Leukocyte-Rich versus Leukocyte-Poor Platelet-Rich Plasma for the Treatment of Knee Osteoarthritis

A Double-Blind Randomized Trial

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Background: Platelet-rich plasma (PRP) is gaining large interest in clinical practice as a minimally invasive injective treatment for knee osteoarthritis (OA). Different preparation methods are available, and the presence of leukocytes, deemed detrimental in some preclinical studies, is one of the most debated aspects regarding PRP efficacy.

Purpose: To compare the safety and effectiveness of leukocyte-rich PRP (LR-PRP) and leukocyte-poor PRP (LP-PRP) for the treatment of knee OA.

Study Design: Randomized controlled trial; Level of evidence, 1.

Methods: A total of 192 patients with symptomatic knee OA (Kellgren-Lawrence grade 1-3) were randomly allocated to 3 weekly injections of LR-PRP or LP-PRP. LP-PRP was obtained with a filter for leukodepletion. LR-PRP and LP-PRP were divided into aliquots of 5 mL, with a mean platelet concentration of 1146.8 $\times$ 10^9/L and 1074.9 $\times$ 10^9/L and a mean leukocyte concentration of 7991.4 $\times$ 10^6/L and 0.1 $\times$ 10^6/L, respectively. Patients were evaluated at baseline and thereafter at 2, 6, and 12 months for the primary outcome, the International Knee Documentation Committee (IKDC) subjective score; and for secondary outcomes, the Knee injury and Osteoarthritis Outcome Score (KOOS) subscales, EuroQol–visual analog scale (EQ-VAS), and Tegner score.

Results: No differences between groups were observed in terms of absolute values or improvement of the clinical scores across all follow-up intervals. The mean IKDC subjective score at baseline and 12 months improved from 45.6 to 60.7 in the LR-PRP group as compared with 46.8 to 62.9 in the LP-PRP group (P = .626). No severe adverse events were described in either group, although 15 mild adverse events (knee pain or swelling) were reported: 12.2% for LR-PRP and 4.7% for LP-PRP (P = .101). No statistically significant difference was also found between LR-PRP and LP-PRP in terms of failures (7.8% vs 3.5%, P = .331).

Conclusion: This double-blind randomized trial showed that 3 intra-articular LR-PRP or LP-PRP injections produced similar clinical improvement in the 12 months of follow-up in patients with symptomatic knee OA. Both treatment groups reported a low number of adverse events, without intergroup differences. The presence of leukocytes did not significantly affect the clinical results of PRP injections.

Registration: NCT02923700 (ClinicalTrials.gov identifier).

Keywords: knee; osteoarthritis; platelet-rich plasma (PRP); leukocytes; injective; intra-articular

Knee osteoarthritis (OA) can be managed by a range of nonoperative approaches, from physical therapies to dietary supplements and pharmacological treatments, as well as intra-articular injections.[19,34] In particular, corticosteroids and hyaluronic acid injections are routinely used to provide clinical benefit, although this effect presents significant variability among patients and the effectiveness is generally short term.[14] This explains the research efforts to find new solutions to address knee OA, looking for products with disease-modifying effect capable of delaying or avoiding surgery. Among the emerging treatment options, injective strategies based on the use of autologous blood derivatives have been the subject of vigorous scientific investigation.[26] In particular, platelet-rich plasma (PRP) is gaining interest in clinical practice as a minimally invasive injective treatment for knee OA, thanks to its safety and the simple preparation technique to obtain its biologically active content.[5]

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PRP efficacy is supported by several randomized controlled trials and meta-analyses, mainly focused on the knee joint, with better results than other injectable options, such as saline, corticosteroids, and hyaluronic acid.[22,35] Moreover, a disease-modifying effect of PRP is supported by the literature in animal OA models, showing that it may reduce synovial inflammation and attenuate cartilage damage progression.[8] However, despite the large clinical application of this appealing innovative approach and the promising findings, many questions remain. In particular, different preparation methods are available for PRP, which can yield products with different compositions and characteristics, and little is known about the most suitable type of concentrate for each clinical indication.[31] In this scenario, the presence of leukocytes is one of the most debated aspects regarding PRP efficacy, and it is used as one of the main discriminants to distinguish PRP products. Some preclinical evidence suggests that leukocytes may be deleterious and impair the overall effects of PRP,[9] while other findings support their use owing to the release of beneficial cytokines.[8] High-level clinical studies are needed to understand the real clinical effects of leukocytes in PRP preparations used to address knee OA.

The aim of this double-blind randomized trial was to evaluate the influence of leukocytes on PRP safety and clinical effectiveness through the comparison between leukocyte-rich PRP (LR-PRP) and leukocyte-poor PRP (LP-PRP) for the intra-articular injective treatment of patients affected by knee OA. The hypothesis was that the presence of leukocytes could affect the clinical outcome obtained with PRP injections, resulting in a lower clinical improvement and a higher rate of adverse events when compared with PRP injections that were poor in leukocytes.

METHODS

Study Design and Patient Selection

This prospective double-blind randomized trial was approved by the hospital ethics committee and internal review board of the Rizzoli Orthopaedic Institute (0035854). The trial was registered at ClinicalTrials.gov (NCT02923700). The study was entirely conducted at a highly specialized referral center for orthopaedic pathologies. Patients were enrolled by orthopaedic clinicians between September 2016 and January 2020 in the research outpatient clinic focused on patients with knee OA. Informed consent was obtained from each patient for study participation. The following inclusion criteria were used for selection: male and female patients affected by monolateral symptomatic knee OA with a history of chronic pain or swelling (at least 4 months), age between 18 and 80 years, imaging findings of knee OA (Kellgren-Lawrence grade 1-3), and failed results after at least 2 months of nonoperative treatment. The following exclusion criteria were used for the selection: age >80 years, Kellgren-Lawrence grade 4, bilateral knee OA, history of trauma or intra-articular injection therapy within 6 months before treatment or knee surgery within 12 months, major axial deviation (varus >5°, valgus >5°), presence of any concomitant knee lesion causing pain or swelling (eg, untreated knee instability, meniscal pathologies, focal chondral or osteochondral lesion requiring surgery), neoplasms, systemic disorders (eg, uncontrolled diabetes), metabolic disorders of the thyroid, severe cardiovascular diseases, rheumatoid arthritis, inflammatory arthropathy, hematological diseases, infections, immunodepression, anticoagulants or antiaggregant therapy, use of nonsteroidal anti-inflammatory drugs in the 5 days before blood harvest, and hemoglobin level <11 g/dL or platelet count <150,000/mm^3 at blood harvest.

After enrollment in the study, patients were randomly allocated to 2 treatment groups: one group received 3 weekly intra-articular injections of LR-PRP; the other received 3 weekly intra-articular injections of LP-PRP. The randomization list was provided by an independent statistician, as generated using a random number generator and then kept in a dedicated office. In particular, progressively numbered sealed envelopes containing the treatment allocation were used by a clinical monitor to inform the hematologist (A.C.). Patients, the physicians administering the treatment, and the clinical investigators who evaluated patients at the follow-up visits were all blinded to the type of PRP administered (only the hematologists preparing the 2 PRP types were aware of the study groups), and the specific group assignment was disclosed to the patient only after the 12-month follow-up.

PRP Preparation Methods and Administration

Both PRP preparations were produced via a manual method in the transfusional unit (no commercial PRP kit), using the CompoFlex Double System and the CompoStop Flex T5003 (Fresenius Kabi). In a sterile manner, a single 300-mL unit of peripheral venous blood was harvested in citrate phosphate dextrose adenine (14 mL/100 mL of whole blood) from each patient at the Hospital Transfusion Medicine Service. Immediately afterward, 2 centrifugations were performed: the first at 1800 rpm for 10 minutes to separate erythrocytes, and the second at 3500 rpm for 10 minutes

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to concentrate platelets. To obtain LP-PRP, the plasma and buffy coat, deprived of most red blood cells, were connected to a polyester filter for the leukodepletion (CompoStop Flex T5003) before the second centrifugation (inline filtration by gravity; filtration efficiency, averaging $<0.1 \times 10^6$/L residual leukocytes).[13,17] After the second centrifugation, the platelet-poor plasma was manually removed in both groups, thus obtaining 16 mL of LR- or LP-PRP. The goal was to concentrate the platelets at 1000×10^9/L ± 20%. The collected amount of LR- or LP-PRP was divided into 3 aliquots of 5 mL and stored at −30°C to be used for treatment after being thawed in a dry thermostat at 37°C for 30 minutes. Frozen PRP was thawed 15 minutes before the injection. After thawing, the PRP sample was transferred directly from the transfusion unit to the outpatient clinic in the same hospital, using a thermal bag and avoiding exposure to light. LR- and LP-PRP preparations were identical in physical characteristics such that the administering physician would be unable to tell the difference. The remaining 1 mL was used to perform the platelet, erythrocyte, and leukocyte counts. Cell counts were performed before freezing in the hospital analysis laboratory, with an automated analytical tool.

All patients of both groups were treated by experienced orthopaedic surgeons (A.D.M. and L.A.) at the outpatient department. The treatment consisted of 3 injections with 1-week intervals. Before the injection, PRP was activated by adding 1 mL of calcium gluconate. The skin was sterilely dressed, and the injection was performed through a classic superolateral approach using a 22-g needle. At the end of the procedure, the patient was encouraged to bend and extend the knee a few times to allow PRP to spread throughout the joint. After the injection, patients were sent home with instructions to restrict use of the leg (avoiding strenuous sports and daily living activities for the treated knee) for at least 24 hours and to use ice or other cold therapy on the affected area to relieve pain. During the injection cycle, rest or mild activities were permitted, weightbearing and range of motion were not restricted, and a gradual resumption of normal sports or recreational activities was allowed as tolerated. No other nonoperative treatments were prescribed during the study period, and the use of oral medications was discouraged, especially in the 5 days before each follow-up visit.

Patient Evaluation

All patients were prospectively evaluated at baseline and then at 2, 6, and 12 months after the last injection. All complications and adverse events were assessed and reported during follow-up visits, evaluating the safety of both injective PRP treatments. Mild adverse events were defined as the presence of significant pain or swelling of the treated knee for >5 days as reported by patients, and severe adverse events were defined as any event that resulted in death, was life-threatening, or required hospitalization or interventions to prevent permanent impairment or damage. The primary clinical outcome was defined as the change in International Knee Documentation Committee (IKDC) subjective score at 12 months after the injections. Moreover, the following scores were used for patient evaluation at baseline and each follow-up visit: Knee injury and Osteoarthritis Outcome Score (KOOS) subscales for subjective functional improvement, EuroQol–visual analog scale (EQ-VAS) for patient self-rated health, and Tegner scale for sport/activity level. Patient judgment of the treatment was also recorded at 6 and 12 months using a specific question: "Compared to the baseline status, how would you rate the treated knee now?" The responses were recorded using a 5-point scale: "much better," "somewhat better," "about the same," "somewhat worse," and "much worse." To guarantee double blinding of the trial, all clinical evaluations were performed by independent physicians (A.B. and I.R.) not involved in the injective procedure and who was blinded to the randomization list. In particular, clinical scores were obtained via paper questionnaires during the clinical visit in the research outpatient clinic. Patients completed the questionnaires, and doctors were available in case of questions. If patients were not available for an in-person visit at the requested follow-up time, a phone interview by a physician was performed to retrieve the questionnaire data.

The treatment was considered failed if the knee needed a new injective or surgical procedure because of symptom persistence or worsening. For failed results, the last clinical evaluation available indicating the negative clinical condition causing the study treatment failure was considered for subsequent follow-ups.

Statistical Analysis

For the sample size calculation, a power analysis was performed for the primary endpoint of IKDC subjective score improvement at the 12-month follow-up. A pilot study (G. Filardo, MD, unpublished data, January 2012) revealed an SD of 15.2 points. With an alpha error of .05, a beta error of 0.2, and a minimal clinically significant difference of 6.7 points corresponding to one-third of the documented mean improvement, the minimum sample size was 83 for each group. With a possible dropout rate of 15%, 96 patients per group were required for a total of 192 patients.

For the results analysis, all continuous data were expressed in terms of mean and standard deviation, and categorical data as proportions or percentages. The Shapiro-Wilk test was performed to test normality of continuous variables. The Levene test was used to assess the homoscedasticity of the data. The repeated-measures general linear model with a Sidak test for multiple comparisons was performed to assess the differences at the follow-up times. The Friedman non-parametric test, followed by the Wilcoxon post hoc pairwise comparison corrected by the Bonferroni method for multiple comparisons, was used to assess the differences at the follow-up times for non-normally distributed scores. Analysis of variance was performed to assess the differences between groups of continuous, normally distributed, and homoscedastic data; otherwise, the Mann-Whitney test was used. Analysis of variance followed by the Scheffè post hoc pairwise comparison was also used to assess the differences between groups of continuous, normally distributed, and homoscedastic data; otherwise, the Kruskal-Wallis test followed by the Mann-Whitney test

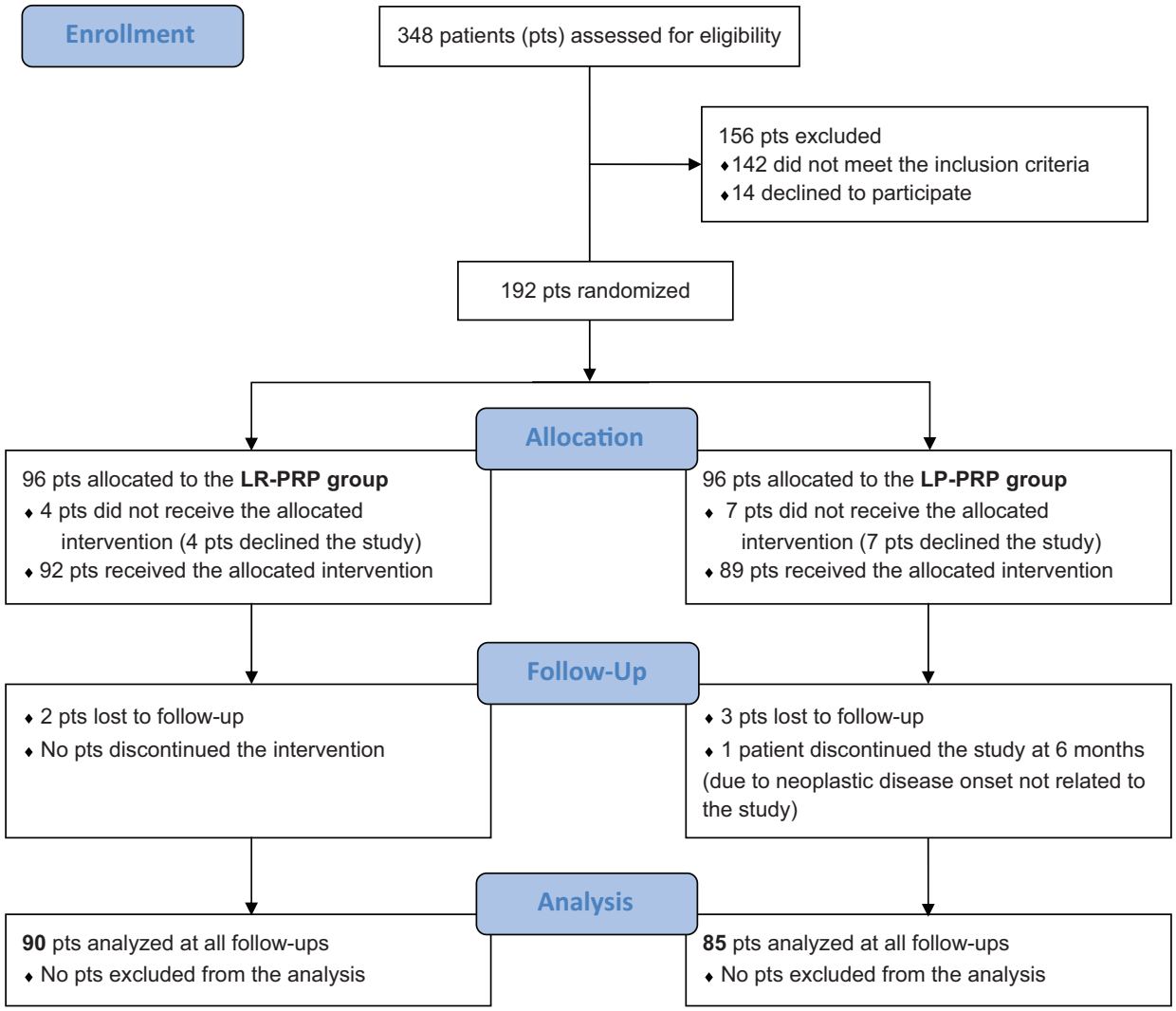

Figure 1. CONSORT (Consolidated Standards of Reporting Trials) flow diagram showing patient inclusion and follow-up. LP, leukocyte poor; LR, leukocyte rich; PRP, platelet-rich plasma.

with the Bonferroni correction for multiple comparison was used. The Spearman rank correlation was used to assess correlations between numerical scores and continuous data, and the Kendall tau correlation was used to assess correlations between ordinal scores and continuous data. For all tests, $P < .05$ was considered significant. All statistical analysis was performed using SPSS 19.0 (IBM Corp).

RESULTS

Patient Characteristics

A total of 192 patients with symptomatic knee OA met the inclusion criteria and were included in the study (Figure 1).[33] Eleven patients did not receive the allocated intervention after being enrolled (for personal reasons), and 6 dropped out for unavailability at follow-up visits. Thus, the study population comprised 90 patients in the LR-PRP group and 85 in the LP-PRP group. Baseline demographics were collected for each patient: sex, age, body mass index (BMI), affected side, symptom duration, previous surgery, and OA grade according to the Kellgren-Lawrence classification. The 2 groups were homogeneous for all baseline characteristics except the activity level (Table 1).

PRP Characteristics

The number of platelets per milliliter increased a mean 4.6 times in LR-PRP and 4.4 times in LP-PRP with respect to baseline whole blood values, with no significant difference between the groups ($P = .071$). The leukocyte concentration was significantly higher in LR-PRP than LP-PRP ($P < .0005$): in LR-PRP, leukocytes were present with a mean concentration of 1.2 times with respect to the whole blood value, while in LP-PRP >99.99% of leukocytes were removed as compared with whole blood values.

TABLE 1
Baseline Demographic Characteristics and Clinical Scores of Patients in Both Groups[a]

	No. or Mean ± SD (Range)		
	LR-PRP (n = 90)	LP-PRP (n = 85)	*P* Value
Sex, male:female	62:28	50:35	.345
Age, y	55.2 ± 9.8	55.7 ± 10.7	.733
Body mass index	26.1 ± 4.5	27.4 ± 4.1	.063
Smoking status, yes:no	10:80	13:72	.413
Side, left:right	39:51	44:41	.291
Symptom duration, mo	56.7 (4-220)	64.5 (4-360)	.395
Kellgren-Lawrence grade			.987
1	10	8	
2	40	40	
3	40	37	
Previous surgery, yes:no	53:37	50:35	.999
IKDC subjective	45.6 ± 15.5	46.8 ± 15.8	.595
Tegner			
Presymptoms	4.2 ± 2.0	3.6 ± 1.8	.017
Pretreatment	3.0 ± 1.6	2.4 ± 1.3	.011
KOOS			
Pain	62.7 ± 17.7	65.2 ± 16.8	.335
Symptoms	62.9 ± 18.2	67.6 ± 15.8	.058
ADL	70.9 ± 20.7	73.8 ± 16.8	.477
Sport/Recreation	47.2 ± 20.2	48.6 ± 18.8	.616
Quality of Life	37.1 ± 16.5	39.0 ± 18.3	.398
EQ-VAS	68.9 ± 16.1	72.4 ± 15.1	.144

[a]ADL, Activities of Daily Living; EQ-VAS, EuroQol–visual analog scale; IKDC, International Knee Documentation Committee; KOOS, Knee injury and Osteoarthritis Outcome Score; LP, leukocyte poor; LR, leukocyte rich; PRP, platelet-rich plasma.

TABLE 2
Platelet, Erythrocyte, and Leukocyte Concentrations in Whole Blood and PRP for the Treatment Groups[a]

	Mean (Range)		
	LR-PRP	LP-PRP	*P* Value
Platelets			
Whole blood	249.5 (153.1-373.0)	245.1 (151.8-393.4)	.656
PRP	1146.8 (799.3-1591.3)	1074.9 (512.4-1652.1)	.071
Erythrocytes			
Whole blood	4942.3 (4030.1-6350.0)	5000.4 (3910.2-7650.1)	.244
PRP	0.2 (0.0-0.6)	0.2 (0.1-0.8)	≥.999
Leukocytes			
Whole blood	6613.3 (4206.7-13,912.0)	6290.5 (3580.2-11,592.3)	.300
PRP	7991.4 (1330.4-16,930.7)	0.1 (0.0-0.6)	<.0005

[a]Platelet and erythrocyte values are expressed as n × 10^9/L; leukocyte values are expressed as n × 10^6/L. LP, leukocyte poor; LR, leukocyte rich; PRP, platelet-rich plasma.

Comparative Analysis

A statistically significant improvement in all clinical scores was documented from baseline to the final follow-up for both groups (Table 2). The mean IKDC subjective score (Figure 2) improved significantly at 12 months, from 45.6 ± 15.5 to 60.7 ± 21.1 for the LR-PRP group ($P < .0005$) and from 46.8 ± 15.8 to 62.9 ± 19.9 for the LP-PRP group ($P < .0005$). The comparative analysis of the primary outcome (change in the IKDC subjective score at 12 months) did not demonstrate a significant difference between the groups ($P = .626$). Moreover, no differences were observed between the groups in terms of absolute values and improvement of the other clinical scores (Table 3, with minimal clinically important differences specified). Also, the 2 groups reported comparable judgment of the treatment, with the knee rated "improved" in 70% (47% as "much better" and 23% as "somewhat better") and 68% (48% as "much better" and 20% as "somewhat better") of patients at 6 and 12 months, respectively, for the LR-PRP group and in 75% (48% as "much better" and 27% as "somewhat better") and 76% (48% as "much better" and 28% as "somewhat better") of patients at 6 and 12 months, respectively, for the LP-PRP group.

Male sex and younger age were correlated with higher clinical improvement in both treatment groups, while no significant correlation was found between clinical outcomes and BMI or Kellgren-Lawrence grade. The subanalyses based on age, sex, BMI, and Kellgren-Lawrence grade did not demonstrate any significant differences between the treatment groups.

No severe adverse events were described for either group, although 15 mild adverse events were reported: knee pain, joint warmth, or swelling that resolved within few days after cold compress and rest. The rate of mild adverse events was 12.2% for the LR-PRP group and 4.7% for the LP-PRP group, without a statistically significant difference ($P = .101$).

Similarly, no statistically significant difference was found between LR-PRP and LP-PRP in terms of failures (7.8% vs 3.5%, respectively; $P = .331$). In the LP-PRP group, 2 patients underwent total knee replacement at 6 and 8 months of follow-up, and 1 patient received a new injection therapy at 6 months. In the LR-PRP group, 3 patients underwent a surgical procedure for persistent symptoms at 6 months (2 total knee replacements and 1 arthroscopic debridement), and 4 patients were treated with another knee injective therapy (2 at 3 months, 1 at 6 months, and 1 at 12 months).

DISCUSSION

The main finding of this double-blind randomized trial is that the presence of leukocytes did not influence the clinical outcome of PRP injective treatment to address patients with knee OA. The LR- and LP-PRP groups reported comparable clinical improvement at all evaluations performed at all follow-ups, without any significant difference in terms of adverse events and treatment failures.

The safety and effectiveness of PRP for knee OA treatment have been supported by several clinical studies, yielding satisfactory results in terms of functional improvement and reduction of pain-related symptoms up to 12 months, especially in young patients and during the early stages of OA.[2,15,16,18,39] Nevertheless, despite the large number of trials in favor of PRP, its use is not yet recommended by the principal guidelines for the management of knee OA.[6,10,24] This is likely related to the lack of a standardized protocol and to the availability of

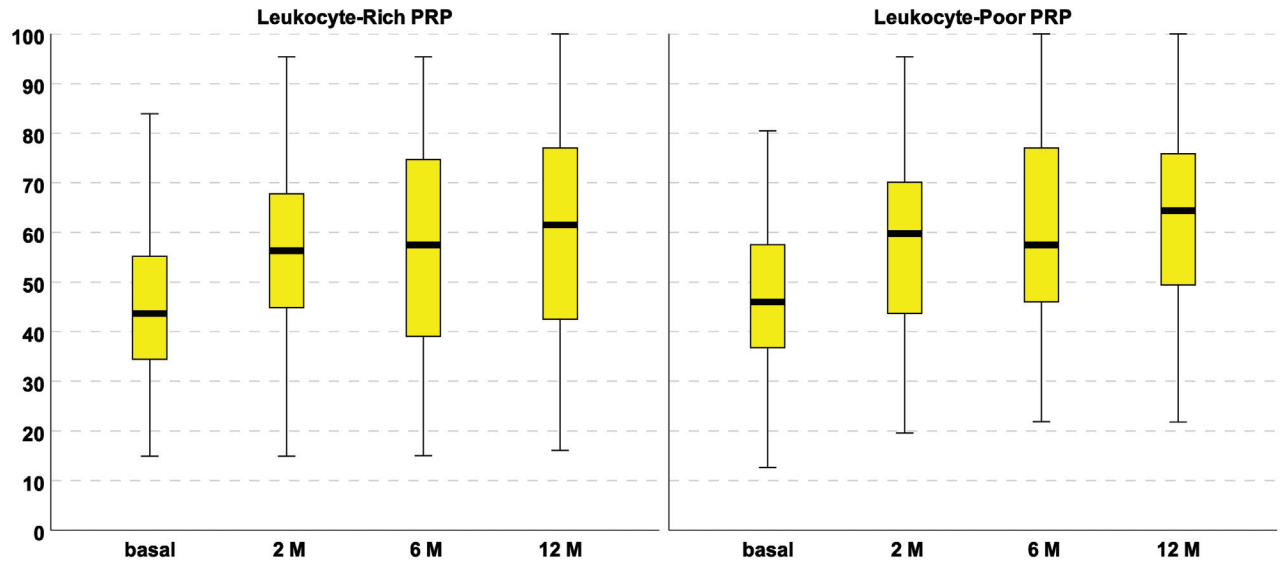

Figure 2. International Knee Documentation Committee subjective score trend in both treatment groups at baseline and 2-, 6-, and 12-month follow-ups. The box-and-whisker plots show median, quartile, and 95% Confidence Interval. PRP, platelet-rich plasma.

TABLE 3
Scores at Baseline and Follow-up in the Treatment Groups[a]

Outcome: Group	Baseline	2 mo	6 mo	12 mo
IKDC subjective				
LR-PRP	45.6 ± 15.5	55.6 ± 18.1[b]	57.9 ± 20.6[b,c]	60.7 ± 21.1[b,c,d]
LP-PRP	46.8 ± 15.8	57.4 ± 18.0[b]	60.0 ± 20.3[b,c]	62.9 ± 19.9[b,c,d]
Tegner				
LR-PRP	3.0 ± 1.6[e]	3.3 ± 1.3	3.4 ± 1.4	3.6 ± 1.5[b]
LP-PRP	2.4 ± 1.3[e]	3.3 ± 1.4[b]	3.3 ± 1.5[b]	3.5 ± 1.5[b]
KOOS				
Pain				
LR-PRP	62.7 ± 17.7	72.4 ± 18.7[b]	75.2 ± 19.3[b,c]	76.2 ± 19.4[b,c]
LP-PRP	65.2 ± 16.8	75.3 ± 15.0[b]	74.9 ± 18.8[b,c]	78.3 ± 18.5[b,c]
Symptoms				
LR-PRP	62.9 ± 18.1	73.3 ± 17.3[b]	74.9 ± 18.9[b,c]	75.7 ± 18.8[b,c]
LP-PRP	67.6 ± 15.8	76.8 ± 14.2[b]	77.5 ± 16.8[b,c]	80.3 ± 16.6[b,c]
ADL				
LR-PRP	70.9 ± 20.7	79.8 ± 18.0[b]	81.7 ± 17.9[b,c]	82.2 ± 17.9[b,c]
LP-PRP	73.8 ± 16.8	83.8 ± 14.2[b]	81.9 ± 18.2[b]	85.2 ± 17.5[b,c]
Sport/Recreation				
LR-PRP	47.2 ± 20.2	57.5 ± 22.2[b]	59.3 ± 24.9[b]	61.7 ± 25.3[b,c]
LP-PRP	48.6 ± 18.8	60.1 ± 21.4[b]	61.6 ± 23.6[b,c]	63.9 ± 23.9[b,c]
Quality of Life				
LR-PRP	37.1 ± 16.5	50.0 ± 22.5[b]	55.0 ± 26.4[b,c]	57.0 ± 27.0[b,c,d]
LP-PRP	39.0 ± 18.3	52.8 ± 21.2[b]	55.2 ± 25.3[b,c]	59.8 ± 25.1[b,c]
EQ-VAS				
LR-PRP	68.9 ± 16.1	75.0 ± 14.4[b]	75.2 ± 15.8[b]	77.0 ± 15.7[b,d]
LP-PRP	72.4 ± 15.1	75.8 ± 14.1[b]	78.9 ± 12.1[b,d]	79.1 ± 13.6[b,d]

[a]ADL, Activities of Daily Living; EQ-VAS, EuroQol–visual analog scale; IKDC, International Knee Documentation Committee; KOOS, Knee injury and Osteoarthritis Outcome Score; LP, leukocyte poor; LR, leukocyte rich; PRP, platelet-rich plasma.

[b]$P < .05$ vs baseline.

[c]Minimal clinically important difference achieved at 6 and 12 months, respectively: IKDC, 8.6 and 8.5; KOOS ADL, 9.0 and 9.2; KOOS Pain, 9.3 and 9.1; KOOS Quality of Life, 10.3 and 10.3; KOOS Sport/Recreation, 12.5 and 11.6; KOOS Symptoms, 8.4 and 8.2.[7]

[d]$P < .05$ vs 2 months.

[e]$P < .05$ in favor of the LR-PRP group.

countless PRP preparation methods, which can yield products with different characteristics. The number of compositions and associated variables can be overwhelming and confusing for clinicians and patients, as well as scientists working in the field. Thus, it is paramount to understand if different products may provide different results in terms of safety and effectiveness, and the field is currently focusing most of the attention on the presence of leukocytes in PRP.[21]

Several in vitro experiments have suggested that leukocytes can alter PRP properties, releasing catabolic and proinflammatory molecules, which could be detrimental to the joint. In human synoviocyte cultures, LR-PRP resulted in significantly greater synoviocyte death and higher production of proinflammatory mediators when compared with LP-PRP, platelet-poor plasma, or saline.[9] Cavallo et al[12] investigated the effect of various PRP formulations on human chondrocytes, stating that PRP with a relatively low concentration of platelets and few leukocytes led to greater cell growth and anabolism in terms of type II collagen and aggrecan production. Conversely, PRP with high concentrations of platelets and leukocytes contained the highest level of growth factors and cytokines and induced a higher hyaluronan production but also promoted catabolic pathways. In a similar comparative setting focused on OA synoviocytes, Assirelli et al[4] confirmed that PRP with a high concentration of platelets and leukocytes can upregulate proinflammatory factors and downmodulate anticatabolic mediators. However, PRP without leukocytes had a limited number of platelets and did not provide superior results as compared with the simple platelet-poor plasma, thus suggesting that the lower concentration of platelets in LP-PRP may lead to a lower secretion of bioactive molecules and therefore lower effects. As such, the complexity of the analyzed composites may go beyond the mere leukocyte component and the simplistic dichotomy between good and bad PRP, based on the leukocyte presence. Moreover, in vitro studies alone cannot mirror the complexity of the joint environment response to PRP intra-articular injections, and in vivo studies are needed to confirm the suggested detrimental effects of leukocytes in PRP.[1]

In a mouse model of posttraumatic knee OA, Jayaram et al[23] compared the intra-articular injection of LR-PRP and LP-PRP to assess the potential to alter disease progression. The authors observed controversial results, indicating better protection from cartilage volume loss at phase-contrast computed tomography analysis after LP-PRP, while LR-PRP demonstrated better protection from thermal hyperalgesia; in addition, the histologic evaluation did not show differences in terms of cartilage degeneration and synovitis. Different results were noted in a rat knee OA model by Araya et al[3] evaluating 3 types of platelet concentrates: pure PRP, LP-PRP, and LR-PRP. All PRP compositions provided improvement of pain-related behavior and histological findings, but pure PRP offered better results in reducing the progression of cartilage degeneration and preventing synovitis and infrapatellar fat pad fibrotic changes. Similarly, Yin et al[41] found better results in preventing cartilage destruction in rabbits with knee OA after pure PRP injections than with LR-PRP. The authors also investigated the effects of both PRPs on synovial fluid biomarkers levels and cited an increase of proinflammatory factors, such as IL-1β and PGE2, after LR-PRP injections, underlining the possible proinflammatory effect of leukocytes.

Overall, the literature inclines toward the secretion of proinflammatory molecules from leukocytes. However, other bioactive molecules are secreted as well, and the final net effect remains to be demonstrated, especially when going from preclinical studies to the clinical setting. With this aim, a biomarker analysis was performed in humans, yielding inconsistent findings as compared with the animal model. Mariani et al[30] assessed synovial fluid and blood samples in patients treated with LR-PRP, stating that proinflammatory cytokine levels did not increase significantly after 1 week from the injection. In this light, the detrimental effects documented in preclinical studies were not confirmed. In addition, although leukocytes may secrete proinflammatory molecules and cause an inflammatory reaction, this seems self-limiting with no inflammatory response detectable 1 week after the injection.

From a clinical results perspective, evidence supporting the influence of leukocytes on PRP effects is lacking. Several studies supported promising clinical results in patients with knee OA treated with intra-articular LR-PRP injections,[25,29] and some products even rely on leukocytes to provide bioactive molecules, with positive findings.[27,28] In the end, a comparative evaluation in humans is needed to understand the advantages and disadvantages of leukocytes in PRP. A network meta-analysis of 1055 patients with knee OA provided an indirect comparison between LR-PRP and LP-PRP. The authors stated that functional outcome scores were at best marginally affected by leukocyte concentration in favor of LP-PRP, while the incidence of local reactions to PRP injections was not affected.[37] However, this meta-analysis presented several concerns related to the paucity of available data and scarce methodology.[32]

Trials directly comparing PRP products are needed to draw meaningful conclusions on the role of leukocytes. The largest available study comparing the safety and efficacy of the 2 PRP preparations evaluated 144 patients treated with 3 injections of PRP prepared with either a single-spinning (LP-PRP) or double-spinning (LR-PRP) procedure. Pain and swelling reaction after the injections were more frequent in the LR-PRP group, thus supporting the negative role played by leukocytes; in the end, this did not affect the final results, with the 2 treatment groups having comparable positive results up to 12 months.[20] More recently, a prospective randomized controlled trial evaluated 90 patients with knee OA treated with 3 injections of LR-PRP, LP-PRP, or hyaluronic acid (30 patients for each group).[40] The authors noted more transient local side effects after the injection of LR-PRP, but the clinical results up to 12 months were even better in these patients than the other 2 groups. However, this study presents significant drawbacks, such as the small sample size and the significantly different platelet concentration for LP-PRP (1.9-fold vs whole blood) than LR-PRP (4.6-fold vs whole blood). The significant difference in platelet concentrations may represent a bias in the evaluation of leukocyte-related results by influencing the outcome and therefore reducing the strength of the study conclusions.

To overcome the limitations of the previous literature and offer clear indications on the role of leukocytes, clinical studies need to go beyond the simple comparison of products with or without leukocytes, as commercial products with leukocytes generally imply a higher number of platelets and likely other differences in terms of composition as compared with leukocyte-free or LP products. The current double-blind randomized trial aimed to produce 2 products as similar as possible before using a filter to isolate the study variable by removing leukocytes. A large number of patients were included, and the double-blind design allowed us to control for the placebo effect of the injective treatment. While demonstrating significant improvement in all scores for both groups, the comparative analysis between LR-PRP and LP-PRP did not show any difference in terms of clinical improvement at all evaluations performed during follow-ups. Moreover, the 2 treatment groups had similar numbers of failures and adverse events, not confirming the findings suggested by previous studies. This is important, given the larger number of patients, stronger study design, and focus of this study to isolate the leukocyte contribution to the final results.

Despite the stronger methodology than previous literature, this study presents some limitations. First is the lack of a control group to evaluate the placebo effect, which was suggested to play an important role in injective treatment results.[36] However, the aim of this study was to investigate the role of leukocytes, rather than to assess PRP efficacy versus placebo, which has already been demonstrated in randomized controlled trials and meta-analyses.[22] A validated filter for leukodepletion was used, but the lack of molecular analysis could not exclude the possibility that the filter could somehow affect the molecular composition of the products. Moreover, the use of an anticoagulant during the blood harvest procedure and the use of freeze-thaw PRP should be acknowledged as a variable that could affect platelets' biologic effects,[38] and further studies should verify the influence of leukocytes in fresh PRP products. Another limitation of this study was the lack of imaging evaluation, although this should not be considered a main outcome for injective therapies for OA at 12 months, a follow-up time mostly focused on homeostatic effects. Moreover, previous studies have already evaluated this aspect, demonstrating no progression of Kellgren-Lawrence grade or cartilage thickness damage at short-term follow-up after PRP injections.[11] Another important aspect would be the molecular characterization of the injected products. Yet, the procedures differed only for the filter application, removing leukocytes while leaving otherwise similar products to be compared, which were characterized in terms of number of platelets, erythrocytes, and leukocytes. The use of the Tegner score could be criticized as well for not being validated for OA, but it has been included as a secondary aspect to provide a more complete description of the study population, given that part of the sample was relatively young and active. Although patients were advised not to take other medications, especially in the 5 days before each visit, it is not possible to fully exclude those patients who independently decided to undergo and not report other treatments or measures (eg, pain medications, weight loss, activity changes) that could affect the level of symptoms. Finally, when compared with previous evaluations in which adverse effects were documented early after treatment, in this study, patients were visited after 2 months from the treatment,[20] which could have underestimated patient reporting of mild adverse events happening immediately after the injection.

Despite these limitations, this study sheds some light on this controversial aspect of platelet concentrates. Leukocytes alone were not confirmed to be as detrimental as previously suggested, thus currently supporting the use of LR- and LP-PRP for knee OA treatment. PRP is a composite product, and many other variables beside leukocytes likely interact, contributing to the final results—among others, the volume of PRP and the number of platelets injected, the preparation method, the activation by different substances, the timing of activation, and the number and time between injections, as well as the type of patient and disease phase that could benefit more from this biological treatment.[26] Unresolved issues related to all these parameters must be addressed to optimize the use of PRP for patients affected by knee OA.

CONCLUSION

This double-blind randomized trial showed that 3 intra-articular LR- or LP-PRP injections provided a significant and similar clinical improvement up to 12 months of follow-up in patients with symptomatic knee OA. Both treatment groups had a low number of failures and adverse events, without significant intergroup differences. Therefore, the presence of leukocytes did not significantly affect the clinical results obtained with PRP injections, and further studies should focus on the interaction of more biological variables, besides leukocytes, to optimize PRP treatment for knee OA.

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