age had an important contribution (Table 8), for the CG, age was already included predominantly in factor 3 (Table 9).

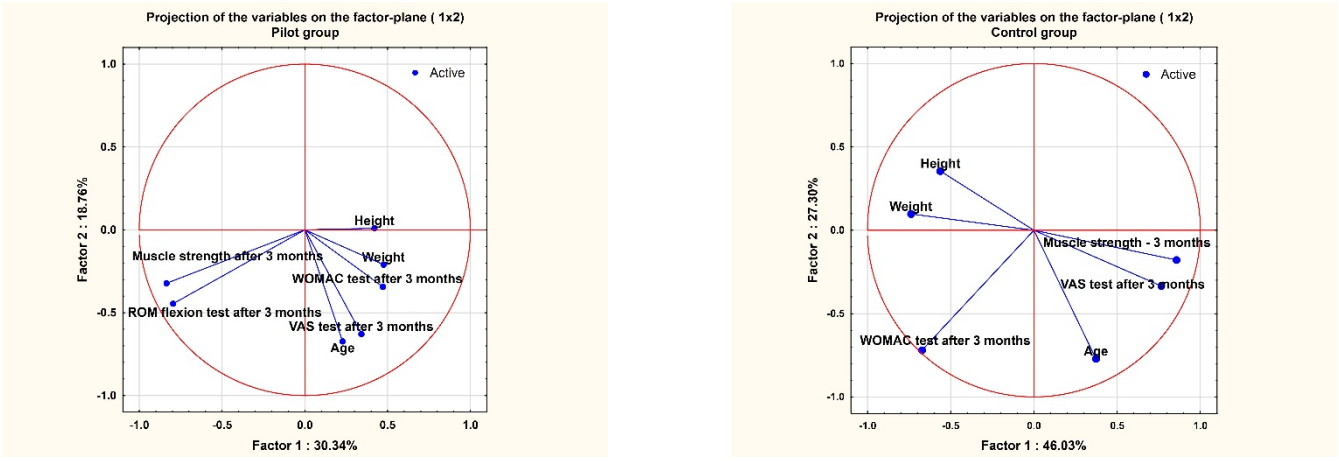
Table 8. Factor coordinates of the variables based on correlations for the pilot group (PG) at the initial stage.

Variable	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 6
Age (years)	−0.17544	−0.12843	0.800838	−0.40152	0.372737	−0.10588
Weight (kg)	−0.38716	0.763855	0.261097	−0.09065	−0.16274	0.38502
Height (cm)	−0.47821	0.638149	−0.0947	0.355641	0.32781	−0.34755
Initial WOMAC test	−0.42858	−0.06762	−0.64829	−0.42846	0.422578	0.161166
Initial VAS test	0.512754	0.487966	−0.17507	−0.56843	−0.24671	−0.2804
Initial muscle strength	0.876851	0.101257	−0.01479	0.093	0.219449	−0.0209
Initial ROM flexion test	0.802037	0.262331	0.026423	0.113245	0.342044	0.243706

Table 9. Factor coordinates of the variables based on correlations for the control group (CG) at the initial stage.

Variable	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 6
Age (years)	−0.40854	−0.77165	0.248408	−0.09066	−0.40941	−0.01051
Weight (kg)	0.64822	0.150963	0.508954	−0.54186	0.048725	−0.04472
Height (cm)	0.583746	0.23023	0.660958	0.366693	−0.06892	0.173656
Initial WOMAC test	0.639288	−0.74257	−0.15343	0.015238	0.10918	0.064899
Initial VAS test	−0.60224	−0.47681	0.526263	0.156451	0.263397	−0.19787
Initial muscle strength	−0.90239	−0.12719	0.112096	−0.19381	0.15468	0.308977
CG initial WOMAC	0.639288	−0.74257	−0.15343	0.015238	0.10918	0.064899

While for the CG the PCA diagram for the first two factors practically remained unchanged (Figure 11b), being only an inversion of axes in relation to Figure 10b, for the PG, there were obvious changes (Figure 11a) in relation to the previous situation. These changes were best seen by analyzing the contribution of factors for each group. Thus, if for both the PG and the CG age was a parameter that was included in the second-order factor, for the PG, the weight and height remained coupled and gave contributions to factor 3 (Table 10). In contrast, for the CG, weight was a factor of prime importance (Table 11), while height was a less important factor. These observed aspects are natural for patients who do not receive effective treatment.



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

Figure 11. PCA analysis results for the (**a**) pilot group and (**b**) control group at 3 months.

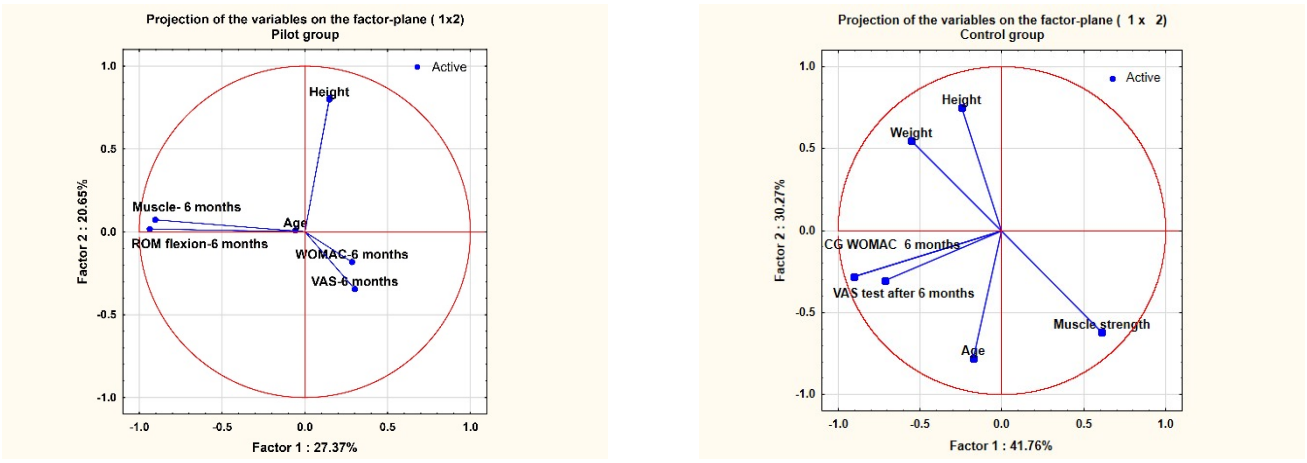
Table 10. Factor coordinates of the variables based on correlations for the pilot group (PG) after 3 months.

Variable	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 6
Age	0.227779	−0.672723	0.236629	0.539305	0.271364	−0.269485
Weight	0.474756	−0.210575	−0.668359	0.184855	0.167216	0.465744
Height	0.419664	0.009419	−0.742912	−0.060786	−0.227288	−0.463873
WOMAC test after 3 months	0.470012	−0.343063	0.100854	−0.681551	0.427492	−0.055478
VAS test after 3 months	0.340695	−0.629027	0.242235	−0.153364	−0.619587	0.148715
Muscle strength after 3 months	−0.836608	−0.323219	−0.253536	−0.227014	−0.022256	−0.069839
ROM flexion test after 3 months	−0.795795	−0.445332	−0.292966	0.003051	0.068194	0.060417

Table 11. Factor coordinates of the variables based on correlations for the control group (CG) after 3 months.

Variable	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 6
Age	0.372638	−0.770865	0.351498	−0.004635	−0.376629	−0.038537
Weight	−0.739012	0.097571	0.392983	0.516199	0.042614	−0.147063
Height	−0.562548	0.355252	0.666723	−0.284778	0.011011	0.177754
WOMAC test after 3 months	−0.671922	−0.720087	−0.100533	−0.036668	0.120227	0.063944
VAS test after 3 months	0.764624	−0.334418	0.375759	−0.175039	0.311154	−0.186721
ROM flexion test after 3 months	−0.671922	−0.720087	−0.100533	−0.036668	0.120227	0.063944
Initial muscle strength	0.856078	−0.178459	0.13093	0.359274	0.118778	0.273779

For the following data set taken after 6 months of monitoring, the PCA diagram for the CG remained practically unchanged (Figure 12b) compared with the previous situations.



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

Figure 12. PCA analysis results for the (**a**) pilot group and (**b**) control group at 6 months.

For the PG, there were obvious changes (Figure 12a) in relation to the previous situation. In Figure 10a, if the PG presented important contributions for factor 2 from the VAS and WOMAC values, after 6 months of monitoring, the contributions of these factors were found in factor 3 (Table 12). For the PG, the height and weight parameters remained coupled and contributed to factor 2. This fact suggests that the body mass index would be extremely useful for analyzing the evolution of this group.

Table 12. Factor coordinates of the variables based on correlations for the pilot group (PG) after 6 months.

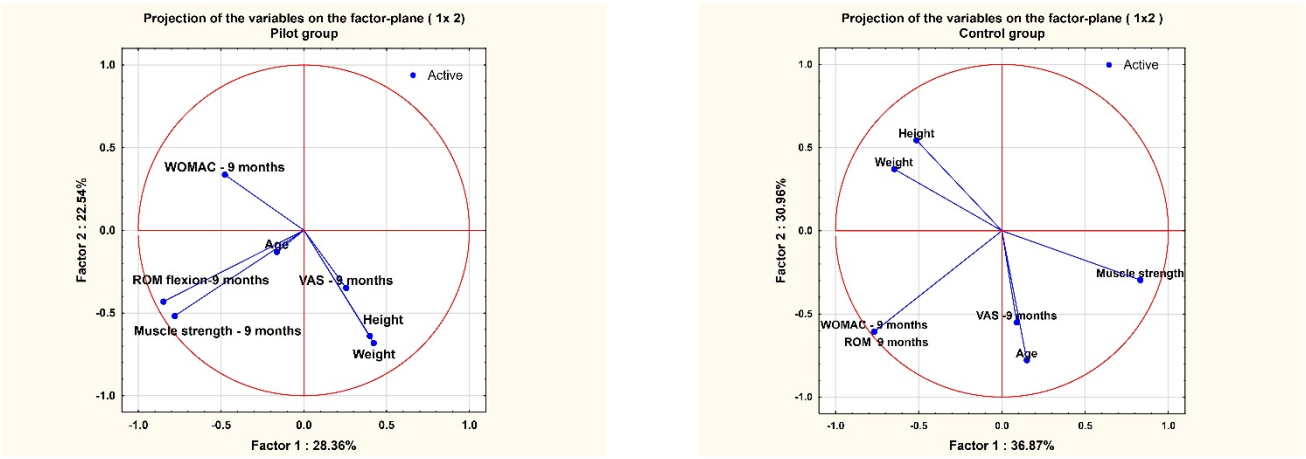
Variable	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 6
Age	−0.057318	0.008466	−0.638977	−0.689853	0.248303	0.225369
Weight	0.149165	0.80625	−0.236736	−0.067622	0.126056	−0.501077
Height	0.146953	0.798468	−0.108744	0.317836	−0.052394	0.472586
WOMAC test after 6 months	0.284124	−0.182385	−0.719375	0.11887	−0.590437	−0.075881
VAS test after 6 months	0.301459	−0.344811	−0.498948	0.545593	0.4917	−0.030827
Muscle strength after 6 months	−0.904867	0.072547	−0.240087	0.203629	0.006427	0.020377
ROM flexion test after 6 months	−0.937252	0.016756	−0.162417	0.096187	−0.03038	−0.072024

For the CG, age was a parameter that was included in the second-order factor, along with the weight and height (Table 13). However, for the CG, the WOMAC, VAS, and ROM size effects had major contributions, being included in the factor 1 component.

Table 13. Factor coordinates of the variables based on correlations for the control group (CG) after 6 months.

Variable	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 6
Age	−0.173219	−0.781599	−0.42766	0.359204	−0.106859	−0.189099
Weight	−0.552026	0.550402	−0.411497	−0.330655	−0.32422	−0.092426
Height	−0.246372	0.752784	−0.393773	0.432213	0.043256	0.169942
WOMAC test after 6 months	−0.906747	−0.284033	0.265738	0.058011	−0.117826	0.096282
VAS test after 6 months	−0.716628	−0.302249	−0.411121	−0.254999	0.399897	0.033566
ROM flexion test after 6 months	−0.906747	−0.284033	0.265738	0.058011	−0.117826	0.096282
Initial muscle strength	0.608334	−0.621005	−0.34678	−0.150183	−0.187282	0.257677

For the next data set taken after 9 months of monitoring, the PCA diagram for the CG showed configurations with very few modifications (Figure 13b) compared with the previous situations (Tables 14 and 15).



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

Figure 13. PCA analysis results for the (**a**) pilot group and (**b**) control group at 9 months.

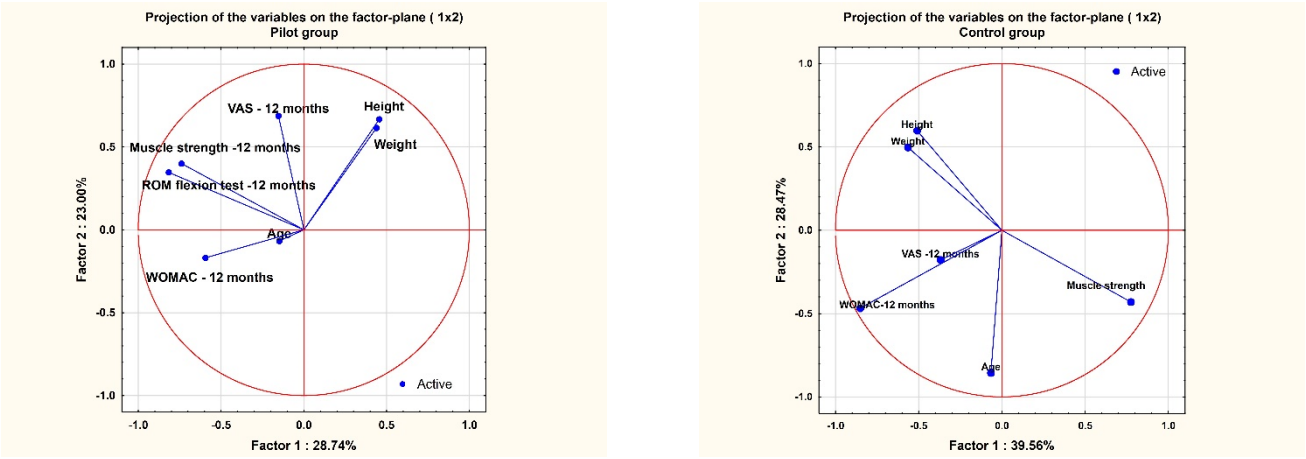
Table 14. Factor coordinates of the variables based on correlations with the pilot group (PG) after 9 months.

Variable	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 6
Age	−0.163722	−0.129883	−0.817701	−0.38458	−0.303894	−0.217337
Weight	0.421275	−0.681319	−0.180406	−0.202216	0.047842	0.531264
Height	0.398624	−0.639181	0.118911	−0.191559	0.463704	−0.407028
WOMAC test after 9 months	−0.477327	0.33645	−0.454268	0.040244	0.655758	0.132361
VAS test after 9 months	0.255099	−0.347793	−0.393474	0.805155	−0.057139	−0.083135
Muscle strength after 9 months	−0.780641	−0.517856	0.201877	−0.006728	−0.099046	−0.054374
ROM flexion test after 9 months	−0.848415	−0.430933	0.075602	0.109443	0.005286	0.065062

Table 15. Factor coordinates of the variables based on correlations with the control group (CG) after 9 months.

Variable	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 6
Age	0.149027	−0.778052	−0.281976	0.458076	0.206861	−0.200724
Weight	−0.648258	0.371211	−0.321741	0.340358	−0.468873	−0.052548
Height	−0.513533	0.544514	−0.499268	0.108515	0.394234	0.152721
WOMAC test after 9 months	−0.767118	−0.606757	0.177542	0.010254	−0.001097	0.108391
VAS test after 9 months	0.088746	−0.550926	−0.642614	−0.506753	−0.137218	0.004974
ROM flexion test after 9 months	−0.767118	−0.606757	0.177542	0.010254	−0.001097	0.108391
Muscle strength	0.830623	−0.295921	−0.112589	0.323617	−0.146676	0.289099

For the PG (Figure 13a), the PCA diagram did not change significantly in relation to the previous situation. In Figure 10a, if the PG presented important contributions for factor 2 from the VAS and WOMAC values placed in the same quadrant, after 9 months of monitoring, the contributions of these factors were found separately, having opposite influences within the same factor (Table 14). After 12 months, for the PG, the height and weight parameters remained coupled (Figure 14a) and contributed as in the previous situations in factor 2 (Table 16).



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

Figure 14. PCA analysis results for the (**a**) pilot group and (**b**) control group at 12 months.

Table 16. Factor coordinates of the variables based on correlations with the pilot group (PG) after 12 months.

Variable	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 6
Age	−0.148961	−0.067687	0.927	−0.096863	0.06868	−0.313573
Weight	0.438348	0.615282	0.381684	−0.025543	0.228492	0.480341
Height	0.455133	0.667716	0.046186	−0.045912	−0.561316	−0.155468
WOMAC test after 12 months	−0.594652	−0.168703	0.257435	0.615788	−0.314365	0.270036
VAS test after 12 months	−0.153677	0.687375	−0.173122	0.544848	0.294779	−0.295345
Muscle strength after 12 months	−0.739748	0.399993	−0.094774	−0.376964	−0.077168	0.06215
ROM flexion test after 12 months	−0.816146	0.346115	−0.007506	−0.231227	0.04065	0.031053

For the CG, age was a parameter that was also included in the second-order factor (Figure 14b) along with height (Table 17). However, the WOMAC, VAS, and ROM sizes had major contributions, being included in the factor 1 component (Table 17).

Table 17. Baseline characteristics of the *t*-test results and factor coordinates of the variables based on correlations with the control group (CG) after 12 months.

Variable	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 6
Age	−0.066009	−0.856533	0.12279	−0.443203	0.111468	0.195097
Weight	−0.564898	0.497323	−0.098491	−0.494398	−0.423191	0.018414
Height	−0.509841	0.59758	0.248188	−0.342513	0.446991	−0.065168
Initial muscle strength	0.774789	−0.430082	0.069126	−0.38836	−0.052896	−0.237342
WOMAC test after 12 months	−0.851222	−0.46791	−0.205264	0.070895	0.016589	−0.095119
VAS test after 12 months	−0.368794	−0.178153	0.878551	0.166963	−0.177382	−0.032566
ROM flexion test after 12 months	−0.851222	−0.46791	−0.205264	0.070895	0.016589	−0.095119

4. Discussion

KOA is considered the tenth largest contributor to global years lived with disabilities [49,50]. The prevalence of OA is very high, and it is expected to affect more than 50 million subjects in the US by 2020 and increase with the aging of the population [51].

Non-pharmacologic strategies are a first-line approach to managing symptoms such as exercise, weight loss, and patient education [52,53]. Physical activity of 150 min/week consisting of moderate-intensity aerobic exercise or 2 days/week of moderate-to-vigorous physical activity in muscle-strengthening exercises is important in maintaining physical function in OA [52].

Pharmacological treatment options include simple analgesics, nonsteroidal NSAIDs, selective COX-2 inhibitors, intra-articular corticosteroid injections, VS, and surgery. However, NSAIDs can cause gastrointestinal problems, renal failure, hypertension, and have a thrombotic potential, especially at high doses [53].

For patients with joint effusions, corticosteroids are usually administered by an IA injection. Systemic administration of corticosteroids, due to severe side effects, is not recommended in OA. Additionally, there are possible adverse events following repeated administration of corticosteroids, such as local tissue atrophy, long-term joint damage due to reduced bone formation, and the risk of infection [54]. Corticoids may produce immunosuppressive and anti-inflammatory effects, reduce production of IL-1, prostaglandins, and leukotriene, and increase joint mobility. IA corticosteroid injections provide a short-term reduction in OA pain and act as key therapy for moderate-to-severe pain relief in patients with OA [54].

For VS with HA, platelet-rich plasma (PRP) injections or combination products such as PRP + HA have been used to improve lubrication, modulate inflammation, and modify the catabolic micro-environment [55]. Contraindications to hyaluronate injections include allergies, joint infections at the injection site, and pregnant and lactating women. Patients with venous or lymphatic stasis in the legs, bleeding disorders, or treatment with anticoagulants are generally contraindicated.

Herein, a two-group parallel randomized trial design was used to evaluate the effectiveness of a single HA-based VS product in combination with PT over a 1-year period.

In this study, the WOMAC score decreased for both groups of patients, with remarkable results obtained at 3 months in the PG followed at some distance by the CG. From this point of view, the PT consistently improved the quality of life of the patients.

The decrease in the VAS pain scores achieved from 5.7 to 2 represents the most important outcome of the combination therapy with Kombihylan® and PT. A VAS value of six is a high value of pain intensity, which is difficult to tolerate by patients, and in

general, medical doctors tend to prescribe long-term analgesic and anti-inflammatory drugs. However, a VAS value of two is associated with mild pain, which is transient and does not require medication. Reaching the VAS value of two for the PG and keeping it steady for 9 months is an important step in reducing drug use and improving the quality of life. With a VAS value of three, the CG stays on the plateau for 6 months, and the descent from the initial point is not as sudden as in the PG but is nonetheless sufficiently satisfactory.

The muscle strength in the PG increased from a value of four, corresponding to a good response, to a value of five, corresponding to a normal muscle strength response. We observed that training through PTE toned the quadriceps muscle and overcame the negative loop phenomenon of arthrogenic inhibition. The fact that in 3 months the strength increased and then was maintained until month 12 showed us that PTE made a significant difference in obtaining good function of the knee extensor when compared with the CG.

Although the values for the knee flexion were relatively small, they fell within the last quadrant, and generally, the last degrees from 110 to 135 are the most difficult to recover. However, the ROM of the PG was improved by an average of 16.6 degrees throughout the period, and the values of the CG marked an average improvement of 8.7 degrees.

Although the results were satisfactory from a clinical perspective, future research is needed to determine the best protocol and sequence of PT steps in combination with IA VS with HA that could lead to the best outcomes for the patient.

There were limitations to this study. An important limitation was the use of only one HA-based product in the same quantity for all patients regardless of age, sex, and weight. The PT procedures were refined before this study and applied according to all patients in the PG. Another limitation of this study was the use of a single 10-day PT protocol, and future studies should focus on extended treatment protocols in different combinations. This study demonstrated the efficacy of VS with HA in combination with PT at several time intervals. Evaluations stopped after a year, and future studies should exceed 1 year to show the effectiveness of these therapies in the medium and long term. Of all the positive aspects discussed above, it is worth mentioning that this study provided specific results, with staggered determinations monitoring the evolution of patients at specific time intervals. On the other hand, the small size of the patient sample, human error, and the lack of a correlation with the biological samples obtained from joint SF are among the few limitations that need to be addressed in the future.

5. Conclusions

We showed that PT plays a key role in the non-surgical treatment of KOA, effectively controlling pain, stiffness, and ROM and improving patients' quality of life. Moreover, the combination of IA VS with an HA-biopolymer and PT can enhance the effect of the VS alone, reducing symptomatology and possibly limiting the KOA progression.

PT is an internationally recognized form of treatment for KOA by physicians and patients as part of treatment guidelines, known as an important clinical tool. It is non-invasive, well tolerated and accepted by patients. The improvement of treatment schemes in KOA is a preoccupation for physiotherapists, especially when IA VS with HA has been performed.

Further studies are needed to evaluate the limitation of joint destruction in KOA using VS with HA in combination with with different PT procedures.

Author Contributions: Study design, I.O., D.M., R.G., D.A.I., D.-P.S. and A.O.; data collection, I.O., R.G. and A.O.; data analysis, D.M., G.D.M. and G.M.; drafting manuscript, I.O., D.M., D.C., D.A.I., I.L.S. and A.-I.G. All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas, and took part in drafting, revising, or critically reviewing the article. All authors have equally contributed to this article as senior authors. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Institutional Review Board (or Ethics Committee) of Micromedica Medical Clinic with no. 32/28 January 2020.

Informed Consent Statement: Informed consent was obtained from all subjects that were involved in the study.

Data Availability Statement: Data are contained within the main text of the article.

Acknowledgments: We would like to thank Luiza Iuliana Hernandez for the technical assistance.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Hootman, J.M.; Helmick, C.G.; Barbour, K.E.; Theis, K.A.; Boring, M.A. Updated Projected Prevalence of Self-Reported Doctor-Diagnosed Arthritis and Arthritis-Attributable Activity Limitation Among US Adults, 2015–2040. *Arthritis Rheumatol.* **2016**, *68*, 1582–1587. [CrossRef]
2. Hamood, R.; Tirosh, M.; Fallach, N.; Chodick, G.; Eisenberg, E.; Lubovsky, O. Prevalence and Incidence of Osteoarthritis: A Population-Based Retrospective Cohort Study. *J. Clin. Med.* **2021**, *10*, 4282. [CrossRef] [PubMed]
3. Berenbaum, F. Osteoarthritis as an Inflammatory Disease (Osteoarthritis Is Not Osteoarthrosis!). *Osteoarthr. Cartil.* **2013**, *21*, 16–21. [CrossRef]
4. Altman, R.D.; Bedi, A.; Karlsson, J.; Sancheti, P.; Schemitsch, E. Product Differences in Intra-Articular Hyaluronic Acids for Osteoarthritis of the Knee. *Am. J. Sports Med.* **2016**, *44*, 2158–2165. [CrossRef] [PubMed]
5. Dahl, L.B.; Dahl, I.M.; Engström-Laurent, A.; Granath, K. Concentration and molecular weight of sodium hyaluronate in synovial fluid from patients with rheumatoid arthritis and other arthropathies. *Ann. Rheum. Dis.* **1985**, *44*, 817–822. [CrossRef]
6. Smith, M.D.; Triantafillou, S.; Parker, A.; Youssef, P.P.; Coleman, M. Synovial membrane inflammation and cytokine production in patients with early osteoarthritis. *J. Rheumatol.* **1997**, *24*, 365–371. [PubMed]
7. Yatabe, T.; Mochizuki, S.; Takizawa, M.; Chijiiwa, M.; Okada, A.; Kimura, T.; Fujita, Y.; Matsumoto, H.; Toyama, Y.; Okada, Y. Hyaluronan Inhibits Expression of ADAMTS4 (Aggrecanase-1) in Human Osteoarthritic Chondrocytes. *Ann. Rheum. Dis.* **2009**, *68*, 1051–1058. [CrossRef]
8. Karna, E.; Miltyk, W.; Pałka, J.A.; Jarzabek, K.; Wołczyński, S. Hyaluronic Acid Counteracts Interleukin-1-Induced Inhibition of Collagen Biosynthesis in Cultured Human Chondrocytes. *Pharmacol. Res.* **2006**, *54*, 275–281. [CrossRef]
9. Yasuda, T. Progress of research in osteoarthritis. Pharmacological effects of hyaluronan. *Clin. Calcium* **2009**, *19*, 1644–1652. [PubMed]
10. Gupta, R.C.; Lall, R.; Srivastava, A.; Sinha, A. Hyaluronic Acid: Molecular Mechanisms and Therapeutic Trajectory. *Front. Veter. Sci.* **2019**, *6*, 192. [CrossRef]
11. Zhang, W.; Moskowitz, R.W.; Nuki, G.; Abramson, S.; Altman, R.D.; Arden, N.; Bierma-Zeinstra, S.; Brandt, K.D.; Croft, P.; Doherty, M.; et al. OARSI Recommendations for the Management of Hip and Knee Osteoarthritis, Part II: OARSI Evidence-Based, Expert Consensus Guidelines. *Osteoarthr. Cartil.* **2008**, *16*, 137–162. [CrossRef] [PubMed]
12. de Rezende, M.U.; de Campos, G.C. Viscosupplementation. *Rev. Bras. Ortop.* **2012**, *47*, 160–164. [CrossRef]
13. Webb, D.; Naidoo, P. Viscosupplementation for Knee Osteoarthritis: A Focus on Hylan G-F 20. *Orthop. Res. Rev.* **2018**, *10*, 73–81. [CrossRef] [PubMed]
14. Boutefnouchet, T.; Puranik, G.; Holmes, E.; Bell, K.M. Hylan GF-20 Viscosupplementation in the Treatment of Symptomatic Osteoarthritis of the Knee: Clinical Effect Survivorship at 5 Years. *Knee Surg. Relat. Res.* **2017**, *29*, 129–136. [CrossRef] [PubMed]
15. Hsieh, Y.-S.; Yang, S.-F.; Lue, K.-H.; Chu, S.-C.; Lu, K.-H. Effects of Different Molecular Weight Hyaluronan Products on the Expression of Urokinase Plasminogen Activator and Inhibitor and Gelatinases during the Early Stage of Osteoarthritis. *J. Orthop. Res.* **2008**, *26*, 475–484. [CrossRef]
16. Liang, J.; Jiang, D.; Noble, P.W. Hyaluronan as a Therapeutic Target in Human Diseases. *Adv. Drug Deliv. Rev.* **2016**, *97*, 186–203. [CrossRef] [PubMed]
17. Ghosh, P.; Guidolin, D. Potential Mechanism of Action of Intra-Articular Hyaluronan Therapy in Osteoarthritis: Are the Effects Molecular Weight Dependent? *Semin. Arthritis Rheum.* **2002**, *32*, 10–37. [CrossRef] [PubMed]
18. Bowman, S.; Awad, M.E.; Hamrick, M.W.; Hunter, M.; Fulzele, S. Recent advances in hyaluronic acid based therapy for osteoarthritis. *Clin. Transl. Med.* **2018**, *7*, 6. [CrossRef]
19. Brandt, K.D.; Block, J.A.; Michalski, J.P.; Moreland, L.W.; Caldwell, J.R.; Lavin, P.T. Efficacy and Safety of Intraarticular Sodium Hyaluronate in Knee Osteoarthritis. ORTHOVISC Study Group. *Clin. Orthop. Relat. Res.* **2001**, *385*, 130–143. [CrossRef] [PubMed]
20. Hunter, D.J. Viscosupplementation for Osteoarthritis of the Knee. *N. Engl. J. Med.* **2015**, *372*, 1040–1047. [CrossRef] [PubMed]
21. Iijima, H.; Eguchi, R.; Shimoura, K.; Yamada, K.; Aoyama, T.; Takahashi, M. Transcutaneous Electrical Nerve Stimulation Improves Stair Climbing Capacity in People with Knee Osteoarthritis. *Sci. Rep.* **2020**, *10*, 7294. [CrossRef] [PubMed]
22. Mota, R.E.M.; Tarricone, R.; Ciani, O.; Bridges, J.F.P.; Drummond, M. Determinants of Demand for Total Hip and Knee Arthroplasty: A Systematic Literature Review. *BMC Health Serv. Res.* **2012**, *12*, 225. [CrossRef]

23. Cherian, J.J.; Harrison, P.E.; Benjamin, S.A.; Bhave, A.; Harwin, S.F.; Mont, M.A. Do the Effects of Transcutaneous Electrical Nerve Stimulation on Knee Osteoarthritis Pain and Function Last? *J. Knee Surg.* **2016**, *29*, 497–501. [CrossRef] [PubMed]
24. Do Carmo Almeida, T.C.; Dos Santos Figueiredo, F.W.; Barbosa Filho, V.C.; de Abreu, L.C.; Fonseca, F.L.A.; Adami, F. Effects of Transcutaneous Electrical Nerve Stimulation on Proinflammatory Cytokines: Systematic Review and Meta-Analysis. *Mediat. Inflamm.* **2018**, *2018*, 1094352. [CrossRef] [PubMed]
25. Tumilty, S.; Munn, J.; McDonough, S.; Hurley, D.A.; Basford, J.R.; Baxter, G.D. Low Level Laser Treatment of Tendinopathy: A Systematic Review with Meta-analysis. *Photomed. Laser Surg.* **2010**, *28*, 3–16. [CrossRef] [PubMed]
26. Smoot, B.; Chiavola-Larson, L.; Lee, J.; Manibusan, H.; Allen, D.D. Effect of Low-Level Laser Therapy on Pain and Swelling in Women with Breast Cancer-Related Lymphedema: A Systematic Review and Meta-Analysis. *J. Cancer Surviv.* **2015**, *9*, 287–304. [CrossRef] [PubMed]
27. Hegedus, B.; Viharos, L.; Gervain, M.; Gálfi, M. The Effect of Low-Level Laser in Knee Osteoarthritis: A Double-Blind, Randomized, Placebo-Controlled Trial. *Photomed. Laser Surg.* **2009**, *27*, 577–584. [CrossRef] [PubMed]
28. Rothenberg, J.B.; Jayaram, P.; Naqvi, U.; Gober, J.; Malanga, G.A. The Role of Low-Intensity Pulsed Ultrasound on Cartilage Healing in Knee Osteoarthritis: A Review. *PM&R* **2017**, *9*, 1268–1277. [CrossRef]
29. Paliwal, S.; Mitragotri, S. Therapeutic Opportunities in Biological Responses of Ultrasound. *Ultrasonics* **2008**, *48*, 271–278. [CrossRef]
30. Schumann, D.; Kujat, R.; Zellner, J.; Angele, M.K.; Nerlich, M.; Mayr, E.; Angele, P. Treatment of Human Mesenchymal Stem Cells with Pulsed Low Intensity Ultrasound Enhances the Chondrogenic Phenotype In Vitro. *Biorheology* **2006**, *43*, 431–443. [PubMed]
31. Zhang, C.; Xie, Y.; Luo, X.; Ji, Q.; Lu, C.; He, C.; Wang, P. Effects of Therapeutic Ultrasound on Pain, Physical Functions and Safety Outcomes in Patients with Knee Osteoarthritis: A Systematic Review and Meta-Analysis. *Clin. Rehabilit.* **2016**, *30*, 960–971. [CrossRef]
32. De Oliveira Perrucini, P.D.; Poli-Frederico, R.C.; de Almeida Pires-Oliveira, D.A.; Dragonetti Bertin, L.; Beltrão Pires, F.; Shimoya-Bittencourt, W.; Martins Santos, V.; Medeiros Coelho, J.; Franco de Oliveira, R. Anti-Inflammatory and Healing Effects of Pulsed Ultrasound Therapy on Fibroblasts. *Am. J. Phys. Med. Rehabilit.* **2019**, *99*, 19–25. [CrossRef] [PubMed]
33. Dantas, L.O.; Moreira, R.D.F.C.; Norde, F.M.; Serrão, P.R.; Alburquerque-Sendín, F.; Salvini, T.F. The Effects of Cryotherapy on Pain and Function in Individuals with Knee Osteoarthritis: A Systematic Review of Randomized Controlled Trials. *Clin. Rehabilit.* **2019**, *33*, 1310–1319. [CrossRef]
34. Barbosa, G.M.; Cunha, J.E.; Cunha, T.M.; Martinho, L.B.; Castro, P.A.T.S.; Oliveira, F.F.B.; Cunha, F.Q.; Ramalho, F.S.; Salvini, T.F. Clinical-like Cryotherapy Improves Footprint Patterns and Reduces Synovial Inflammation in a Rat Model of Post-Traumatic Knee Osteoarthritis. *Sci. Rep.* **2019**, *9*, 14518. [CrossRef] [PubMed]
35. Dantas, L.O.; Jorge, A.E.; Regina Mendes da Silva Serrão, P.; Aburquerque-Sendín, F.; de Fatima Salvini, T. Cryotherapy Associated with Tailored Land-Based Exercises for Knee Osteoarthritis: A Protocol for a Double-Blind Sham-Controlled Randomised Trial. *BMJ Open* **2020**, *10*, e035610. [CrossRef]
36. Hou, Y.; Yang, Z.; Lin, J.; Chen, Y.; Xing, D.; Wang, B. Systematic Review of Clinical Practice Guidelines on Exercise Therapy for Knee Osteoarthritis and Critical Appraisal Using AGREEII Instrument. *Osteoarthr. Cartil.* **2018**, *26*, S324–S325. [CrossRef]
37. Song, Q.; Shen, P.; Mao, M.; Sun, W.; Zhang, C.; Li, L. Proprioceptive neuromuscular facilitation improves pain and descending mechanics among elderly with knee osteoarthritis. *Scand. J. Med. Sci. Sports* **2020**, *30*, 1655–1663. [CrossRef] [PubMed]
38. Noyes, F. *Noyes' Knee Disorders: Surgery, Rehabilitation, Clinical Outcomes*, 1st ed.; Saunders: Philadelphia, PA, USA, 2009; ISBN 978-1-4377-2118-8.
39. Schulz, K.F.; Altman, D.G.; Moher, D. CONSORT 2010 statement: Updated guidelines for reporting parallel group randomized trials. *BMC Med.* **2010**, *8*, 18. [CrossRef] [PubMed]
40. Faiz, K.W. VAS—Visual Analog Scale. *Tidsskr. Nor. Laegeforen* **2014**, *134*, 323. [CrossRef] [PubMed]
41. Alghadir, A.H.; Anwer, S.; Iqbal, A.; Iqbal, Z.A. Test-Retest Reliability, Validity, and Minimum Detectable Change of Visual Analog, Numerical Rating, and Verbal Rating Scales for Measurement of Osteoarthritic Knee Pain. *J. Pain Res.* **2018**, *11*, 851–856. [CrossRef]
42. Silman, A.; Smolen, J.; Weinblatt, M.; Weisman, M.; Hochberg, M.; Gravallese, E. *Rheumatology, 2-Volume Set*, 7th ed.; Elsevier: Amsterdam, The Netherlands, 2018; ISBN 978-0-7020-7306-9.
43. Brown, M.; Avers, D. Daniels and Worthingham's Muscle Testing, 10th ed.Saunders: Philadelphia, PA, USA, 2018; ISBN 978-0-323-56956-9.
44. Rice, D.A.; McNair, P.J. Quadriceps Arthrogenic Muscle Inhibition: Neural Mechanisms and Treatment Perspectives. *Semin. Arthritis Rheum.* **2010**, *40*, 250–266. [CrossRef] [PubMed]
45. Becker, R.; Berth, A.; Nehring, M.; Awiszus, F. Neuromuscular quadriceps dysfunction prior to osteoarthritis of the knee. *J. Orthop. Res.* **2004**, *22*, 768–773. [CrossRef] [PubMed]
46. Norkin, C.C.; White, D.J. *Measurement of Joint Motion: A Guide to Goniometry*; FA Davis: New York, NY, USA, 2009; ISBN 978-0-8036-2347-7.
47. Benner, R.W.; Shelbourne, K.D.; Bauman, S.N.; Norris, A.; Gray, T. Knee Osteoarthritis: Alternative Range of Motion Treatment. *Orthop. Clin. N. Am.* **2019**, *50*, 425–432. [CrossRef] [PubMed]
48. Murariu, G.; Popa, P.; Timofti, M.; Georgescu, L.P. Multivariate statistical analyses of Danube river water quality at Galati, Romania. *Environ. Eng. Manag. J.* **2018**, *17*, 1249–1266. [CrossRef]

49. Kyu, H.H.; Abate, D.; Abate, K.H.; Abay, S.M.; Abbafati, C.; Abbasi, N.; Abbastabar, H.; Abd-Allah, F.; Abdela, J.; Abdelalim, A.; et al. Global, regional, and national disability-adjusted life-years (DALYs) for 359 diseases and injuries and healthy life expectancy (HALE) for 195 countries and territories, 1990–2017: A systematic analysis for the Global Burden of Disease Study 2017. *Lancet* **2018**, *392*, 1859–1922. [CrossRef]
50. March, L.; Cross, M.; Lo, C.; Arden, N.; Gates, L.; Leyland, K.; Hawker, G.; King, L. Osteoarthritis: A Serious Disease. OARSI org. 2016, pp. 1–103. Available online: https://oarsi.org/sites/default/files/docs/2016/oarsi_white_paper_oa_serious_disease_121416_1.pdf (accessed on 1 March 2022).
51. Cross, M.; Smith, E.; Hoy, D.; Nolte, S.; Ackerman, I.; Fransen, M.; Bridgett, L.; Williams, S.; Guillemin, F.; Hill, C.L.; et al. The global burden of hip and knee osteoarthritis: Estimates from the Global Burden of Disease 2010 study. *Ann. Rheum. Dis.* **2014**, *73*, 1323–1330. [CrossRef] [PubMed]
52. Chae, K.J.; Choi, M.J.; Kim, K.Y.; Ajayi, F.F.; Chang, I.S.; Kim, I.S. *Osteoarthritis: Careand Management: Clinical Guideline*; National Institute for Health and Care, 2014. Available online: https://www.nice.org (accessed on 1 March 2022).
53. Bannuru, R.R.; Osani, M.C.; Vaysbrot, E.E.; Arden, N.K.; Bennell, K.; Bierma-Zeinstra, S.M.A.; Kraus, V.B.; Lohmander, L.S.; Abbott, J.H.; Bhandari, M.; et al. OARSI guidelines for the non-surgical management of knee, hip, and polyarticular osteoarthritis. *Osteoarthr. Cartil.* **2019**, *27*, 1578–1589. [CrossRef] [PubMed]
54. Rozental, T.D.; Sculco, T.P. Intra-articular corticosteroids: An updated overview. *Am. J. Orthop.* **2000**, *29*, 18–23.
55. Pourcho, A.M.; Smith, J.; Wisniewski, S.J.; Sellon, J.L. Intraarticular platelet-rich plasma injection in the treatment of knee osteoarthritis: Review and recommendations. *Am. J. Phys. Med. Rehabilit.* **2014**, *93*, S108–S121. [CrossRef]