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

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Purpose: To prospectively compare the efficacy of intra-articular injections of platelet-rich plasma (PRP) and hyaluronic acid (HA) with a sham control group (normal saline solution [NS]) for knee osteoarthritis in a randomized, dose-controlled, placebo-controlled, double-blind, triple-parallel clinical trial. **Methods:** A total of 87 osteoarthritic knees (53 patients) were randomly assigned to 1 of 3 groups receiving 3 weekly injections of either leukocyte-poor PRP (31 knees), HA (29 knees), or NS (27 knees). The Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) score and International Knee Documentation Committee (IKDC) subjective score were collected at baseline and at 1, 2, 6, and 12 months after treatment. Data were analyzed using generalized estimating equations. **Results:** All 3 groups showed statistically significant improvements in both outcome measures at 1 month; however, only the PRP group sustained the significant improvement in both the WOMAC score (63.71 ± 20.67, increased by 21%) and IKDC score (49.93 ± 17.74, increased by 40%) at 12 months. For the intergroup comparison, except for the first month, there was a statistically significant difference between the PRP and NS groups in both scores throughout the study duration (regression coefficients of 8.72 [$P = .0015$], 7.94 [$P = .0155$], and 11.92 [$P = .0014$] at 2, 6, and 12 months, respectively, for WOMAC score, and 9.1 [$P = .0001$], 10.28 [$P = .0002$], and 13.97 [$P < .0001$], respectively, for IKDC score). There was no significant difference in both functional outcomes between the HA and NS groups at any time point. Only the PRP group reached the minimal clinically important difference in the WOMAC score at every evaluation (15%, 21%, 18%, and 21% at 1, 2, 6, and 12 months, respectively) and the minimal clinically important difference in the IKDC score at 6 months (improvement of 11.6). **Conclusions:** Intra-articular injections of leukocyte-poor PRP can provide clinically significant functional improvement for at least 1 year in patients with mild to moderate osteoarthritis of the knee. **Level of Evidence:** Level I, randomized controlled single-center trial.

See commentary on page 118

The prevalence of knee osteoarthritis (OA) has climbed swiftly because of an increase in life expectancy and physical activity of the population; in addition, obesity has been indicated as a robust risk factor for knee OA in several systematic reviews and meta-analyses.[1-5] The efficacy of intra-articular hyaluronic acid (HA) injection for the treatment of OA knees remains a matter of conflict. A Cochrane review concluded

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Table 1. Subject Eligibility Criteria

Inclusion criteria
Age between 20 and 80 yr
Ability to provide informed consent
Unilateral or bilateral knee VAS pain score ≥ 4 of 10 (worst possible pain) for >4 mo
Diagnosis of Ahlbäck OA stage I-III by radiography (triple film of bilateral lower limb, standard knee anterior-posterior view in full extension, lateral view in 30° of flexion, and 45° Merchant view)
No prior PRP injection in knee
No prior surgical procedure in participating knee
Exclusion criteria
Ahlbäck OA stage IV
Major axial deviation (varus > 5°, valgus > 5°)
Any concomitant symptomatic knee disorder (i.e., ligamentous or meniscal injury)
Systemic inflammatory arthropathy
Hematologic disease
Severe cardiovascular disease
Neurologic disorder
Active infection
Immunocompromised
Therapy with anticoagulant or antiaggregant
Use of NSAID and/or chondroprotective supplement, such as glucosamine and chondroitin sulfate, within 7 d before trial
Recent intra-articular injection of corticosteroid (within 30 d) and prior treatment with HA in past 6 mo
Hb level < 11 g/dL, platelet count < 150,000/mm^3

HA, hyaluronic acid; Hb, hemoglobin; NSAID, nonsteroidal anti-inflammatory drug; OA, osteoarthritis; PRP, platelet-rich plasma; VAS, visual analog scale.

that HA had beneficial effects on pain, function, and patient global assessment, especially in the 5- to 13-week postinjection period[6]; and a recent systematic review and meta-analysis of randomized controlled trials showed that the use of HA injections that have been approved in the United States is safe and effective in knee OA.[7] However, several meta-analyses contrarily reported that intra-articular HA injections were not clinically effective and might even be associated with a greater risk of adverse effects.[8-12] Although the American Academy of Orthopaedic Surgeons guideline does not recommend the use of HA in patients with symptomatic OA knees,[13] it remains a widely used intra-articular injecting modality for orthopaedic clinicians today.

Interest in intra-articular injections of platelet-rich plasma (PRP) for knee OA has been rapidly growing. PRP is an autologous derivative of whole blood that contains high concentrations of growth factors through which PRP has shown an agonistic effect on chondrogenesis and mesenchymal stem cell proliferation.[14] Furthermore, PRP was shown to have antinociceptive and anti-inflammatory activities to reduce pain and modulate the OA process.[15] Among the different preparation methods, several studies with high levels of evidence have shown that leukocyte-poor PRP is better than leukocyte-rich PRP regarding clinical and laboratory outcomes.[10,16-19] Despite the wide clinical interest in orthopaedic and sports medicine, current guidelines from the American Academy of Orthopaedic Surgeons indicate an inability to "recommend for or against" the use of PRP for the treatment of knee OA.[13]

Several randomized controlled trials[20-25] and meta-analyses[26-29] have compared the efficacy of intra-articular injections of PRP and HA in the treatment of knee OA, and most have shown favorable results for PRP; however, there is a paucity of studies that have implemented a sham control group in comparing the true efficacies of PRP and HA without possible bias from placebo effects.[21,23] The aim of this study was thus to prospectively compare the efficacy of intra-articular injections of PRP and HA with a sham control group (normal saline solution [NS]) for knee OA in a randomized, dose-controlled, placebo-controlled, double-blind, triple-parallel clinical trial. The primary outcome measure was the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) score, and the secondary outcome measure was the International Knee Documentation Committee (IKDC) subjective knee evaluation score. We hypothesized that PRP would be both statistically and clinically superior to HA and placebo in providing symptomatic relief for mild to moderate OA knees.

Methods

Patient Selection

This study was approved in March 2014 by the hospital institutional review board (Kaohsiung Veterans General Hospital, VGHKS14-CT2-15) before the first patient enrollment. Evidence from the literature and the methods for PRP and HA injection in this trial, as well as benefits and possible adverse effects, were included in the written consent form to be signed by each participant. Subject screening was performed in the outpatient department, where the senior author and chairman of the orthopaedics department (J-H.R.) evaluated patients' eligibility for study inclusion through history taking, physical examination, laboratory testing, and imaging studies (Table 1). Nonsteroidal anti-inflammatory drugs and chondroprotective supplements were prohibited from being taken during the duration of the trial. Paracetamol was permitted during the study but had to be discontinued 72 hours before each follow-up assessment.

All participants and study staff were unaware of the group assignments because the participating knees were randomly subjected to a standardized injection protocol of 3 different agents, which were disclosed to the participants only after a follow-up period of 12 months. They were assessed with Internet-based (www.orthopaedicscores.com) WOMAC and IKDC functional outcome questionnaires (each with a score of 0-100, with a higher score meaning a better

outcome) for each participating knee before the treatment and at 4 time points (1, 2, 6, and 12 months) after the treatment by a blinded observer (C-C.Y.) who was not involved in the injection procedure.

To determine an adequate sample size for the study, we performed a power analysis using free software (G*Power).[30] A minimum total sample size requirement of 57 knees (or 19 knees per treatment arm) was calculated based on a study power of 80% ($\beta = .2$), a false-positive rate of 5% ($\alpha = .05$), and an effect size (Cohen f) of 0.15. This study was then designed to enroll approximately 30 knees per group at baseline in anticipation of a possible dropout rate of 20%.

Randomization

Through a computer-generated simple randomization system, participating knees of subjects who met all the inclusion criteria were randomly assigned at a 1:1:1 ratio to 1 of 3 treatment groups to receive 3 weekly intra-articular injections: group 1, PRP (RegenKit-THT; Regen Lab, Le Mont-sur-Lausanne, Switzerland); group 2, HA (Hyruan Plus, 20 mg/2 mL; molecular weight > 2,500 kDa; LG Chem, Seoul, Republic of Korea); and group 3, NS (placebo group).

Blinding and Intervention Protocol

To keep the participants blinded to their assigned treatments, all had 10 mL of blood drawn and spun before each injection regardless of their group assignment; thus, the time each participant spent in the office (trial locale) during each intervention was comparable (about 30-40 minutes). All intra-articular injections were performed through an anteromedial approach aseptically by the first author (K-Y.L.) in a curtained cubicle specifically designed to keep him from knowing the identity of the patient, as well as to keep the patient blinded to the type of injection he or she received. Each patient was instructed to sit in a chair with the knees in 90° of flexion traversed through an opaque curtain separating the patient from the injector. Furthermore, some subjects may have received bilateral knee injections of different materials, and we aimed to keep the joint-filling sensation of each knee as equivocal as possible to maintain blinding; thus, a uniformed protocol of 2 mL of the assigned treatment material (PRP, HA, or placebo) was administered during each injection. Local anesthesia was not used during any injection to avoid its possible deleterious effect on the activation of platelets by changing the pH of the environment.[31]

Preparation of PRP

PRP was prepared using RegenKit-THT, which required 10 mL of blood to be drawn and single spun at 1,500 rpm for 8 minutes. This would yield an average of 5.0 ± 0.5 mL of PRP with approximately 90% of platelets recovered for a concentration of 1.81 ± 0.34 times that of the baseline value.[32] The product is considered leukocyte-poor PRP because nearly 70% of the white blood cells are removed during each centrifugation (Study Report—Regen THT Tube Performance Testing; U.S. Food and Drug Administration 510(k) No. BK090048, Regen Lab, unpublished data, 2010). To avoid potential degranulation of alpha granules during a PRP freeze-thaw process,[23,33] all subjects in this study had their blood freshly drawn and spun before each injection. The total preparation and injection time occurred within 20 minutes of blood drawing; thus, the use of anticoagulants was not warranted.

Statistical Methods

Baseline demographic characteristics of the 3 study cohorts were compared using the 1-way analysis of variance (ANOVA) test for continuous variables and Pearson χ-square test for categorical variables. Evaluations of the within-group and intergroup functional outcome scores were conducted using generalized estimating equations (GEEs)[34] with robust variance estimates. Because of the correlated nature of the data and repeated observations taken for each participating knee, a GEE was used as a multivariate analysis to investigate the temporal effects of different intra-articular injection treatments on the clinical outcomes while controlling for important patient characteristics such as sex, age, body mass index (BMI), and severity of OA (Ahlbäck stage); relations between and among such variables at different time points were analyzed simultaneously. All the analyses were conducted with SAS software for Windows (version 9.4; SAS Institute, Cary, NC) by a statistician who was blinded to the control and intervention groups in the data set. Statistical significance was set at $P < .05$ throughout.

Results

From April to August 2014, a total of 194 patients were assessed for eligibility. Of these patients, 85 did not meet the inclusion criteria and 56 declined to participate or specifically requested either PRP or HA treatment (Fig 1). Thus, a total of 87 knees in 53 patients were enrolled in the study; we randomly allocated 31 knees to group 1 (PRP), 29 to group 2 (HA), and 27 to group 3 (NS). There were no withdrawals during the injection period; however, 1 knee in the PRP group and 1 knee in the HA group (the same patient with bilateral enrollment) missed the 2-month follow-up evaluation, whereas 1 knee in the HA group and 1 knee in the placebo group (another patient with bilateral enrollment) missed the 6-month follow-up evaluation. There were no significant demographic differences among the 3 groups across sex proportion, age, and Ahlbäck stage for OA, as well as pretreatment WOMAC and IKDC scores. A small but significant difference in BMI was found, being higher in the HA group (Table 2). No serious adverse effects related to the intra-articular injection were reported among the 3 groups

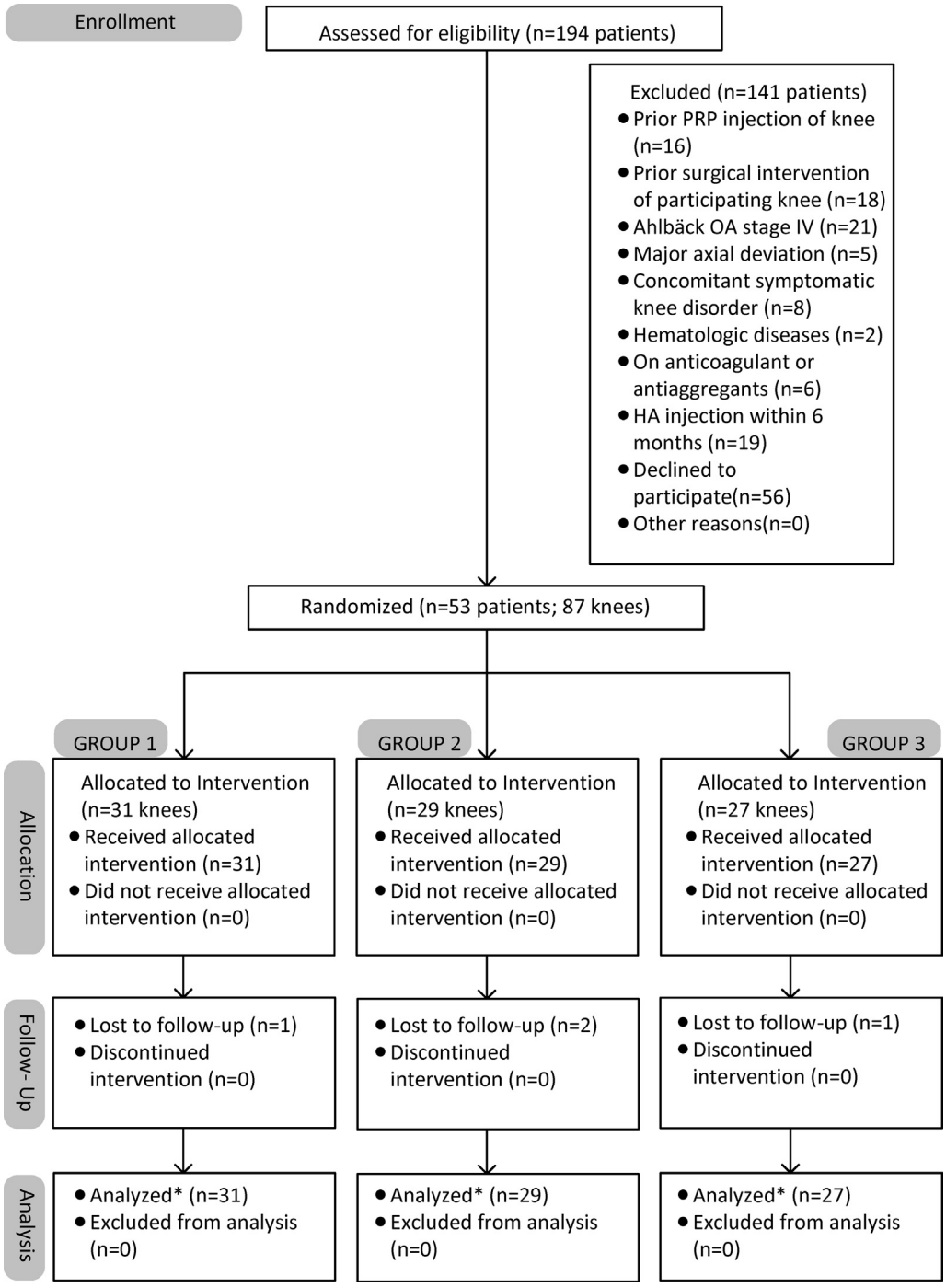

Fig 1. Consolidated Standards of Reporting Trials (CONSORT) flow diagram. All allocated participating knees were included in the final analysis because the generalized estimating equation methodology does not exclude missing data (asterisks). (HA, hyaluronic acid; OA, osteoarthritis; PRP, platelet-rich plasma.)

throughout the study duration except for some transient localized pain at the injection site, which spontaneously resolved uneventfully within a few hours.

Primary Outcome: WOMAC Score

For the within-group comparison of WOMAC scores, all 3 groups showed statistically significant improvements from their respective baseline scores at the 1-month follow-up (improvements by 15%, 14%, and 12% in PRP, HA, and NS groups, respectively) (Table 3). However, this significant amelioration was sustained throughout the rest of the study duration in only the PRP group (improvements by 21%, 18%, and 21% at 2, 6, and 12 months' follow-up respectively; $P < .05$), whereas both the HA and NS groups showed declining WOMAC scores without statistically significant differences from their respective basal evaluations after the first month (Fig 2A).

Table 2. Baseline Demographic Characteristics of Study Participants

	Group 1: PRP (n = 31)	Group 2: HA (n = 29)	Group 3: NS (n = 27)	*P* Value
Sex				.775
Male	9 (29.03)	10 (34.48)	10 (37.04)	
Female	22 (70.97)	19 (65.52)	17 (62.96)	
Age, mean (SD), yr	61.17 (13.08)	62.53 (9.9)	62.23 (11.71)	.8932
BMI, mean (SD)	23.98 (2.62)	26.26 (2.99)	24.98 (3.12)	.0127
Ahlbäck stage				.9448
I	5 (16.12)	6 (20.69)	4 (14.81)	
II	16 (51.61)	14 (48.28)	12 (44.44)	
III	10 (32.25)	9 (31.03)	11 (40.74)	
Baseline WOMAC score, mean (SD)	52.81 (18.14)	52.67 (18.06)	48.59 (16.92)	.6013
Baseline IKDC score, mean (SD)	35.71 (13.77)	35.93 (12.71)	33.3 (10.52)	.6838

NOTE. Data are presented as number of knees (percentage) unless otherwise indicated. Continuous variables were analyzed by analysis of variance; categorical variables were analyzed by the χ-square test. Statistical significance was set at $P < .05$.

BMI, body mass index; HA, hyaluronic acid; IKDC, International Knee Documentation Committee; NS, normal saline solution; PRP, platelet-rich plasma; SD, standard deviation; WOMAC, Western Ontario and McMaster Universities Osteoarthritis Index.

For the intergroup comparison (Table 4), in which the NS group was used as the reference, while controlling for all the confounding factors, such as sex, age, BMI, and Ahlbäck stage, the PRP group, except at the 1-month follow-up evaluation, consistently showed better WOMAC outcome measures with statistical significance throughout the study duration (regression coefficients of 8.72 [95% confidence interval (CI), 3.32-14.12] with $P = .0015$, 7.94 [95% CI, 1.51-14.37] with $P = .0155$, and 11.92 [95% CI, 4.59-19.25] with $P = .0014$ at 2, 6, and 12 months, respectively); in contrast, there were no statistically significant differences in the WOMAC scores between the HA and NS groups at any follow-up times. On analysis of possible confounding effects of other variables on the WOMAC score (Table 5), only age and sex had statistically significant influences, with better functional outcomes being seen in younger subjects (regression coefficient, −0.44 [95% CI, −0.73 to 0.14]; $P = .0044$) and male subjects (regression coefficient for female subjects, −8.33 [95% CI, −14.93 to −1.72]; $P = .0135$). BMI had no statistically significant effect on the WOMAC score (regression coefficient, −0.78 [95% CI, −1.87 to 0.31]; $P = .1595$), nor did OA severity. Compared with subjects with Ahlbäck stage III, those with Ahlbäck stage II achieved better yet insignificant mean WOMAC scores (regression coefficient, 7.62 [95% CI, −0.27 to 15.51]; $P = .0582$) whereas those with Ahlbäck stage I showed less improvement in WOMAC scores (regression coefficient, −5.51 [95% CI, −13.63 to 2.62]; $P = .184$).

Secondary Outcome: IKDC Score

For the within-group comparison of IKDC scores, all 3 groups showed statistically significant improvements from their respective baseline scores at the 1-month follow-up (improvements by 22%, 21%, and 16% in PRP, HA, and NS groups, respectively) (Table 3). However, again, these statistically significant improvements were sustained throughout the rest of the study in only the PRP group (improvements by 34%, 33%, and 40% at 2, 6, and 12 months' follow-up, respectively), whereas both the HA and NS groups showed declining IKDC scores after the first month (Fig 2B).

For the intergroup comparison (Table 4), in which the NS group was used as the reference, while controlling for all the covariates, there were no significant differences among the 3 study groups at 1 month after treatment (PRP vs NS, $P = .2363$; HA vs NS, $P = .36$). Thereafter, the PRP group showed persistent statistically significant superiority to the NS group throughout the rest of the study (regression coefficients of 2.35 [95% CI, −1.54 to 6.24] with $P = .0001$, 9.1 [95% CI, 4.42-13.79] with $P = .0002$, and 10.28 [95% CI, 4.87-15.7] with $P < .0001$ at 2, 6, and 12 months, respectively), whereas there were no statistically significant differences between the IKDC scores of the HA and NS groups at any of the remaining follow-up times. On analysis of possible correlations of other variables with the IKDC score (Table 5), age was found to be a statistically significant factor in outcomes, with younger patients exhibiting higher IKDC scores (regression coefficient, −0.37 [95% CI, −0.59 to −0.15]; $P = .0009$), whereas both sex and BMI were not influential factors ($P = .0516$ and $P = .3908$, respectively). Compared with knees with Ahlbäck stage III, knees with Ahlbäck stage II had statistically significantly greater improvement (regression coefficient, 7.64 [95% CI, 1.88-13.4]; $P = .0093$) whereas knees with stage I did not (regression coefficient, 2.31 [95% CI, −3.9 to 8.52]; $P = .4661$). A post hoc power analysis showed that with a sample size of 87 knees, under the same setting of an effect size (f) of 0.15 and α error of .05, the actual power of this clinical trial reached 0.96.

Discussion

The most significant finding of this study was that better clinical results were achieved with PRP than with

Table 3. WOMAC and IKDC Clinical Scores for PRP, HA, and NS Groups

	Baseline (T1)	1 mo (T2)	2 mo (T3)	6 mo (T4)	12 mo (T5)	Post Hoc Test[†] ($P < .05$)
WOMAC score						
PRP	52.81 ± 18.14	60.91 ± 17.35	63.84 ± 17.86[‡§‖]	62.28 ± 18.47[‡§‖]	63.71 ± 20.67[‡§‖]	T2 > T1; T3 > T1; T3 > T2; T4 > T1; T5 > T1
Change from baseline, %	—	15[¶]	21[¶]	18[¶]	21[¶]	
HA	52.67 ± 18.06	60.29 ± 20.95	57.32 ± 21.85	52.9 ± 19.76	49.33 ± 21.51	T2 > T1; T2 > T4; T2 > T5
Change from baseline, %	—	14[¶]	9	0	−6	
NS	48.59 ± 16.92	54.26 ± 17.16	49.79 ± 17.47	49.7 ± 15.81	46.94 ± 16.74	T2 > T1; T2 > T4; T2 > T5
Change from baseline, %	—	12[¶]	2	2	−3	
IKDC score						
PRP	35.71 ± 13.77	43.61 ± 14.86	47.83 ± 15.85[‡]	47.33 ± 16.24[‡§‖]	49.93 ± 17.74[‡§]	T2 > T1; T3 > T2; T4 > T2; T5 > T2
Change from baseline, %	—	22	34	33[¶]	40[¶]	
HA	35.93 ± 12.71	43.57 ± 15.67	42.29 ± 17.18	40.29 ± 15.76	38.64 ± 16.09	T2 > T1; T2 > T4; T2 > T5
Change from baseline, %	—	21	18	12	8	
NS	33.3 ± 10.52	38.65 ± 11.07	35.56 ± 11.35	34.2 ± 11.11	32.96 ± 11.15	T2 > T1; T2 > T4; T2 > T5
Change from baseline, %	—	16	7	3	−1	

NOTE. Data are presented as mean ± standard deviation unless otherwise indicated. The minimal clinically important difference for the WOMAC score was defined as 12% of the baseline value or 6% of the maximal value. The minimal clinically important difference for the IKDC score was defined as an absolute change of 6.3 at 6 months and 16.7 at 12 months.

HA, hyaluronic acid; IKDC, International Knee Documentation Committee subjective knee evaluation; NS, normal saline solution; PRP, platelet-rich plasma; WOMAC, Western Ontario and McMaster Universities Osteoarthritis Index.

[†]Generalized estimating equation followed by post hoc analysis of within-group differences in WOMAC and IKDC clinical scores for PRP, HA, and NS groups (controlling for age, sex, BMI, and Ahlbäck stage).

[‡]Statistically significant difference ($P < .05$) from placebo (NS) group.

[§]Minimal clinically important difference from placebo (NS) group.

[‖]Minimal clinically important difference of PRP group from HA group.

[¶]Minimal clinically important difference from baseline within each group.

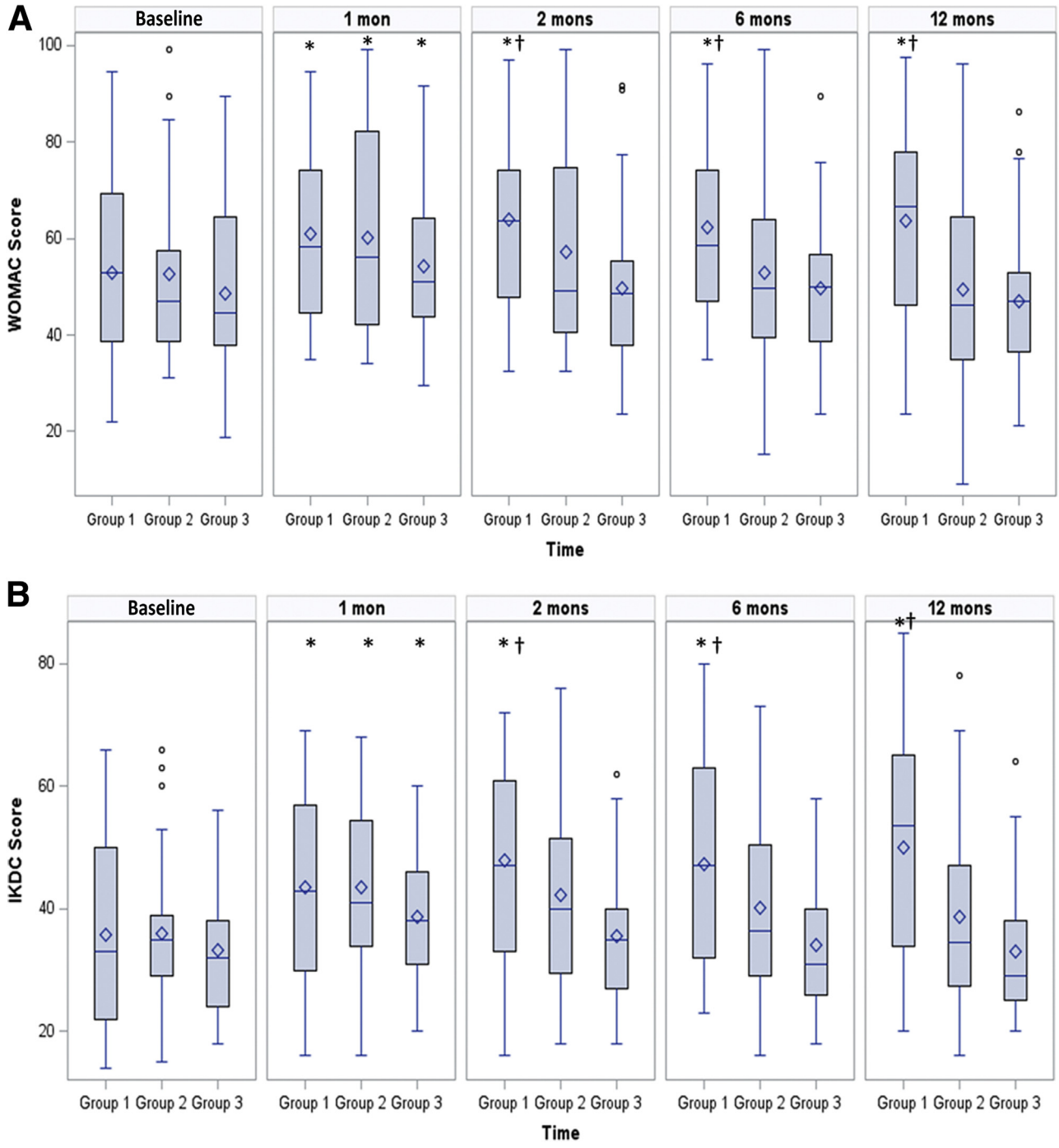

Fig 2. Box-and-whisker plots showing treatment effect of platelet-rich plasma (group 1), hyaluronic acid (group 2), and normal saline solution (group 3) on Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) scores (A) and International Knee Documentation Committee (IKDC) scores (B) over time. In each box, the solid line and diamond delineate the median and mean, respectively. The bottom and top of each box represent the 25th and 75th percentiles, respectively. The whiskers represent the high and low values, excluding the outliers. Outliers that are between 1.5 and 3.0 box lengths from the median are depicted as circles. Asterisks denote significant within-group difference ($P < .05$) compared to baseline. Daggers denote significant between-group difference ($P < .05$) when Group 1 and Group 2 are each compared to Group 3. (mon, month.)

HA or NS in the treatment of OA knees. Our clinical results showed that all 3 groups had statistically significant improvements from their respective baseline scores in both the primary and secondary outcome evaluations 1 month after the intervention; the PRP group was able to sustain such significant within-group improvement throughout the study, with both its mean WOMAC and IKDC scores improving from baseline by 21% and 40%, respectively, at the end of 12 months, whereas both the HA and NS groups did not show statistically significant

Table 4. Treatment Effect Analysis of Between-Group Comparison

	WOMAC Score			IKDC Score		
Interaction*	Regression Coefficient	95% CI	*P* Value	Regression Coefficient	95% CI	*P* Value
PRP × 1 mo	1.95	−3.7 to 7.61	.4981	2.35	−1.54 to 6.24	.2363
PRP × 2 mo	8.72	3.32 to 14.12	.0015	9.1	4.42 to 13.79	.0001
PRP × 6 mo	7.94	1.51 to 14.37	.0155	10.28	4.87 to 15.7	.0002
PRP × 12 mo	11.92	4.59 to 19.25	.0014	13.97	8.19 to 19.74	<.0001
HA × 1 mo	1.99	−3.46 to 7.43	.4745	2.09	−2.38 to 6.55	.36
HA × 2 mo	2.72	−2.5 to 7.94	.3075	4.11	−1.1 to 9.32	.1224
HA × 6 mo	−2.24	−7.55 to 3.07	.408	3.1	−1.94 to 8.13	.2282
HA × 12 mo	−3.37	−9.62 to 2.87	.2898	2.48	−3.23 to 8.18	.3949

NOTE. Statistical significance was set at $P < .05$.

CI, confidence interval; HA, hyaluronic acid; IKDC, International Knee Documentation Committee; PRP, platelet-rich plasma; WOMAC, Western Ontario and McMaster Universities Osteoarthritis Index.

*Group × time interaction (PRP vs normal saline solution and HA vs normal saline solution) at each follow-up time.

improvements from their pretreatment WOMAC and IKDC evaluations after the 1-month evaluation. It is interesting that our sham control group exhibited a statistically significant improvement from baseline at the 1-month follow-up for both the primary and secondary outcome measures. This phenomenon strongly suggests a positive placebo effect and further gives credence to our study measures of the WOMAC and IKDC scores being patient reported, as well as avoiding investigator bias. For the intergroup comparison of PRP and HA with respect to placebo, both the primary and secondary outcome measures showed that although there was no statistically significant difference among the 3 groups at 1 month after treatment, the PRP group showed statistically significant superiority to the placebo group throughout the rest of the study; in contrast, the HA group did not show its distinction statistically from the placebo group in both outcome measures at any time point.

In the analysis of possible confounding effects from other covariates, GEEs showed that age had a statistically significant effect on both our primary and secondary measures: the younger the patient, the better the outcome. This finding is contrary to what was reported by Patel et al.[35] and Raeissadat et al.[24] because age was not a statistically significant factor regarding patient outcomes in their respective clinical trials. The Ahlbäck OA grade did not prove to have a statistically significant influence on the WOMAC outcome, which coincides with the results of Cerza et al.[20] in a randomized trial but is contrary to what was noted in other studies, in which patients with less severe OA changes had better clinical results.[24,33,35,36] There is also a lack of consensus in the literature on the effect of BMI on patient outcomes after HA and PRP injections, with some studies showing superior outcomes in patients with a low BMI[33,36] but others showing no difference[35]; our result coincides with the latter. In addition, although the sex difference had no influence on the IKDC evaluation, it was a statistically significant factor regarding the WOMAC score, with male patients exhibiting a more favorable outcome; this result is contrary to what was found by Patel et al.,[35] who stated that the sex difference was not a significant factor in the WOMAC evaluation.

To our knowledge, there is a paucity of prospective, double-blind, randomized controlled trials that have compared the efficacy of intra-articular injections of PRP and HA with implementation of a sham control

Table 5. Effect of Interaction Between Variables and Functional Outcome Measures

	WOMAC Score			IKDC Score		
	Regression Coefficient	95% CI	*P* Value	Regression Coefficient	95% CI	*P* Value
Age	−0.44	−0.73 to 0.14	.0044	−0.37	−0.59 to −0.15	.0009
Sex						
Male	Ref			Ref		
Female	−8.33	−14.93 to −1.72	.0135	−4.71	−9.45 to 0.03	.0516
BMI	−0.78	−1.87 to 0.31	.1595	−0.37	−1.21 to 0.47	.3908
Ahlbäck stage						
I	−5.51	−13.63 to 2.62	.184	2.31	−3.9 to 8.52	.4661
II	7.62	−0.27 to 15.51	.0582	7.64	1.88 to 13.4	.0093
III	Ref			Ref		

NOTE. Statistical significance set at $P < .05$.

BMI, body mass Index; CI, confidence interval; IKDC, International Knee Documentation Committee; Ref, reference category; WOMAC, Western Ontario and McMaster Universities Osteoarthritis Index.

group over a course of 52 weeks in the literature. Several randomized controlled trials have investigated the efficacy of PRP for OA knees, with most showing PRP to be more likely to decrease pain and improve functionality than viscosupplementation[20-24,36,37]; however, 1 common limitation cited among these Level I studies is the lack of a placebo group in such 2-armed trials. Studies have shown that placebo effects are genuine biopsychosocial phenomena attributable to the overall therapeutic context.[38,39] Beecher[38] claimed that about 35% of patients responded positively to placebo treatment in a proto—meta-analysis. Thus, to prove that the efficacy of a treatment is above and beyond the psychological results of a simple belief in the ability of the drug to cure, the placebo-controlled trial is the gold standard for testing the efficacy of treatments.[40] Gormeli et al.[23] conducted a 6-month randomized controlled trial that incorporated a placebo group, showing that multiple injections of PRP were more efficacious in treating early OA knees than either a single injection of PRP or multiple injections of HA. However, there were several limitations to their study; first, unlike our study, there was a lack of uniformity in the injection amount (5 mL for PRP, 2 mL for HA, and unspecified for saline solution), which may have rendered performance bias to a true blinding effect. Second, their use of leukocyte-rich PRP might have contributed to the nonsignificant difference between the functional scores of the 1-dose injection PRP group and HA group. Several prior clinical studies have suggested that leukocyte-poor PRP contributed to superior clinical outcomes and less of an adverse local reaction possibly because of the less deleterious effects of proteases and reactive oxygen released from the white blood cells.[10,19,20,25,33,35-37]

This clinical trial not only statistically affirmed the findings of the previous randomized controlled trials that PRP has superior efficacy in treating OA knees than HA or NS[20,25,35-37,41] but also attested to the clinically meaningful differences in both the WOMAC and IKDC scores among the 3 injection therapies. Angst et al.[42] stated that the minimal difference that patients and their physicians consider clinically important (the so-called minimal clinically important difference [MCID]) in the WOMAC measure is 12% of the baseline value or 6% of the maximal value. Our results showed that only the PRP group reached such clinical significance at each follow-up time (improvements by 15%, 21%, 18%, and 21% from baseline at 1, 2, 6, and 12 months, respectively) whereas both the HA and NS groups did not show such significant differences after the 1-month follow-up time (Table 3). For the intergroup comparison, the PRP group clearly surpassed the MCID (difference of 6 points) compared with the HA or NS group at each time point after the first post-treatment evaluation, whereas there was no such difference between the HA and NS groups throughout the study except at 2 months (57.32 vs 49.79). Similarly, Greco et al.[43] showed that the MCID in the IKDC score is an absolute change of 6.3 at 6 months and 16.7 at 12 months. Our results showed that at the 6-month follow-up, only the PRP group reached such clinical significance, with a change of 11.62 from baseline (from 35.71 to 47.33), while approaching clinical significance at 12 months, with an absolute difference of 14.22 (from 35.71 to 49.93) (Table 3). For between-group comparisons, the PRP group showed clinically significant ascendancy compared with the HA group, with a change of 7.04 (47.33 vs 40.29) at 6 months, and likewise compared with the NS group, with differences of 13.13 (47.33 vs 34.2) and 16.97 (49.93 vs 32.96) at 6 months and 12 months, respectively; in contrast, the HA group again did not show such clinically significant differences compared with the NS group at both evaluation points. Overall, the data in this study failed to support HA as being either statistically or clinically more effective than NS for the intra-articular treatment of OA knees, which is in line with the findings of a recent meta-analysis and randomized controlled study.[12,44]

A strength of our study is the application of GEEs in analyzing longitudinal data. We believed a GEE may be the most appropriate analytical methodology in such a randomized controlled trial because it allows the estimation of treatment effects (i.e., group differences) across multiple time points within a single statistical model and it can more accurately explore the overall average effects by focusing more on the average changes in response over time and the impact of covariates on these changes.[34] Such an approach minimizes the accumulation of type I errors from multiple endpoint comparisons. Several prior randomized controlled trials comparing the efficacies of PRP and HA used cross-sectional significance tests (e.g., χ-square and Student t tests) at every time point to compare group differences[20,24,35,37]; this approach might be subject to 2 methodologic hazards: (1) inflation of a type I error and (2) failure to account for measurements taken at different time points as coming from the same individual.[45] Moreover, unlike repeated-measures ANOVA, another commonly used analytical tool in previous randomized controlled trials,[20,23-25] GEEs do not require the outcome variable to have a specific distribution. This feature can greatly benefit studies in which data are skewed or the distribution of data is difficult to verify owing to a small sample size. Furthermore, missing data are practically inevitable in longitudinal studies, thus leading to unbalanced designs. If a subject has any missing values, repeated-measures ANOVA will exclude the individual from the analysis entirely; in contrast, GEEs take all available data into account in an unbalanced design and use maximum-likelihood estimation to make a

more efficient effect estimate (i.e., treatment effect).[34] Thus, we believe the results of our clinical trial were given further credence by the use of GEEs in the statistical analysis.

Limitations

Several limitations to this study warrant discussion. First, the sample size of patients with Ahlbäck stage I OA in each group was small; however, this realistically reflects the ratio of subjects with different indexes of OA severity in the outpatient department because more symptomatic patients are more inclined to seek medical treatment and vice versa. Second, most enrolled subjects had bilateral knees that participated; however, this is also a pragmatic reflection of the phenomenon seen in the outpatient department. Although knee OA was historically considered an asymmetrical disease, several cross-sectional studies have shown that bilateral knee pain is a frequent problem in the community.[46-48] Thus, by including bilateral knee OA patients, our study design closely reflects actual clinical practice and further validates the application of our results to a larger clinical patient population; furthermore, each subject was adamantly instructed to reply to both the WOMAC and IKDC questionnaires for each participating knee independent of the other. Third, the mean BMI of the HA group was statistically higher than the BMIs of the other groups; however, this possible confounding effect from the skewed distribution was rescinded using GEEs, which analyzed the clinical outcomes of the 3 tested groups while controlling for their important covariates such as sex, age, BMI, and severity of OA (Ahlbäck stage). Finally, this trial did not include imaging studies, such as radiography or magnetic resonance imaging, for the evaluation of joint cartilage or perform intra-articular biological analyses for possible differences in the concentrations of various biomarkers after each injection modality. Future long-term studies of larger sample sizes encompassing all stages of degeneration with the inclusion of imaging evaluation and biomarker analysis of the knee joints are warranted to further elucidate our findings.

Conclusions

Intra-articular injections of leukocyte-poor PRP can provide clinically significant functional improvement for at least 1 year in patients with mild to moderate OA of the knee.

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