Journal of Cartilage & Joint Preservation® 3 (2023) 100129

Contents lists available at ScienceDirect

Journal of Cartilage & Joint Preservation®

journal homepage: www.elsevier.com/locate/jcjp

Original Research

# Platelet-rich plasma is similar to platelet-rich plasma plus hyaluronic acid for the treatment of knee osteoarthritis at 2 years: a randomized controlled trial

Eric A. Branch[a], Joshua J. Cook[a], Achraf Cohen[b], Hillary Plummer[a], Alireza Emami[a], Jessica Truett[a], and Adam W. Anz[a,*]

[a] Andrews Institute Center for Regenerative Medicine, Department of Research, Andrews Research & Education Foundation, Gulf Breeze, FL, USA
[b] Department of Mathematics and Statistics, University of West Florida, Pensacola, FL, USA

ARTICLE INFO

*Keywords:*
Chondrodegeneration
Hyaluronic acid
Inflammatory biomarkers
Knee osteoarthritis
Platelet-rich plasma
Viscosupplementation

ABSTRACT

*Introduction:* Autologous platelet-rich plasma (PRP) and hyaluronic acid (HA) are common treatments for knee osteoarthritis (OA). Several studies have demonstrated PRP as a safe and effective treatment for OA and, in some studies, produces a better outcome than HA.
*Objective:* The primary objective of this study was to determine if HA injected with PRP would improve the efficacy of treatment of symptomatic knee OA compared to PRP alone. We hypothesized that the addition of HA would improve the efficacy of (PRP).
*Methods:* A total of 64 participants with Kellgren-Lawrence (KL) Grades 1 to 4 knee OA were randomized into 2 groups and scheduled to receive 3-injections. Group 1 [PRP] received injections of PRP. Group 2 [HA+PRP] received the same treatment, with an additional HA injection for the first 2 injections. Both groups were blindfolded for the first 2 injections. To evaluate the efficacy of the regimen, participants completed patient reported outcome measures (PROMs). PROMs were completed prior to injections and at 1, 3, 6, 12, 18, and 24 months after the final injection.
*Results:* Participants in both groups felt pain consistent with standard-of-care HA injection regimens with no difference in perceived pain. No infections or complications were observed. All PROM scores demonstrated improvement over baseline from 1-24 months post injections. There were no statistically significant differences between the groups.
*Conclusions:* For the treatment of OA, PRP alone or PRP concomitantly with HA performed similarly out to 24 months. PRP in combination with HA was not superior to PRP alone.

## Introduction

Osteoarthritis (OA), the most common type of arthritis, is a chronic musculoskeletal condition caused by excessive joint degeneration and inflammation that affects an estimated 27 million adults in the United States. Primary symptoms of OA include pain, aching, stiffness, swelling, and decreased range of motion within and around the damaged joint. Unfortunately, there are currently no cures for OA, leaving treatment of symptoms as the primary option for healthcare professionals.[1] Nonsurgical treatment options include medications such as nonsteroidal anti-inflammatory drugs, injectables, physical treatments such as rehabilitative therapy, unloading braces, and activity modifications (ie, canes or walkers).[2] The most common injectables are steroid, hyaluronic acid (HA) and platelet-rich plasma (PRP). HA is a naturally occurring glycosaminoglycan found ubiquitously around the body and specifi-

* Adam W. Anz, Andrews Research & Education Foundation, 1020 Gulf Breeze Pkwy, Gulf Breeze, FL 32561.
*Email address:* adam.anz@bhcpns.org (A.W. Anz).

https://doi.org/10.1016/j.jcjp.2023.100129
Received 17 October 2022; Revised 5 March 2023; Accepted 18 March 2023
Available online 23 March 2023

cally in synovial fluid.[3] There are several types of HA that are commonly defined by molecular weight, including low molecular weight HA, medium molecular weight HA, and high molecular weight HA.[4] High molecular weight HA specifically demonstrates viscoelastic biomechanical properties that have led to several theorized mechanisms of action that may impact joint health, including proteoglycan/glycosaminoglycan synthesis, reduction of nociceptor response and inflammatory pathways, and most commonly, chondroprotection through joint lubrication and CD44 binding.[4] Intra-articular injections of HA have been developed and proven as a safe and effective way to treat knee OA.[5,6] Although mixed results have been reported, several studies have found HA to possess a statistically significant difference in decreasing pain and improving function when compared with placebo.[3,7-9] PRP is a biologic product obtained from centrifuging autologous peripheral blood to separate and concentrate platelets (PLTs), leukocytes, growth factors, and other plasma proteins from the remaining blood components.[10] PRP is characterized as leukocyte-rich PRP or leukocyte-poor PRP, depending on neutrophil concentration.[11] Leukocyte-poor PRP is associated with anti-inflammatory effects, specifically in studies treating knee OA.[12-19] Although the therapeutic mechanism of action is not completely understood, PRP contains many anti-inflammatory and anabolic proteins that have in vitro evidence of chondroprotection, chondrogenesis, and the ability to decrease chondrocyte apoptosis.[16,20] Clinically, several randomized studies and meta-analyses have demonstrated that PRP is a safe and effective treatment for OA and, in some studies, has had better outcomes when compared directly with HA.[13,15,17-19,21-23] Clinical use of PRP for knee arthritis typically involves a 3-injection series over 3 weeks (ie, an injection once per week for 3 weeks), as a basic science study and 2 clinical studies have suggested superiority of a 3-injection series over a single injection.[15,24,25]

Concomitant treatments of PRP and HA should have a synergistic therapeutic effect, as they have distinct and independent mechanisms of action.[26,27] While theoretically plausible, the increased cost of this approach necessitates a clear advantage of combination therapy as compared with monotherapy before its use is recommended widely. Several recent clinical trials have studied combination therapy with PRP and HA for knee OA.[28-35] Thus, the primary objective of this study was to determine if HA injected at the same time as PRP would improve the efficacy of treatment compared to PRP alone in the treatment of symptomatic knee OA with a long-term follow up of 24 months. We hypothesized that the addition of HA would improve the efficacy PRP.[26,27] We also aimed to quantify the cellular content of the PRP samples.

## Methods

### *Participants*

This was a double-blinded, randomized controlled trial with 2 groups conducted in accordance with Consolidated Standards of Reporting Trials 2010 guidelines Fig. 1.[36] Institutional review board approval was obtained (number 1379083-1, Baptist Hospital Institutional Review Board) and the study was posted on clinicaltrials.gov (NCT03889925). Enrollment started March 2019 and completed May 2021 and was executed in the outpatient setting at the primary institute (Andrews Research & Education Foundation). Follow-up completed in July 2021, and final data analysis was completed by June 2022. No changes were made to methods, protocols, nor outcome measures after trial commencement. Patients between the ages of 30 and 80 years who had symptomatic and radiographic evidence of unilateral or bilateral OA in the tibiofemoral or patellofemoral compartment of the target knee (KL Grades 1-4) were screened for participation in this study by members of the research team. Inclusion criteria included documented diagnosis of primary OA for at least 6 weeks, as confirmed by radiographic imaging. Exclusion criteria included prior viscosupplementation, surgery, or biological treatments in the involved knee in the past 6 months, corticosteroid injection in any joint within 3 months prior to screening, a diagnosis of gout or other rheumatologic disease, and any inflammatory conditions. Potential participants were informed that the study may offer them direct benefit in the form of OA treatment via the injection regimen. Potential participants were also instructed not to take any prescription or over the counter nonsteroidal anti-inflammatory drugs or anti-inflammatory or antiplatelet medications for 1 week prior to the first injection and until after the final injection. If eligible and interested in participating, the potential participants went through the informed consent process (enrollment) with a member of the research team. No specific advertising nor recruitment materials were utilized. No compensation was given to participants in this study.

### *Randomization and injection regimen*

A 1:1 ratio, computer-generated simple randomization method was used for group allocation using sequentially numbered containers. The randomization method was generated and accessible by the research director, but not by members of the study team nor the principal investigator. The participants were randomized into the 2 study groups by the research director and subsequently scheduled by the research team to receive a series of 3 injections. Group 1 [PRP] received 3 intra-articular injections of liquid PRP dosed at 1-week intervals. Group 2 [HA+PRP] received 3 intra-articular injections of liquid PRP dosed at 1-week intervals, with 3 mL high molecular weight hyaluronic acid (Hymovis, Fidia Pharma) injected concomitantly for the first 2 injections. During the concomitant injections, group 2 [HA+PRP] received the PRP injection first, then received a separate HA injection. The Arthrex ACP System was used to prepare all PRP injections. All injections were prepared and delivered using the described regimens immediately at the point of care in the clinic (within approximately 20 minutes of the initial blood draw). Participants were blinded to injection randomization. Both groups wore a blindfold for the first 2 injections and were informed that multiple injections may be required due to the volume of the injection treatment.

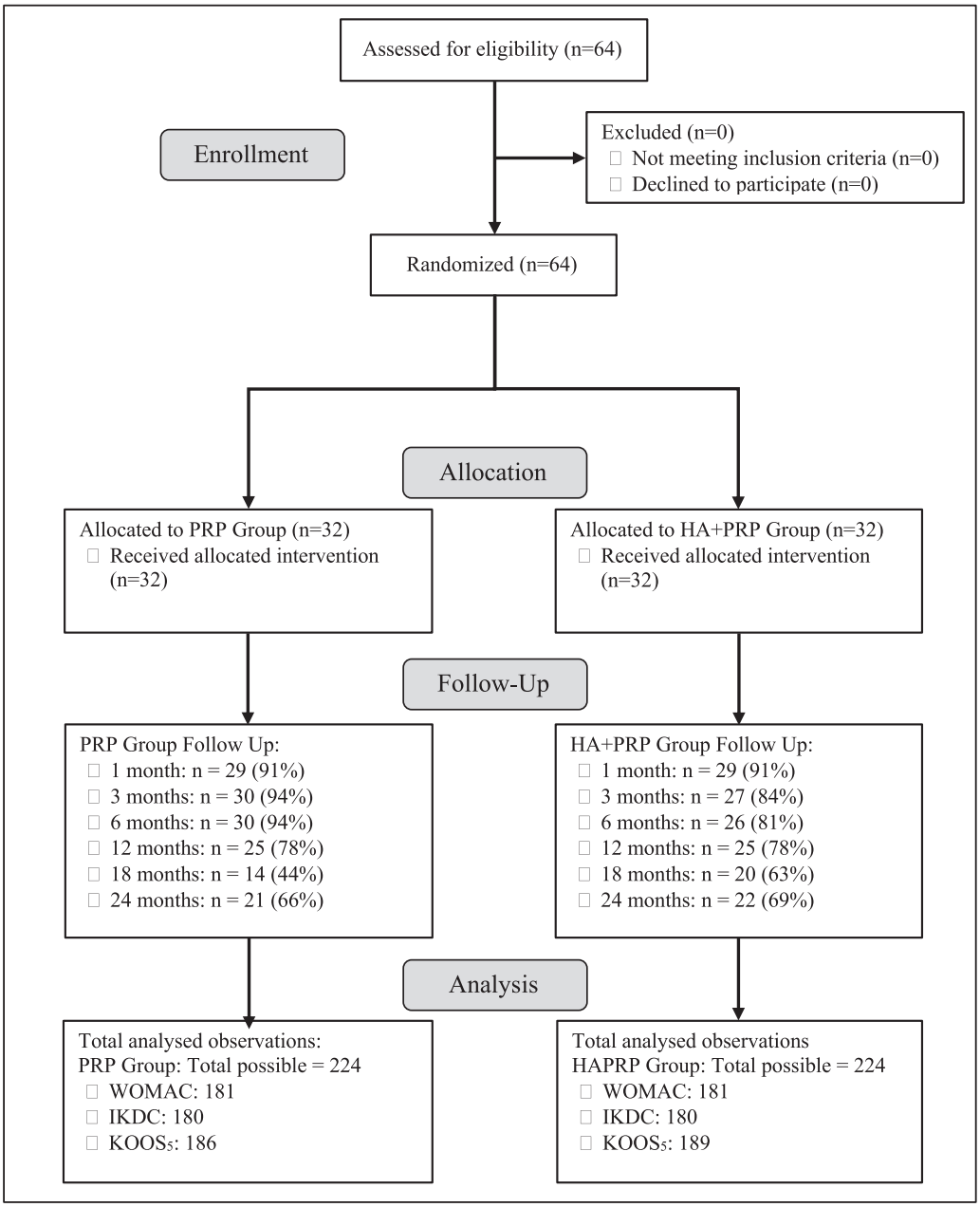


**Fig. 1.** Flowchart of participant assessment, randomization, allocation, follow-up, and analysis. HA, hyaluronic acid; IKDC, International Knee Documentation Committee; $KOOS_5$, Knee Injury and Osteoarthritis Outcome Score; PRP, platelet-rich plasma; WOMAC, Western Ontario and McMaster Universities Osteoarthritis Index.

*PRP preparation*

PRP was prepared with an Arthrex ACP System and Centrifuge (Arthrex, Inc.).[37] The ACP System has a reported PLT recovery rate of 200% compared to whole blood.[37] This involved harvesting 15 mL of whole blood from the patient's forearm following standard venipuncture technique. The blood was immediately centrifuged at 1500 rpm for 5 minutes at room temperature within the Arthrex Centrifuge (Horizon 24 Flex). The top layer of the soft stack was carefully aspirated into the inner injection syringe as standard in the ACP preparation, technique which produced 4 to 5 mL of a liquid leukocyte poor PRP. The bottom layer (containing mostly red blood cells [RBCs]) was disposed of according to standard operating procedures. The inner syringe was then unscrewed and removed, making the ACP ready for use at the point of care. About 100 uL was set aside in a microcentrifuge tube during injections for quantification of the PRP with an automated hematocytometer (XN-350, Sysmex). The remaining liquid PRP (approximately 4-5

mL) was used for the appropriate injection regimen. Due to the immediate use of the PRP at the point of care, anticoagulant citrate dextrose solution A was not used during preparation. Neither PRP nor whole blood samples were stored, and instead all PRP samples were immediately transferred to the Andrews Research & Education Foundation Regenerative Medicine Center for analysis.

*PRP quantification*

Within the Regenerative Medicine Center, all PRP samples were kept at room temperature while a complete blood cell (CBC) count was performed using the automated hematocytometer (XN-350). About 50 uL of each PRP sample was loaded into the hematocytometer and internally mixed with Cellpack DCL Diluent (Sysmex), Lysercell WDF Reagent (Sysmex), Sulfolyser Hematology Reagent (Sysmex), and Fluorocell WDF Fluorescent Reagent (Sysmex). This allowed for quantification of the following: white blood cell (WBC), RBC, hemoglobin, hematocrit PLT, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration (MCHC), mean platelet volume (MPV), RBC distribution width coefficient of variation, neutrophils, lymphocytes (Lymphs), monocytes (Monos), eosinophil, and basophil (Basos).

*PROMs and safety follow-up*

To evaluate the efficacy of the injection regimens, participants completed PROMs, including the WOMAC, which was the primary outcome measure in this study. Secondary PROMs included the IKDC and KOOS. For the KOOs a composite score was created incorporating all 5 subscores into one weighted composite score as described in prior study; it is this composite score which will be referred to as $KOOS_5$.[38,39] PROMs were completed prior to their injections and at 1, 3, 6, 12, 18, and 24 months after the final injection in the 3-injection series.

*Power analysis*

A power analysis was performed to determine the minimum sample size needed for this study based upon recent clinical trials comparing PRP to HA for the treatment of OA. An a priori power analysis using a power of 0.8 and alpha of .05 revealed that a minimum sample size of 25 participants in each group was needed to find clinically significant effects. As the study was designed with WOMAC as the primary outcome measure, the study was likewise powered to reflect its suggested sample size. Therefore, the enrollment was set at 64 participants to appropriately power for WOMAC and allow for expected losses to follow-up.

*Statistical analysis*

All participant data was de-identified prior to being analyzed by a statistician, allowing them to remain blinded. All data was entered into the Research Electronic Data Capture System and descriptive data was compiled before being exported for statistical analysis. Measures of central tendency (means) and measures of spread (SDs) were calculated for quantitative variables (age, body mass index [BMI]). A linear mixed-effects model was used to quantify the effects over time and the difference between the groups regarding the WOMAC, $KOOS_5$ and IKDC PROM scores. This model is also known as a multilevel linear model or hierarchical linear model.[40] The advantage of using a linear mixed-effects model is to provide a flexible approach to handle correlated longitudinal data and outcomes that are missing completely at random.[41,42] Additionally, this model was appropriate given the normal residuals of the outcome variables. The model is presented as follows:

$$y_{ijk} = \beta_0 + \beta_1 t_k + \beta_2 groups_i + \text{random effects} + \varepsilon_{ijk}$$

where $y_{ijk}$ is the score at time $t_k$ ($k = 1, \ldots, 8$) for patient $j$ in group $i$ (ie, $i = 1$ for PRP), $\beta_i$ ($i = 0,1,2$) are fixed effects. The group (PRP and HA+PRP) is a fixed effect, testing if a statistical difference exists between the groups' mean scores. Time was defined as a categorical variable, allowing comparisons to be made between follow-up time points and baseline PROM scores.

## Results

The study sample included 64 total participants. Participants were randomized using a computer-generated sequence into 2 groups: PRP ($n = 32$) and HA+PRP ($n = 32$). Group 1 (PRP) participants had an average age of 55.78 ± 11.42 years, sex ratio of 12:20 male to female, BMI of 27.80 ± 4.97, and were identified as KL Grades 1 (58%), 2 (30%), 3 (6.2%), and 4 (6.2%). Group 2 (HA+PRP) participants had an average age of 60.66 ± 8.99 years, sex ratio of 16:16 male to female, BMI of 28.87 ± 8.62, and were identified as KL Grades 1 (66%), 2 (25%), 3 (3.1%), and 4 (6.2%). There was no statistically significant difference in any demographic characteristic between groups 1 and 2 (Table 1). Participants in both groups felt pain consistent with standard-of-care HA injection regimens and no difference in perceived pain was noted by the injectors. None of the participants showed any indication of infection nor complication for the duration of the study.

All participants received the allocated treatment and completed the baseline questionnaires. All scores in both groups demonstrated improvement over baseline from 1 to 24 months postinjection series with improvement plateauing at 3 months for both ($P < .001$, Table 2). Despite both PRP and HA+PRP groups demonstrating overall improvement, there were no statistically significant differences between the 2 treatment groups. Table 3 contains the results of the linear mixed effects model; including estimates, associated 95% CIs and $P$ values.

**Table 1**
Participant demographics.

| Characteristic | Overall, $N = 64$ | Treatment | | $P$ value* |
|---|---|---|---|---|
| | | PRP, $N = 32$ | PRP+HA, $N = 32$ | |
| Sex, n(%) | | | | .3 |
| Female | 36 (56) | 20 (62) | 16 (50) | |
| Male | 28 (44) | 12 (38) | 16 (50) | |
| Affected knee, n(%) | | | | >.9 |
| Left | 32 (50) | 16 (50) | 16 (50) | |
| Right | 32 (50) | 16 (50) | 16 (50) | |
| KL Grade (affected knee), n(%) | | | | .6 |
| 1 | 37 (58) | 16 (50) | 21 (66) | |
| 2 | 19 (30) | 11 (34) | 8 (25) | |
| 3 | 4 (6.2) | 3 (9.4) | 1 (3.1) | |
| 4 | 4 (6.2) | 2 (6.2) | 2 (6.2) | |
| Age at treatment (y), mean ± SD | 58.22 ± 10.49 | 55.78 ± 11.42 | 60.66 ± 8.99 | .10 |
| Height (in), mean ± SD | 67.10 ± 3.58 | 67.12 ± 3.57 | 67.07 ± 3.65 | >.9 |
| Weight (lbs), mean ± SD | 181.73 ± 45.98 | 179.19 ± 39.09 | 184.28 ± 52.49 | >.9 |
| BMI, mean ± SD | 28.33 ± 7.00 | 27.80 ± 4.97 | 28.87 ± 8.62 | .7 |

Abbreviations: BMI, body mass index; HA, hyaluronic acid; KL, Kellgren and Lawrence System for Classification of Osteoarthritis; PRP, platelet-rich plasma.

* Comparison between PRP, PRP+HA Groups. $t$ test (numerical variables, normal distribution), Wilcoxon rank-sum test (numerical variables, non-normal distribution), $\chi^2$ test of independence (categorical variables). An asterisk indicates significant differences among tissue types at $P < .05$.

**Table 2**
Patient reported outcome measures data.*

| PROM | Pretreatment | 1 mo | 3 mo | 6 mo | 12 mo | 18 mo | 24 mo |
|---|---|---|---|---|---|---|---|
| IKDC | | | | | | | |
| PRP | 39.2 ± 10.4 | 50.1 ± 17.3 | 50.4 ± 17.6 | 53.1 ± 16.0 | 50.6 ± 19.1 | 57.0 ± 23.6 | 50.9 ± 20.1 |
| Δ Baseline, % | | 27.8% | 28.6% | 35.5% | 29.1% | 45.4% | 29.8% |
| HA+PRP | 38.6 ± 14.5 | 53.7 ± 14.1 | 56.9 ± 17.6 | 53.1 ± 19.9 | 55.7 ± 21.5 | 53.4 ± 24.5 | 51.8 ± 21.9 |
| Δ Baseline, % | | 39.1% | 47.4% | 37.6% | 44.3% | 38.3% | 34.2% |
| WOMAC | | | | | | | |
| PRP | 40.0 ± 18.6 | 24.9 ± 15.6 | 26.1 ± 20.1 | 21.9 ± 15.3 | 25.9 ± 18.7 | 21.0 ± 15.1 | 26.7 ± 17.8 |
| Δ Baseline, % | | −37.8% | −34.8% | −45.3% | −35.3% | −47.5% | −33.3% |
| HA+PRP | 40.5 ± 18.7 | 22.4 ± 15.2 | 22.3 ± 16.3 | 22.5 ± 16.5 | 22.7 ± 19.3 | 27.1 ± 23.8 | 29.2 ± 23.3 |
| Δ Baseline, % | | −44.7% | −44.9% | −44.4% | −44.0% | −33.1% | −27.9% |
| $KOOS_5$ | | | | | | | |
| PRP | 42.7 ± 12.7 | 59.4 ± 13.5 | 59.0 ± 18.8 | 64.2 ± 16.3 | 61.8 ± 17.7 | 65.3 ± 18.2 | 59.8 ± 19.5 |
| Δ Baseline, % | | 39.1% | 38.2% | 50.4% | 44.7% | 52.9% | 40.0% |
| HA+PRP | 44.3 ± 14.4 | 62.7 ± 12.5 | 62.9 ± 20.5 | 62.5 ± 18.1 | 61.8 ± 20.4 | 60.1 ± 25.4 | 59.0 ± 24.4 |
| Δ Baseline, % | | 41.5% | 42.0% | 41.1% | 39.5% | 35.7% | 33.2% |

Abbreviations: PROM, patient reported outcome measure; HA, hyaluronic acid; IKDC, International Knee Documentation Committee; $KOOS_5$, Knee Injury and Osteoarthritis Outcome Score; PRP, platelet-rich plasma; WOMAC, Western Ontario and McMaster Universities Osteoarthritis Index.

* Values are reported as mean ± SD unless otherwise noted.

**Table 3**
Linear mixed-effects models for WOMAC, IKDC, and $KOOS_5$ scores.

| Predictors* | WOMAC | | | IKDC | | | $KOOS_5$ | | |
|---|---|---|---|---|---|---|---|---|---|
| | Estimates | 95% CI | $P$ | Estimates | 95% CI | $P$ | Estimates | 95% CI | $P$ |
| (Intercept) | 40.11 | 34.13 to 46.09 | <.001 | 40.14 | 35.16 to 45.11 | <.001 | 44.33 | 39.67 to 49.00 | <.001 |
| Time 1 mo | −15.35 | −19.45 to −11.25 | <.001 | 12.10 | 8.55 to 15.65 | <.001 | 16.82 | 13.18 to 20.46 | <.001 |
| Time 3 mo | −14.64 | −18.76 to −10.52 | <.001 | 13.52 | 9.88 to 17.15 | <.001 | 16.79 | 12.45 to 21.12 | <.001 |
| Time 6 mo | −16.40 | −20.55 to −12.2 | <.001 | 13.08 | 9.30 to 16.85 | <.001 | 18.61 | 15.04 to 22.18 | <.001 |
| Time 12 mo | −12.76 | −17.06 to −8.46 | <.001 | 10.73 | 6.27 to 15.20 | <.001 | 16.29 | 12.21 to 20.37 | <.001 |
| Time 18 mo | −12.28 | −17.18 to −7.38 | <.001 | 11.69 | 5.95 to 17.43 | <.001 | 15.59 | 10.75 to 20.44 | <.001 |
| Time 24 mo | −10.59 | −15.11 to −6.08 | <.001 | 10.90 | 4.55 to 17.25 | <.001 | 13.50 | 7.30 to 19.69 | <.001 |
| Group [PRP] | 0.28 | −7.52 to 8.08 | .943 | −2.43 | −9.01 to 4.15 | .463 | −1.62 | −7.60 to 4.36 | .590 |

Abbreviations: HA, hyaluronic acid; IKDC, International Knee Documentation Committee; $KOOS_5$, Knee Injury and Osteoarthritis Outcome Score; PRP, platelet-rich plasma; WOMAC, Western Ontario and McMaster Universities Osteoarthritis Index.

* "Group" is a fixed effects variable. Each "Time" variable is a fixed effect, categorical variable representing time intervals 1, 3, 6, 12, 18, and 24 months.

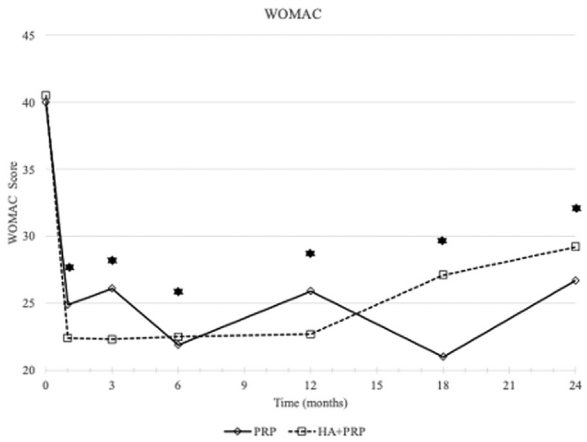


**Fig. 2.** Mean Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) scores over time. An asterisk indicates significant differences among each group to their baseline value, at $P < .05$. No differences were detected between the 2 groups. HA, hyaluronic acid; PRP, platelet-rich plasma.

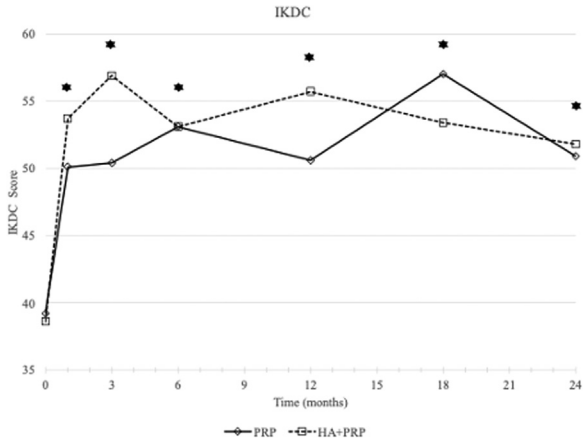


**Fig. 3.** Mean International Knee Documentation Committee (IKDC) scores over time. An asterisk indicates significant differences among each group to their baseline value, at $P < .05$. No differences were detected between the 2 groups. HA, hyaluronic acid; PRP, platelet-rich plasma.

Compliance for the PROMs was consistent out to 12 months postinjection series for both PRP ($n = 25$, 78%) and HA+PRP ($n = 25$, 78%). Of note, compliance was at its lowest at 18 months for PRP ($n = 14$, 44%) and HA+PRP ($n = 20$, 63%), which resulted in a temporary decreased compliance rate compared to 24 months. At 24 months compliance rate increased for both groups PRP ($n = 21$, 66%) and HA+PRP ($n = 22$, 69%). The total number of planned observations for each PROM score was 448, or 224, for each group. WOMAC total survey observations were 362, representing 81% capture rate, $KOOS_5$ observations were 375 or an 84% capture rate, and IKDC observations were 360 for an 80% capture rate.

The average WOMAC score decreased from baseline in both groups. For the WOMAC scores, the effect of time was statistically significant ($P < .001$). Comparing PRP vs HA+PRP groups, there was no evidence of significant difference between the 2 groups ($P = .943$). The average $KOOS_5$ score increased from baseline for both groups. For the $KOOS_5$ scores, the effect of time was statistically significant ($P < .001$). There was no significant difference between the means of $KOOS_5$ scores for the PRP and HA+PRP groups ($P = .590$). The average IKDC score increased from baseline for both groups. For the IKDC scores, the effect of time was statistically significant ($P < .001$). There was no significant difference between the means of IKDC scores for the PRP and HA+PRP groups ($P = .463$). All PROM score values (mean ± SD) are included in Table 2. WOMAC, IKDC, and $KOOS_5$ longitudinal data comparisons are illustrated in Figures 2-4.

Results from the CBC analysis of the PRP samples are presented in Table 4, which show significant differences between the content of PRP used in the PRP or the HA+PRP groups only in terms of MPV, % neutrophils, % Lymphs, % Monos, % Basos, and absolute Basos. Importantly, all samples from both groups were analyzed but many samples had no reading for the marker of interest and were recorded as “Samples with No Value.”

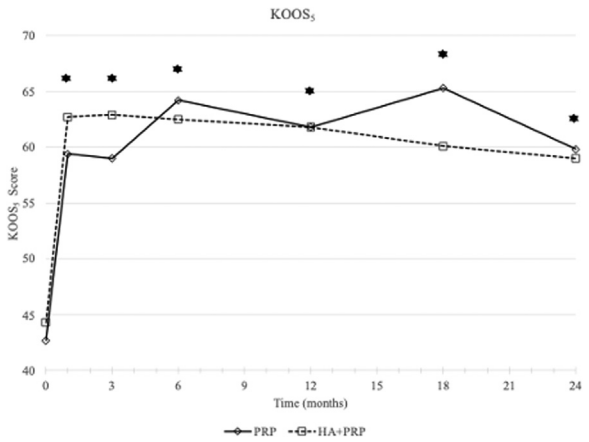


**Fig. 4.** Mean Knee Injury and Osteoarthritis Outcome Score ($KOOS_5$) scores over time. An asterisk indicates significant differences among each group to their baseline value, at $P < .05$. No differences were detected between the 2 groups. HA, hyaluronic acid; PRP, platelet-rich plasma.

## Discussion

In general, the lack of increased pain, infections, and adverse events was expected given the existing safety studies on HA and PRP. However, a recent systematic review by Zhang et al[43] reviewing the efficacy and safety of PRP compared to PRP+HA suggested that PRP+HA was generally safer than standalone PRP injections. This review indicated that across 9 studies, PRP represented 54 adverse events while PRP+HA represented 28.[43] Similar to our study, 2 of the 9 studies had 0 adverse events associated with PRP and 3 of the 9 had 0 adverse events associated with PRP+HA.[43] The most important finding of this study was that a combination of HA+PRP was not superior to PRP alone and both groups demonstrated improvement over baseline PROMs out to 24 months, with overall improvement plateauing and remaining constant at 3 months postinjection series. The HA+PRP group demonstrated a trend of increased improvement in PROMs compared to the PRP group at 1 and 3 months postinjection series despite not being statistically different than the PRP group. This may suggest that the HA+PRP group benefitted from a faster clinical effect that is only sustained out to 3 months postinjection. Specifically regarding the WOMAC (which was adequately powered out to 12 months postinjection series), our study demonstrated a statistically significant and minimal clinically important difference (MCID) change from baseline of −11.3 and −13.3 (negative indicating a decrease in score and thus an improvement in condition) at 24 months for HA+PRP and PRP groups respectively. Comparisons of the WOMAC at baseline to 24 months were not adequately powered due to the decrease in compliance after the 12-month follow-up but followed the same trend as the previous timepoints nonetheless. The decrease in PROM compliance toward the end of the study was expected given the compliance patterns of long-term (ie, past 6-12 months) studies despite the fact that we had no explicit drop-outs nor withdrawals. Additionally, this study quantified the PRP contents being injected and found variation between the 2 groups in MPV, % neutrophils, % Lymphs, % Monos, % Basos, and absolute Basos. We do not believe these markers represent confounding variables since no one group had higher or lower concentrations of a specific biomarker. Importantly, several markers of interest in the CBC analyses were observed to be largely absent in PRP, such as MCHC and red blood cell distribution width coefficient of variation. Other values, such as IG and subtypes of WBCs, were absent in some but not most of the PRP samples.

In 3 studies evaluating the WOMAC postinjection of PRP or HA+PRP, the MCID was found to be −7.86.[44-46] These studies observed improvements in the WOMAC for each group but did not observe a statistically significant nor MCID improvement between PRP and PRP+HA groups at 3 months postinjection.[44-46] However, a statistically significant difference between the groups was observed at 6 months postinjection.[44-46] These studies did not continue into long-term follow-up, which explains their overall lower rate of PROM compliance issues and supports our presented study. Furthermore, our study supports these results since both the PRP and HA+PRP groups improved separately out to 24 months postinjection series despite not showing a significant difference nor MCID between the 2 groups. Importantly, our study differs from the previous literature by showing a MCID in both groups separately (−11.3 and −13.3 at 24 months for HA+PRP and PRP) compared to the historical value of −7.86. Our study also showed separate improvements in the IKDC and KOOS out to 24 months postinjection compared to baseline for each group. This is in-line with studies by Jacob et al and Chen-Rong et al who observed separate statistical and MCID improvements in the IKDC score 6 months postinjection for the PRP and HA+PRP groups, despite only detecting statistical differences but not MCID between the 2 groups.[29,44] For the KOOS, our results are similar to Abate et al and Huang et al who observed separate statistical and MCID improvements in the KOOS score 3 months and 6 months postinjection for the PRP and HA+PRP groups, but only detected group statistical differences at 3 months postinjection.[31,47] Although our study supports the results of others regarding changes in the IKDC and KOOS, our study was underpowered for these PROMs. Thus, we are specifically emphasizing the results of the WOMAC as our adequately-powered, primary endpoint up to 12 months of follow-up with a consistent trend being observed out to 24 months. To further support the PROMs findings and ameliorate the issue of random missing data, a linear mixed-effects model was used to quantify the effects over time and the difference between groups regarding each of the PROMs scores. The linear mixed-effects model is very similar to traditional repeated measures analysis

**Table 4**
Platelet-rich plasma product analysis.

| Characteristic* | Overall, *N* | Treatment | | *P* value† |
|---|---|---|---|---|
| | | PRP, *N* = 91 | PRP+HA, *N* = 91 | |
| WBC (K/uL), mean ± SD | 1.28 ± 2.19 | 1.33 ± 2.51 | 1.22 ± 1.84 | >.9 |
| *Samples with no value* | 3 | 1 | 2 | |
| RBC (MIL/uL), mean ± SD | 0.03 ± 0.12 | 0.05 ± 0.17 | 0.02 ± 0.02 | .2 |
| *Samples with no value* | 3 | 1 | 2 | |
| HGB (g/dL), mean ± SD | 0.06 ± 0.37 | 0.10 ± 0.52 | 0.02 ± 0.05 | .6 |
| *Samples with no value* | 3 | 1 | 2 | |
| HCT (%), mean ± SD | 0.21 ± 1.06 | 0.33 ± 1.49 | 0.09 ± 0.13 | .2 |
| *Samples with no value* | 3 | 1 | 2 | |
| PLT (K/uL), mean ± SD | 404.04 ± 116.53 | 406.32 ± 108.96 | 401.74 ± 124.29 | .9 |
| *Samples with no value* | 3 | 1 | 2 | |
| MCV (fL), mean ± SD | 41.42 ± 28.54 | 42.19 ± 29.87 | 40.63 ± 27.26 | .7 |
| *Samples with no value* | 21 | 9 | 12 | |
| MCH (pg), mean ± SD | 9.35 ± 25.88 | 7.00 ± 17.10 | 11.79 ± 32.53 | >.9 |
| *Samples with no value* | 21 | 9 | 12 | |
| MCHC (g/dL), mean ± SD | 15.20 ± 34.61 | 14.22 ± 29.83 | 16.17 ± 38.98 | .7 |
| *Samples with no value* | 59 | 30 | 29 | |
| MPV (fL), mean ± SD | 9.72 ± 0.69 | 9.82 ± 0.68 | 9.63 ± 0.68 | .047** |
| *Samples with no value* | 3 | 1 | 2 | |
| RDW-CV (%), mean ± SD | 32.99 ± 19.06 | 29.42 ± 21.85 | 37.45 ± 16.85 | .4 |
| *Samples with no value* | 173 | 86 | 87 | |
| % Neutrophils, mean ± SD | 15.85 ± 10.87 | 18.15 ± 11.33 | 13.47 ± 9.92 | .007** |
| *Samples with no value* | 56 | 27 | 29 | |
| % Lymphs, mean ± SD | 69.40 ± 13.16 | 67.39 ± 14.10 | 71.49 ± 11.86 | .043** |
| *Samples with no value* | 56 | 27 | 29 | |
| % Monos, mean ± SD | 13.41 ± 4.69 | 12.54 ± 4.09 | 14.31 ± 5.12 | .040** |
| *Samples with no value* | 56 | 27 | 29 | |
| % EOS, mean ± SD | 0.30 ± 0.57 | 0.35 ± 0.55 | 0.25 ± 0.59 | .2 |
| *Samples with no value* | 56 | 27 | 29 | |
| % Basos, mean ± SD | 0.53 ± 0.57 | 0.71 ± 0.65 | 0.34 ± 0.41 | <.001** |
| *Samples with no value* | 56 | 27 | 29 | |
| % Imm. Granulocytes, mean ± SD | 0.13 ± 0.45 | 0.13 ± 0.44 | 0.13 ± 0.47 | .9 |
| *Samples with no value* | 56 | 27 | 29 | |
| Absolute Lymph (K/uL), mean ± SD | 1.20 ± 1.43 | 1.17 ± 1.35 | 1.23 ± 1.52 | >.9 |
| *Samples with no value* | 56 | 27 | 29 | |
| Absolute Monos (K/uL), mean ± SD | 0.24 ± 0.34 | 0.23 ± 0.33 | 0.25 ± 0.35 | .7 |
| *Samples with no value* | 56 | 27 | 29 | |
| Absolute EOS (K/uL), mean ± SD | 0.01 ± 0.04 | 0.01 ± 0.05 | 0.00 ± 0.01 | .3 |
| *Samples with no value* | 56 | 27 | 29 | |
| Absolute Basos (K/uL), mean ± SD | 0.01 ± 0.02 | 0.01 ± 0.02 | 0.01 ± 0.01 | .012** |
| *Samples with no value* | 56 | 27 | 29 | |
| Absolute Imm. Granulocytes (K/uL), mean ± SD | 0.00 ± 0.02 | 0.01 ± 0.03 | 0.00 ± 0.00 | .7 |
| *Samples with no value* | 56 | 27 | 29 | |

Abbreviations: Basos, basophils; Imm. Granulocytes, immature granulocytes; EOS, eosinophil; HA, hyaluronic acid; HCT, hematocrit; HGB, hemoglobin; Lymphs, lymphocytes; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; Monos, monocytes; MPV, mean platelet volume; PLT, platelet; PRP, platelet-rich plasma; RBC, red blood cell; RDW-CV, red blood cell distribution width coefficient of variation; WBC, white blood cell.

* Samples with no value were analyzed but had no reading for the marker of interest.

† Comparison between PRP, PRP+HA Groups; T-test (numerical variables, normal distribution), Wilcoxon rank-sum test (numerical variables, non-normal distribution), $\chi^2$ test of independence (categorical variables).

** Significant differences among tissue types at $P < .05$.

of variance, but it is better suited to handle unbalanced groups and missing data, which was the case during the later follow-up time points. Our model defined patients and treatment group as fixed effects with time as a categorical variable, allowing us to quantify the effects over time for each of the PROM scores. The effect of time was statistically significant to the PROM score at each timepoint but the treatment group had no effect. This finding suggested that the follow-up time was impacting the PROM score, with point estimates expecting a gradual statistical improvement as time progressed. This was observed in our study, despite the aforementioned issues regarding power and lack of differences between the treatment groups.

PRP has benchtop and clinical evidence that supports its use for the indication of OA. In benchtop studies, PRP has a clear mechanism of action to improve the catabolic and inflammatory environment of OA.[48] Clinical trials in humans have established safety and efficacy in case series, comparative cohort and randomized controlled trials,[49-52] and systematic reviews of the literature.[53,54] Recent systematic reviews and meta-analyses have concluded greater clinical improvements in pain and function with PRP injections as compared with controls, with only one review concluding that there may be an increased risk of local adverse reactions after multiple PRP injections.[53,54] Ding et al,[45] in a meta-analysis of 14 RCTs, reported significant reduction of pain and improvement

of functional outcomes from 3 to 12 months when comparing PRP with steroid, HA, and placebo. This study supported the existing literature by showing that PRP, and PRP combined with HA both lead to a significant reduction of pain and improvement of functional outcomes up to 24 months postinjection series. Meta-analysis by Belk et al reported PRP to result in significant improvements in pain and function when compared with HA at an average follow-up of 11.1 months.[21] When treating patients with OA, one randomized study has found a trend toward improved efficacy of PRP in patients with KL grades lower than 2.[55] This implies there could be a higher efficacy in the lower KL grades. Unfortunately, this study did not include a wide enough distribution of KL grades to subgroup our results. Evaluation of point-of-care biologic therapeutic products have been plagued by heterogeneity of quality and content. Over the last several years great strides have been made in defining terminology and defining product cellular quantity as well as quality. This study quantified PRP using a common and reliable measure, a CBC count with differential, via a hemocytometer. Not all values measured by the CBC count are present as they are in whole blood, but many key parameters such as RBC, WBC, and PLT can be accurately measured. This method aimed to reduce the heterogeneity and ambiguity of PRP quantification in a common, inexpensive, and reproducible manner.

There is growing evidence of PRP's superiority to HA in direct head-to-head trials and meta-analyses. Therefore, for studies showing that PRP+HA is better than HA alone, it is possible that they are demonstrating just the superior effect of PRP over HA rather than a synergistic effect. This is supported by the fact that the studies that controlled for PRP by using it as monotherapy failed to demonstrate that the addition of HA provided consistent benefit.[56] Lana et al[28] provides the best study design to test this hypothesis, which compared PRP monotherapy vs HA monotherapy vs combination therapy. The PRP group had significantly greater reduction in visual analog scale scores at 30, 90, 180, and 360 days and significantly greater WOMAC physical activity improvement at 360 days compared to the HA group. Combining HA and PRP resulted in significantly less pain and less functional limitation compared to HA alone up to 1 year after treatment. HA+PRP combination also resulted in significantly more physical function early in the treatments compared to PRP alone, however only out to 1 month and 3 months, respectively.[28] A major strength of our study design compared to those in the literature is the inclusion of long-term follow-up timepoints, out to 24 months postinjection series. Contrasting studies follow to 12 months at most and thus may fail to identify longer term treatment outcome trends, and falsely extrapolate conclusions on short term improvement. Importantly, short-term benefits did seem to be present for the HA+PRP group in our study and we confirmed that they are sustained out to 24 months postinjection series despite a difference between groups not being detected.

## Limitations

The limitations of this study include the lack of a true placebo group, only measurement of clinical symptomatology without radiological evidence, lack of KL grade comparisons, and underpowered PROMs. This study did not include a true placebo group, such as the use of a saline injection, because several studies have already compared HA and PRP separately to a true placebo as well as intragroup comparisons.[57,58] The use of placebo groups also come with 2 limitations of their own. First, placebo groups have been suggested as a cause for low clinical trial enrollment rates, which directly limit the completion and generalizability of studies. Second, it has been suggested that the use of a true placebo in light of a proven and available treatment option may have ethical implications with regards to patient safety.[59] Due to this, our findings should be compared to the available historical data of other studies that involved true placebos, albeit we do recognize that this external requirement is a limitation of our study. Future studies should capture both PROMs and measure improvement radiologically or by objective, quantitative methods. Additionally, efficacy of treatment may differ in mild KL 1-2 compared to KL 3-4, but this study did not have a large enough distribution of KL grades to study this further. However, a prior study has demonstrated improved outcomes in patients with KL 1-2 when using PRP.[55] Finally, the study was appropriately powered and designed for WOMAC as the primary outcome measure out to 12 months postinjection series, but is underpowered past 12 months due to compliance issues. A power analysis was not performed for the IKDC and KOOS, so it is likely that these PROMs are underpowered and were treated as such during the study.

## Conclusion

For the treatment of OA, PRP alone or PRP administered concomitantly with HA performed similarly out to 24 months. PRP administered in combination with HA was not superior to PRP alone.

## Funding

This project was funded as an investigator-initiated, in-house study within the Andrews Research & Education Foundation.

## Ethics approval

Complete written informed consent was obtained from the patients for the publication of this study and accompanying images.

## Declaration of competing interest

All authors have no conflicts of interest to declare.

## Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jcjp.2023.100129.

## References


1. Cunningham LS, Kelsey JL. Epidemiology of musculoskeletal impairments and associated disability. *Am J Public Health*. 1984;74(6):574–579. doi:10.2105/ajph.74.6.574.
2. Robson EK, Hodder RK, Kamper SJ, et al. Effectiveness of weight-loss interventions for reducing pain and disability in people with common musculoskeletal disorders: a systematic review with meta-analysis. *J Orthop Sports Phys Ther*. 2020;50(6):319–333.
3. Altman R, Manjoo A, Fierlinger A, Niazi F, Nicholls M. The mechanism of action for hyaluronic acid treatment in the osteoarthritic knee: a systematic review. *BMC Musculoskelet Disord*. 2015;16(1):1–10.
4. Iturriaga V, Vásquez B, Bornhardt T, Del Sol M. Effects of low and high molecular weight hyaluronic acid on the osteoarthritic temporomandibular joint in rabbit. *Clin Oral Investig*. 2021;25(7):4507–4518. doi:10.1007/s00784-020-03763-x.
5. Bannuru R, Natov N, Dasi U, Schmid C, McAlindon T. Therapeutic trajectory following intra-articular hyaluronic acid injection in knee osteoarthritis–meta-analysis. *Osteoarthr Cartil*. 2011;19(6):611–619.
6. Bannuru RR, Vaysbrot EE, Sullivan MC, McAlindon TE. Relative efficacy of hyaluronic acid in comparison with NSAIDs for knee osteoarthritis: a systematic review and meta-analysis. *Semin Arthritis Rheum*. 2014;43(5):593–599.
7. Bowman S, Awad ME, Hamrick MW, Hunter M, Fulzele S. Recent advances in hyaluronic acid based therapy for osteoarthritis. *Clin Transl Med*. 2018;7(1):1–11.
8. Singh H, Knapik DM, Polce EM, et al. Relative efficacy of intra-articular injections in the treatment of knee osteoarthritis: a systematic review and network meta-analysis. *Am J Sports Med*. 2022;50(11):3140–3148.
9. Jevsevar DS, Shores PB, Mullen K, Schulte DM, Brown GA, Cummins DS. Mixed treatment comparisons for nonsurgical treatment of knee osteoarthritis: a network meta-analysis. *J Am Acad Orthop Surg*. 2018;26(9):325–336.
10. Alves R, Grimalt R. A review of platelet-rich plasma: history, biology, mechanism of action, and classification. *Skin Appendage Disord*. 2018;4(1):18–24. doi:10.1159/000477353.
11. Le ADK, Enweze L, DeBaun MR, Dragoo JL. Current clinical recommendations for use of platelet-rich plasma. *Curr Rev Musculoskelet Med*. 2018;11(4):624–634. doi:10.1007/s12178-018-9527-7.
12. Cerza F, Carnì S, Carcangiu A, et al. Comparison between hyaluronic acid and platelet-rich plasma, intra-articular infiltration in the treatment of gonarthrosis. *Am J Sports Med*. 2012;40(12):2822–2827.
13. Cole BJ, Karas V, Hussey K, Merkow DB, Pilz K, Fortier LA. Hyaluronic acid versus platelet-rich plasma: a prospective, double-blind randomized controlled trial comparing clinical outcomes and effects on intra-articular biology for the treatment of knee osteoarthritis. *Am J Sports Med*. 2017;45(2):339–346.
14. Dallari D, Stagni C, Rani N, et al. Ultrasound-guided injection of platelet-rich plasma and hyaluronic acid, separately and in combination, for hip osteoarthritis: a randomized controlled study. *Am J Sports Med*. 2016;44(3):664–671.
15. Görmeli G, Görmeli CA, Ataoglu B, Çolak C, Aslantürk O, Ertem K. Multiple PRP injections are more effective than single injections and hyaluronic acid in knees with early osteoarthritis: a randomized, double-blind, placebo-controlled trial. *Knee Surg, Sports Traumatol, Arthrosc*. 2017;25(3):958–965.
16. Moussa M, Lajeunesse D, Hilal G, et al. Platelet rich plasma (PRP) induces chondroprotection via increasing autophagy, anti-inflammatory markers, and decreasing apoptosis in human osteoarthritic cartilage. *Exp Cell Res*. 2017;352(1):146–156.
17. Patel S, Dhillon MS, Aggarwal S, Marwaha N, Jain A. Treatment with platelet-rich plasma is more effective than placebo for knee osteoarthritis: a prospective, double-blind, randomized trial. *Am J Sports Med*. 2013;41(2):356–364.
18. Sánchez M, Fiz N, Azofra J, et al. A randomized clinical trial evaluating plasma rich in growth factors (PRGF-Endoret) versus hyaluronic acid in the short-term treatment of symptomatic knee osteoarthritis. *Arthroscopy*. 2012;28(8):1070–1078.
19. Smith PA. Intra-articular autologous conditioned plasma injections provide safe and efficacious treatment for knee osteoarthritis: an FDA-sanctioned, randomized, double-blind, placebo-controlled clinical trial. *Am J Sports Med*. 2016;44(4):884–891.
20. Muir SM, Reisbig N, Baria M, Kaeding C, Bertone AL. The concentration of plasma provides additional bioactive proteins in platelet and autologous protein solutions. *Am J Sports Med*. 2019;47(8):1955–1963.
21. Belk JW, Kraeutler MJ, Houck DA, Goodrich JA, Dragoo JL, McCarty EC. Platelet-rich plasma versus hyaluronic acid for knee osteoarthritis: a systematic review and meta-analysis of randomized controlled trials. *Am J Sports Med*. 2021;49(1):249–260.
22. Wu Q, Luo X, Xiong Y, et al. Platelet-rich plasma versus hyaluronic acid in knee osteoarthritis: a meta-analysis with the consistent ratio of injection. *J Orthop Surg*. 2020;28(1):2309499019887660.
23. Xu Z, Luo J, Huang X, Wang B, Zhang J, Zhou A. Efficacy of platelet-rich plasma in pain and self-report function in knee osteoarthritis: a best-evidence synthesis. *Am J Phys Med Rehabil*. 2017;96(11):793–800.
24. Chouhan DK, Dhillon MS, Patel S, Bansal T, Bhatia A, Kanwat H. Multiple platelet-rich plasma injections versus single platelet-rich plasma injection in early osteoarthritis of the knee: an experimental study in a guinea pig model of early knee osteoarthritis. *Am J Sports Med*. 2019;47(10):2300–2307.
25. Subramanyam K, Alguvelly R, Mundargi A, Khanchandani P. Single versus multi-dose intra-articular injection of platelet rich plasma in early stages of osteoarthritis of the knee: a single-blind, randomized, superiority trial. *Arch Rheumatol*. 2021;36(3):326.
26. Andia I, Abate M. Knee osteoarthritis: hyaluronic acid, platelet-rich plasma or both in association? *Exp Opin Biol Ther*. 2014;14(5):635–649.
27. Yu W, Xu P, Huang G, Liu L. Clinical therapy of hyaluronic acid combined with platelet-rich plasma for the treatment of knee osteoarthritis. *Exp Therap Med*. 2018;16(3):2119–2125.
28. Lana JFSD, Weglein A, Sampson SE, et al. Randomized controlled trial comparing hyaluronic acid, platelet-rich plasma and the combination of both in the treatment of mild and moderate osteoarthritis of the knee. *J Stem Cells Regen Med*. 2016;12(2):69–78. doi:10.46582/jsrm.1202011.
29. Jacob G, Shetty V, Shetty S. A study assessing intra-articular PRP vs PRP with HMW HA vs PRP with LMW HA in early knee osteoarthritis. *J Arthrosc Joint Surg*. 2017;4(2):65–71.
30. Guo Y, Yu H, Yuan L, et al. Treatment of knee osteoarthritis with platelet-rich plasma plus hyaluronic acid in comparison with platelet-rich plasma only. *Int J Clin Exp Med*. 2016;9(6):12085–12090.
31. Abate M, Verna S, Schiavone C, Di Gregorio P, Salini V. Efficacy and safety profile of a compound composed of platelet-rich plasma and hyaluronic acid in the treatment for knee osteoarthritis (preliminary results). *Eur J Orthop Surg Traumatol*. 2015;25(8):1321–1326.
32. Saturveithan C, Premganesh G, Fakhrizzaki S, et al. Intra-articular hyaluronic acid (HA) and platelet rich plasma (PRP) injection versus hyaluronic acid (HA) injection alone in patients with grade III and IV knee osteoarthritis (OA): a retrospective study on functional outcome. *Malays Orthop J*. 2016;10(2):35.
33. Barac B, Damjanov N, Zekovic A. The new treatment approach in knee osteoarthritis: efficacy of cellular matrix combination of platelet rich plasma with hyaluronic acid versus two different types of hyaluronic acid (HA). *Int J Clin Rheumatol*. 2018;13(5):289–295.
34. Kurapati K, Tapadia S, Rao M, Anbarasu K, Verma VK, Beevi SS. Efficacy of intra-articular injection of platelet rich plasma and hyaluronic acid in early knee osteoarthritis case series. *Eur J Mole Clin Med*. 2018;5(1):30–36.
35. Abbassy AA, Trebinjac S, Kotb N. The use of cellular matrix in symptomatic knee osteoarthritis. *Bosn J Basic Med Sci*. 2020;20(2):271.
36. Schulz KF, Altman DG, Moher D, for the CONSORT Group. CONSORT 2010 statement: updated guidelines for reporting parallel group randomised trials. *BMJ*. 2010;340:c332. doi:10.1136/bmj.c332.
37. Arthrex. *ACP® Double-Syringe System (Autologous Conditioned Plasma)*; 2014. vAPT2470.
38. Roos EM, Engelhart L, Ranstam J, et al. ICRS recommendation document: patient-reported outcome instruments for use in patients with articular cartilage defects. *Cartilage*. 2011;2(2):122–136.

39. Frobell RB, Roos EM, Roos HP, Ranstam J, Lohmander LS. A randomized trial of treatment for acute anterior cruciate ligament tears. *N Engl J Med*. 2010;363(4):331–342.
40. Finch WH, Bolin JE, Kelley K. *Multilevel Modeling Using R*. Crc Press; 2019.
41. Hedeker D, Gibbons RD. Application of random-effects pattern-mixture models for missing data in longitudinal studies. *Psychol Meth*. 1997;2(1):64.
42. Rubin LH, Witkiewitz K, Andre JS, Reilly S. Methods for handling missing data in the behavioral neurosciences: don't throw the baby rat out with the bath water. *J Undergrad Neurosci Educ*. 2007;5(2):A71.
43. Zhang Q, Liu T, Gu Y, Gao Y, Ni J. Efficacy and safety of platelet-rich plasma combined with hyaluronic acid versus platelet-rich plasma alone for knee osteoarthritis: a systematic review and meta-analysis. *J Orthop Surg Res*. 2022;17(1):499. doi:10.1186/s13018-022-03398-6.
44. Chen-Rong KE, Rui Z Ji-Xin X. Clinical efficacy of autologous platelet-rich plasma combined with intra-articular hyaluronic acid injection for knee osteoarthritis. *Chin J Gen Pract*. 2016;14(11):1810–1812.
45. Ding Q, Lv S, Shen X, Tong P. A prospective randomized controlled study on platelet-rich plasma (PRP) combined with sodium hyaluronate (HA) intra-articular injection in the treatment of knee osteoarthritis. *Shanghai Med Pharmaceut J*. 2017;38(5):25–28.
46. Sun SF, Lin GC, Hsu CW, Lin HS, Liou IS, Wu SY. Comparing efficacy of intraarticular single crosslinked Hyaluronan (HYAJOINT Plus) and platelet-rich plasma (PRP) versus PRP alone for treating knee osteoarthritis. *Sci Rep*. 2021;11(1):140. doi:10.1038/s41598-020-80333-x.
47. Huang K, Wu Z, Zhang Z, Chang W, Wang G, Sun Q. Hyaluronic acid, platelet rich plasma and the combination of both in the treatment of osteoarthritis of the knee. *Chin J Osteoporos*. 2019;25(12):1707–1711.
48. Khatab S, van Buul GM, Kops N, et al. Intra-articular injections of platelet-rich plasma releasate reduce pain and synovial inflammation in a mouse model of osteoarthritis. *Am J Sports Med*. 2018;46(4):977–986.
49. Filardo G, Di Matteo B, Di Martino A, et al. Platelet-rich plasma intra-articular knee injections show no superiority versus viscosupplementation: a randomized controlled trial. *Am J Sports Med*. 2015;43(7):1575–1582.
50. Kon E, Mandelbaum B, Buda R, et al. Platelet-rich plasma intra-articular injection versus hyaluronic acid viscosupplementation as treatments for cartilage pathology: from early degeneration to osteoarthritis. *Arthroscopy*. 2011;27(11):1490–1501.
51. Riboh JC, Saltzman BM, Yanke AB, Fortier L, Cole BJ. Effect of leukocyte concentration on the efficacy of platelet-rich plasma in the treatment of knee osteoarthritis. *Am J Sports Med*. 2016;44(3):792–800.
52. Spaková T, Rosocha J, Lacko M, Harvanová D, Gharaibeh A. Treatment of knee joint osteoarthritis with autologous platelet-rich plasma in comparison with hyaluronic acid. *Am J Phys Med Rehabil*. 2012;91(5):411–417.
53. Dai W-L, Zhou A-G, Zhang H, Zhang J. Efficacy of platelet-rich plasma in the treatment of knee osteoarthritis: a meta-analysis of randomized controlled trials. *Arthroscopy*. 2017;33(3):659–670. e1.
54. Meheux CJ, McCulloch PC, Lintner DM, Varner KE, Harris JD. Efficacy of intra-articular platelet-rich plasma injections in knee osteoarthritis: a systematic review. *Arthroscopy*. 2016;32(3):495–505.
55. Filardo G, Kon E, Di Martino A, et al. Platelet-rich plasma vs hyaluronic acid to treat knee degenerative pathology: study design and preliminary results of a randomized controlled trial. *BMC Musculoskelet Disord*. 2012;13(1):1–8.
56. Baria MR, Vasileff WK, Borchers J, et al. Treating knee osteoarthritis with platelet-rich plasma and hyaluronic acid combination therapy: a systematic review. *Am J Sports Med*. 2022;50(1):273–281.
57. Lin KY, Yang CC, Hsu CJ, Yeh ML, Renn JH. Intra-articular Injection of platelet-rich plasma is superior to hyaluronic acid or saline solution in the treatment of mild to moderate knee osteoarthritis: a randomized, double-blind, triple-parallel, placebo-controlled clinical trial. *Arthroscopy*. 2019;35(1):106–117. doi:10.1016/j.arthro.2018.06.035.
58. Khurana A, Goyal A, Kirubakaran P, Akhand G, Gupta R, Goel N. Efficacy of autologous conditioned serum (ACS), platelet-rich plasma (PRP), hyaluronic acid (HA) and steroid for early osteoarthritis knee: a comparative analysis. *Indian J Orthop*. 2021;55(suppl 1):217–227. doi:10.1007/s43465-020-00274-5.
59. Temple R, Ellenberg SS. Placebo-controlled trials and active-control trials in the evaluation of new treatments. Part 1: ethical and scientific issues. *Ann Intern Med*. 2000;133(6):455–463. doi:10.7326/0003-4819-133-6-200009190-00014.