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The Effectiveness of Leukocyte-Poor Platelet-Rich Plasma Injections for Symptomatic Mild to Moderate Osteoarthritis of the Knee With Joint Effusion or Bone Marrow Lesions in a Japanese Population

A Randomized, Double-Blind, Placebo-Controlled Clinical Trial

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Background: Intra-articular platelet-rich plasma (PRP) injections have been proposed for the treatment of knee osteoarthritis (OA); however, their effectiveness in Japanese patients remains unclear.

Purpose: To investigate whether 3 intra-articular injections of leukocyte-poor PRP (LP-PRP) improve symptoms and joint function in symptomatic Japanese patients with mild to moderate knee OA.

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

Methods: Of 72 patients screened, 30 were included and randomized to receive LP-PRP (n = 15) or saline (placebo; n = 15) injections between March 2019 and February 2023. Patients attended a screening visit and 3 treatment visits at 1 week apart, followed by 3 follow-up visits (at 4, 12, and 24 weeks) after the initial treatment visit. The primary efficacy outcome measure was the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) score, whereas the secondary efficacy outcome measures were the proportion of patients showing a visual analog scale (VAS) improvement of ≥50%. Magnetic resonance imaging was performed to evaluate joint effusion and bone marrow lesions using the Whole-Organ Magnetic Resonance Imaging Score. Patients were followed for 24 weeks.

Results: Patients in the PRP group (mean age, 65.9 years) had a mean hip-knee-ankle angle of 5.1°, with 7 and 8 patients demonstrating Kellgren-Lawrence grade 2 and 3 knee OA, respectively. Patients in the placebo group (mean age, 67.9 years) had a mean hip-knee-ankle angle of 3.8°, with 6 and 9 patients showing Kellgren-Lawrence grade 2 and 3 knee OA, respectively. No significant differences were identified in any baseline factors. The percentage change in Western Ontario and McMaster Universities Osteoarthritis Index scores from baseline to 24 weeks was significantly different (P= .032) between the PRP (median, 75.9%; quantile 1 [Q1], 49.6; quantile 3 [Q3], 94.1]) and placebo (median, 27.7%; Q1, –9.4; Q3, 80.9]) groups. Overall, 73.3% and 28.6% of the PRP group and placebo group, respectively, exhibited an improvement in visual analog scale scores of ≥50%, with a significant improvement observed in the PRP group (*P* = .027). Changes in bone marrow lesions from baseline to 24 weeks, as assessed on magnetic resonance imaging, significantly differed between groups (*P* = .017), with no significant differences in other secondary endpoints.

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Conclusion: In Japanese patients with knee OA, 3 intra-articular LP-PRP injections led to clinical improvements at 24-week follow-up and significant functional improvements and pain relief after 24 weeks.

Keywords: Japanese; knee; osteoarthritis; platelet-rich plasma

Japan has transitioned into a superaged society, with the elderly population exceeding 21% in 2007 and currently approaching 30%. This demographic shift has brought forth the challenge of locomotive syndrome, characterized by a decline in mobility due to musculoskeletal disorders, particularly knee osteoarthritis (OA). Knee OA is a major degenerative disease affecting approximately 8 million patients and manifests as knee joint pain, stiffness, and swelling.[27] It has substantial individual and societal effects, making it an urgent issue in Japan. Radiological joint changes are observed in approximately 25 million people, with a prevalence of approximately 55% among those aged ≥40 years. Approximately 18 million patients are symptomatic, with a 6-fold increased risk of requiring caregiving.[29] Knee OA progression varies, with symptoms such as knee joint pain and stiffness gradually worsening over several years. Some patients respond well to existing nonoperative treatment approaches and experience favorable outcomes without progressing to surgery, whereas others do not, suffering advanced deformities, pain, and reduced joint function and necessitating surgical interventions.[35]

Platelet-rich plasma (PRP) is plasma containing a high concentration of platelets obtained by whole-blood centrifugation. The presumed mechanism of action of this treatment lies in the complex action of various growth factors within concentrated platelet α-granules as well as growth factors, adhesion factors, and glycoproteins present in plasma.[2] Preparation methods vary; the biological activity of the final product differs depending on various factors, such as platelet concentration and activation methods.[18] PRP contains 2 of 3 major elements required for tissue regeneration (ie, cells, growth factors, and scaffolds) and is applied to soft tissue and other injuries to replicate the initial stages of physiological tissue repair.[36,47] A recent meta-analysis showed that PRP treatment for knee OA led to sustained relief of knee joint pain and improvement in knee joint function for 6 months to 1 year compared with intra-articular hyaluronic acid injections and placebos.[7,14,37,39] Nevertheless, that report was based on clinical results of patients outside of Japan; it remains uncertain whether a similar effectiveness can be achieved in Japanese patients with knee OA who receive comprehensive orthopaedic care, including diagnosis, nonoperative treatment, and surgery, under the national health insurance system. Accordingly, phase 1 and 2a clinical trials were conducted to evaluate the safety and feasibility of intra-articular PRP injections in Japanese patients with knee OA.[42,43]

As PRP treatment is a new modality, it is important to apply it to groups in which its therapeutic effects are expected to be maximized, especially in situations in which conventional treatment approaches yield limited results and disease progression is anticipated. Clinical manifestations of knee OA–related inflammation include knee joint pain and joint effusion, which are associated with functional impairments and serve as predictive factors for future knee replacement surgery.[12] Additionally, magnetic resonance imaging (MRI) findings, such as synovitis, joint fluid accumulation, and bone marrow lesions (BMLs), are reportedly associated with knee OA progression.[6,34]

We aimed to compare the effectiveness of intra-articular PRP injections versus intra-articular saline injections (ie, placebo) for knee OA in patients exhibiting joint effusion or BMLs on MRI. We hypothesized that multiple intra-articular injections of leukocyte-poor PRP (LP-PRP) would effectively improve knee joint function and alleviate pain in symptomatic patients with knee OA with joint effusion or BMLs.

METHODS

Study Design and Patient Selection

This single-center, double-blind randomized controlled trial was approved by the local ethics committee and conducted in accordance with the 2014 "Act on the Safety of Regenerative Medicine" and "Pharmaceuticals, Medical Devices and Other Therapeutic Products Act,"[22] which, to our knowledge, represent the strictest regulatory framework for the production and therapeutic use of platelet concentrates worldwide (NA8150002). The trial was registered at the University Hospital Medical Information Network (No. UMIN000034517) and the Japan Registry of Clinical Trials (No. jRCTb032190218). Each patient provided informed consent before enrollment.

Patients were recruited from October 16, 2018 to February 14, 2023. Treatment was administered from March 2019 to February 2023. Participants were enrolled at the

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TABLE 1
Inclusion and Exclusion Criteria[a]

Inclusion Criteria	Exclusion Criteria
Patients who met diagnostic criteria for knee OA	Bilateral knee OA requiring infiltration in both knees
Male and female patients aged 20-79 y	Polyarticular disease
History of chronic (at least 3 mo) knee joint pain ≥35 mm on VAS (0-100 mm)	Severe mechanical deformity of varus or valgus (HKA angle ≥10°)
KL grades 1-3 (patellofemoral OA cases with KL grade 4 excluded)	Meniscal degeneration or injury with mechanical symptoms (locking, catching, etc)
Concomitant joint effusion or BMLs on MRI	Knee surgery in last 6 mo
BMI of 18.5-29.9	Intra-articular hyaluronic acid injection in last 1 mo
Willingness/availability to be observed during follow-up period	History of infections or current infection in affected joint
	Skin disease around knee
	Compromised host (diabetes mellitus, immunosuppressive therapy, etc)
	Systemic autoimmune disease (rheumatoid arthritis, etc)
	Gout or pseudogout
	Blood disorders (thrombopathy, thrombocytopenia, anemia with hemoglobin <9.0 g/dL)
	Treatment with anticoagulant drugs (aspirin, warfarin, etc)
	Treatment with steroids in last 3 mo
	Treatment with NSAIDs in last 2 wk
	Malignancy in last 5 y
	Lack of consent
	Patients with dementia or psychiatric disorders who could potentially present high safety risk for study treatment and interfere with assessment of endpoints (such as ethical and scientific aspects)

[a]BMI, body mass index; BML, bone marrow lesion; HKA, hip-knee-ankle; KL, Kellgren-Lawrence; MRI, magnetic resonance imaging; NSAID, nonsteroidal anti-inflammatory drug; OA, osteoarthritis; VAS, visual analog scale.

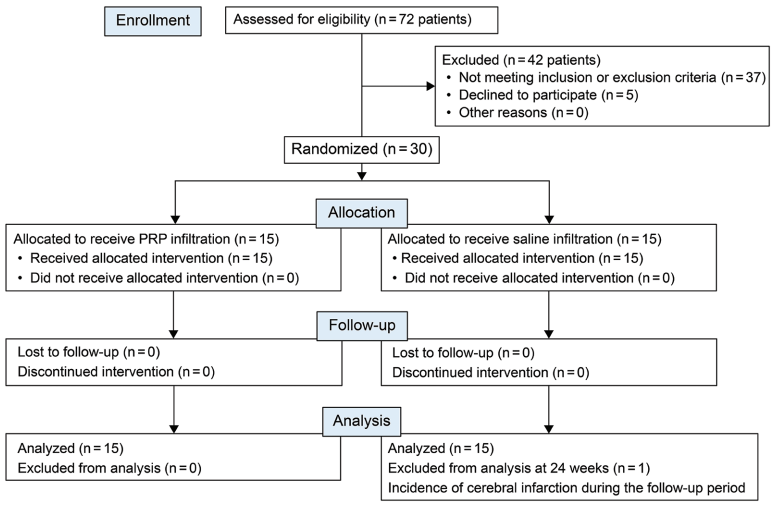

Figure 1. Consolidated Standards of Reporting Trials (CONSORT) flow diagram. PRP, platelet-rich plasma.

outpatient clinic of the University of Tsukuba Hospital and were evaluated for eligibility according to inclusion and exclusion criteria (Table 1).

Figure 1 presents the Consolidated Standards of Reporting Trials (CONSORT) flow diagram for this trial.[28] A total of 72 patients with symptomatic knee OA met the inclusion criteria; the most common reasons for exclusion were a hip-knee-ankle (HKA) angle[11] of ≥10° and polyarthralgia (n = 7), diabetes mellitus (n = 6), and refusal to participate in the trial (n = 5). After inclusion, 30 patients were randomized to receive 3 weekly intra-articular injections of PRP (n = 15) or saline (placebo; n = 15). This study was designed to evaluate the efficacy of 3 intra-articular PRP injections at 1-week intervals over a 24-week period. Patients in both treatment groups were allowed to take acetaminophen only for breakthrough pain. Patients attended a screening

TABLE 2
Baseline Patient Characteristics and Clinical Findings[a]

	PRP (n = 15)	Placebo (n = 15)
Age, y	65.9 ± 8.0	67.9 ± 10.7
Male/female sex, n	3/12	6/9
Left/right side, n	6/9	7/8
BMI	24.0 ± 3.3	22.9 ± 1.4
KL grade		
2	7 (47)	6 (40)
3	8 (53)	9 (60)
Primary arthritis	12 (80)	14 (93)
HKA angle, deg	5.1 ± 2.3	3.8 ± 3.0
Alignment, n		
Varus	12	12
Valgus	3	3
VAS score, mm	62.6 ± 14.6	50.1 ± 19.8
Normalized WOMAC score		
Pain	43.7 ± 17.8	37.0 ± 18.8
Stiffness	49.5 ± 23.5	45.6 ± 31.4
Physical function	40.0 ± 21.7	37.0 ± 15.5
Total	41.6 ± 19.6	37.7 ± 15.4
WORMS score		
Effusion		
1	8 (53)	5 (33)
2	5 (33)	8 (53)
3	2 (13)	2 (13)
BMLs	5.0 ± 4.5	3.9 ± 4.4

[a]Data are presented as mean ± SD or n (%) unless otherwise indicated. BMI, body mass index; BML, bone marrow lesion; HKA, hip-knee-ankle; KL, Kellgren-Lawrence; PRP, platelet-rich plasma; VAS, visual analog scale; WOMAC, Western Ontario and McMaster Universities Osteoarthritis Index; WORMS, Whole-Organ Magnetic Resonance Imaging Score.

visit and 3 treatment visits at 1 week apart, followed by 3 follow-up visits (at 4, 12, and 24 weeks) after the initial treatment visit (Figure 1).

Randomization and Blinding

Patients were randomly assigned to 2 treatment groups using a computer-generated randomization system with stratified allocation at a 1:1 ratio. Group 1 (investigation arm) received 3 intra-articular PRP injections, and group 2 (control arm) received 3 intra-articular saline injections, spaced out once per week, with the stratification factor set as Kellgren-Lawrence (KL) grade 1/2 or KL grade 3. Non-blinded personnel were responsible for PRP preparation, peripheral blood collection, and intra-articular PRP/saline injection administration, ensuring consistency across patients. A senior orthopaedic surgeon (A.K.) acted as a blinded evaluator who conducted outpatient assessments at 4, 12, and 24 weeks after treatment. Patient blinding was achieved by placing patients in the supine position during the injection process, rendering a visual inspection impossible. Furthermore, the injection syringe was shielded with aluminum foil, and the injection site was concealed using a screen to block visibility.

Patient Characteristics

Both groups were homogeneous, with similar baseline characteristics (Table 2).

PRP Procedure

Patients who met inclusion criteria were scheduled for the first visit and received either of the 2 active study treatment modalities—namely, infiltration of the affected knee with PRP (3 injections per week) or infiltration of the affected knee with saline (3 injections per week; Otsuka Normal Saline [Otsuka])—depending on previously performed randomization. Additionally, 36 mL of peripheral blood was obtained from the median cubital vein using a 22-gauge needle and injected into four 9.0-mL sterile extraction tubes containing 3.8% trisodium citrate.[23,24,42,43] Blood was centrifuged once at 2100 rpm for 8 minutes at room temperature using PRGF-Endoret (BTI Biotechnology Institute). A line was drawn 5.0 mm above the buffy coat layer. Plasma above this line was divided into 2 parts: platelet-poor plasma (upper part) and LP-PRP (lower part). After platelet-poor plasma removal, 8.0 mL of LP-PRP was extracted: 6.0 mL was injected into the knee joint without activation, 1.0 mL was stored at −80°C for growth factor and plasma protein measurements, and the remaining 0.5 mL was used for hematological analysis. Patients were positioned supine with their knees flexed at 20°. Subsequently, the presence of joint fluid was confirmed by ultrasound, and a puncture was performed; a single orthopaedic surgeon (T.Y.) injected LP-PRP without using ultrasound.

Under sterile conditions, 6 mL of the prepared LP-PRP was injected into the suprapatellar pouch using a 21-gauge needle via the lateral parapatellar approach. Ultrasound was used to assess joint effusion just before the PRP injection in all cases, although ultrasound-guided injections were not universally performed. This study targeted patients with joint effusion or BMLs. For those with joint effusion, needle insertion into the joint space was facilitated by an enlarged joint capsule due to effusion. Needle replacement was omitted after the aspiration of joint effusion to ensure precise PRP delivery into the joint space. Patients without joint effusion but with BMLs underwent ultrasound after intra-articular injections to confirm the presence of a low echoic area within the joint space. After injections, no restrictions were imposed on daily activities. If necessary, patients were allowed to take acetaminophen; however, nonsteroidal anti-inflammatory medications were prohibited. Patients were also not allowed to participate in active or heavy sports for 72 hours.

Hematological Analysis

Cellular composition analysis of white blood cells (WBCs), erythrocytes, and platelets in peripheral whole-blood samples and LP-PRP was performed using an automated cell count analyzer (KX-21N; Sysmex). The platelet concentration ratio (PRP platelet concentration/peripheral whole-

blood platelet concentration or platelet concentration in the final injectate), platelet recovery rate (percentage; number of platelets in 8.0 mL of PRP compared with the platelet count in 36 mL of peripheral blood), leukocyte contamination rate (percentage; number of LP-PRP WBCs/number of whole-blood WBCs), and quantity of injected platelets (intra-articular injection volume of PRP: 6.0 mL × PRP platelet concentration) were calculated. Sterility testing involved submitting a 0.5-mL portion of PRP intended for injections to a culture test at the hospital, which confirmed it as culture-negative. Furthermore, PRP was classified using the PAW classification system.[13]

Outcome Measures

The primary outcome measure was the percentage change in the total Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) score[8] from baseline to 24 weeks, calculated as (baseline score – 24-week score)/baseline × 100%. Secondary outcome measures were as follows: (1) proportion of patients with an improvement of ≥50% on the visual analog scale (VAS; 0-100 mm) at 24 weeks compared with baseline; (2) proportion of patients who met the Outcome Measures in Rheumatology Committee and Osteoarthritis Research Society International Standing Committee for Clinical Trials Response Criteria Initiative (OMERACT-OARSI)–restricted responder criteria (either the WOMAC pain or physical function score improved by ≥50% and by at least 20 points), as defined by Pham et al[32]; and (3) MRI assessment using the Whole-Organ Magnetic Resonance Imaging Score (WORMS).[31] Outcomes were evaluated using the WOMAC VA3.1 questionnaire and compared with those at baseline based on OMERACT-OARSI criteria. The WOMAC comprises 24 items divided into 3 subscales: pain, stiffness, and physical function. Patients answered the questions and received a cumulative score for each of the 3 domains (pain, 0-500; stiffness, 0-200; physical function, 0-1700), with higher scores indicating greater pain and stiffness and worsened physical capability.

Secondary efficacy outcomes were (1) WOMAC subscale scores for pain, stiffness, and physical function; (2) the percentage of OMERACT-OARSI–restricted responders; and (3) the amount of acetaminophen in milligrams per day. Pain was assessed using the VAS measured on a 100-mm line, with 100 indicating the worst possible pain, 50 indicating moderate pain, and 0 indicating no pain. Acetaminophen was the only medication permitted during the clinical trial. The use of rescue medications was recorded daily in patients' diaries.

MRI Assessment

Structural changes were assessed on MRI from baseline to 24 weeks and at 4, 12, and 24 weeks after the first injection. Knee MRI was conducted on each patient using a 1.5-T whole-body scanner with a dStream Knee 16ch coil (Ingenia Evolution; Philips). There were 2 features of the treated knees that were examined on MRI using the WORMS: joint effusion and BMLs.[31] Overall, 2 experienced orthopaedic surgeons (N.A. and H.S.) performed an imaging evaluation by blindly assessing and reviewing the images. Joint effusion was graded on a scale from 0 to 3 based on the estimated maximal distention of the synovial cavity: 0, normal; 1, <33% of maximum potential distention; 2, 33% to 66% of maximum potential distention; and 3, >66% of maximum potential distention. BMLs and subarticular bone marrow abnormalities were defined as poorly marginated areas of increased signal intensity in healthy fatty marrow on fat-suppressed T2-weighted fast spin echo images. This feature was graded from 0 to 3 based on the extent of regional involvement (0, no signal increase; 1, <25% of the area; 2, 25%-50% of the area; and 3, >50% of the area) for each of 14 articular surface regions and for the region of the tibia beneath the tibial spine. Effusion was assessed for improvement, no change, or progression at 24 weeks compared with baseline, whereas BMLs were evaluated for changes in quantity from baseline to 24 weeks.

Sample Size Calculation

In a previous double-blind randomized trial comparing the efficacy of PRP and saline, Smith[40] reported that the mean percentage change in the total WOMAC score from baseline to 6 months was 75% (95% CI, 55-95). Conservatively, this study assumed a mean of 55% for the PRP group. The mean percentage change was set to 4% for the placebo group based on the study by Smith.[40] The number of patients required was 12 per group based on the Student *t* test, with an SD of 36%, significance level of 0.05, and power of 0.9. Considering potential dropouts, the target number of enrolled patients was set to 15 per group (30 patients in total), which was the same sample size used by Smith.[40]

Statistical Analysis

Analyses were performed with a full data set based on the intention-to-treat principle. Continuous variables are presented as mean ± SD or as median (interquartile range), whereas categorical variables are expressed as number (percentage). The 2 groups were compared using the Student *t* test for continuous data and the Fisher exact test for categorical data. The primary outcome was the percentage change in the total WOMAC score from baseline to 24 weeks, which was calculated as 100 × (baseline value – value at 24 weeks)/baseline value. Outcomes were compared between the PRP and placebo groups using mixed-effects models for repeated measures, incorporating group and time as factors along with their interaction.

RESULTS

One participant assigned to the placebo group experienced a cerebral infarction 4 days after the 12-week assessment.

TABLE 3
Characteristics of Injected PRP[a]

	Value
Volume, mL	6.0
Platelet concentration rate, %	2.0 ± 0.2
Concentration of platelets, /μL	475.4 ± 106.7 × 10^3
Recovery rate in platelets, %	43.9 ± 4.4
Leukocyte contamination rate, %	0.0007 ± 0.003
Quantity of injected platelets, × 10^9	2.9 ± 0.6
Sterility, %	100

[a]Data are presented as mean ± SD unless otherwise indicated. PRP, platelet-rich plasma.

Subsequently, hospitalization and anticoagulant therapy were required, resulting in alterations in activities of daily living. Consequently, the final 24-week evaluation results for this patient were excluded (Figure 1). The number of patients who took acetaminophen as a rescue medication during the study period, including the dosage administered (in milligrams per day), did not significantly differ between the PRP (n = 5; 42.0 ± 59.5 mg/d) and placebo (n = 4; 37.7 ± 15.4 mg/d) groups.

Cellular Composition and Concentration Analysis

Table 3 presents the biological characteristics of injected PRP. The mean platelet purity was high at 98.5% ± 1.7%, with erythrocyte and leukocyte amounts of 1.5% ± 1.7% and 0.0009% ± 0.004%, respectively. Cellular composition analysis results (with relative composition in parentheses [percentage]) were as follows: platelet count, 240.2 ± 42.8 × 10^3/μL (5.0 ± 0.8) for whole blood and 475.4 ± 106.7 × 10^3/μL (98.5 ± 1.7) for PRP; WBC count, 5.1 ± 0.8 × 10^3/μL (0.1 ± 0.02) for whole blood and 0.004 ± 0.02 × 10^3/μL (0.0009 ± 0.004) for PRP; and erythrocyte count, 4.5 ± 0.2 × 10^6/μL (94.9 ± 0.8) for whole blood and 0.007 ± 0.008 × 10^6/μL (1.5 ± 1.7) for PRP. The total amount of platelets injected was 2.9 ± 0.6 × 10^9. The preparation process led to the observation of PRP having good reproducibility in terms of platelet concentration rate, purity, increase factor in platelets, and recovery rate. PRP according to the PAW classification system[13] was P2-Bβ.

Clinical Outcomes

Appendix Table A1 (available in the online version of this article) summarizes results for the primary and secondary outcome measures at 24 weeks in the entire study population, including all WOMAC and VAS scores. The percentage change in Western Ontario and McMaster Universities Osteoarthritis Index scores from baseline to 24 weeks was significantly different (P= .032) between the PRP (median, 75.9%; quantile 1 [Q1], 49.6; quantile 3 [Q3], 94.1]) and placebo (median, 27.7%; Q1, –9.4; Q3, 80.9]) groups. (see Appendix Table A1 and Figure 2).

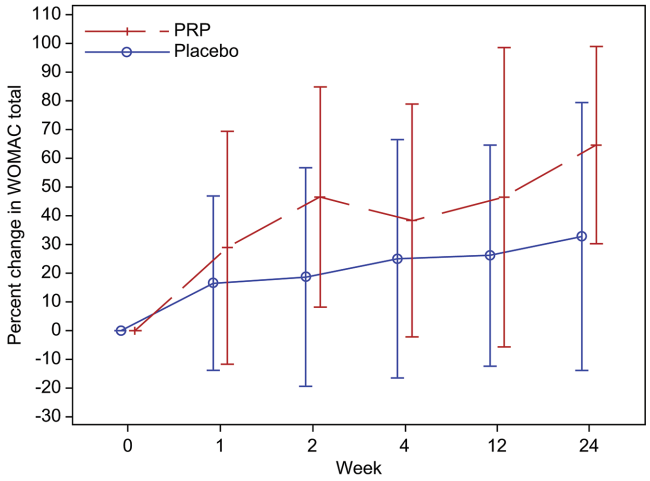

Figure 2. Percentage change in the total Western Ontario and McMaster Universities Osteoarthritis Index score over time in the platelet-rich plasma (PRP) and placebo treatment groups. A significant difference was observed at 24 weeks (*P* = .032).

Analysis of the secondary outcome (ie, proportion of patients with a ≥50% improvement in the VAS score from baseline to 24 weeks) showed that the rate for the PRP group (73.3% [11/15]) was significantly higher than that for the placebo group (28.6% [4/14]) (*P* = .027). The OMERACT-OARSI–restricted responder rate of PRP (66.7% [10/15]) was higher than that of placebo (28.6% [4/14]), albeit not significantly (*P* = .066) (see Appendix Table A1).

Imaging Outcomes

The baseline prevalence of effusion was 100% in both groups. When comparing from baseline to 24 weeks, 4 (27%) and 0 (0%) patients in the PRP and placebo groups, respectively, showed improvement, whereas 11 (73%) and 13 (93%) patients in the respective groups displayed no change; furthermore, 0 (0%) and 1 (7%) patient in the PRP and placebo groups, respectively, exhibited progression (Table 4). However, no significant differences were observed between the groups.

The baseline prevalence of BMLs was 87% (13/15) and 80% (12/15) in the PRP and placebo groups, respectively. When comparing from baseline to 24 weeks, changes in quantity were 1.3 ± 2.1 and −0.4 ± 1.9 in the PRP (Figure 3) and placebo groups, respectively (*P* = .017).

DISCUSSION

In this study, clinical outcomes (ie, WOMAC and VAS scores) and structural outcomes (ie, BMLs) were significantly better with 3 weekly intra-articular LP-PRP injections than with the same number of injections of saline

TABLE 4
Whole-Organ Magnetic Resonance Imaging Score (WORMS) Results[a]

	Baseline	4 wk	12 wk	24 wk	*P* Value
Effusion (0-3)					.100
PRP					
Improvement		2 (13)	2 (13)	4 (27)	
No change	15 (100)	12 (80)	11 (73)	11 (73)	
Progression		1 (7)	2 (13)	0 (0)	
Placebo[b]					
Improvement		0 (0)	0 (0)	0 (0)	
No change	15 (100)	14 (93)	13 (93)[c]	13 (93)	
Progression		1 (7)	1 (7)	1 (7)	
BMLs (0-45)					.017
PRP	5.0 ± 4.5	4.1 ± 3.9	3.9 ± 4.0	3.7 ± 4.0	
Change from baseline		0.9 ± 1.5	1.1 ± 2.1	1.3 ± 2.1	
Placebo	3.9 ± 4.4	4.5 ± 4.5	3.9 ± 4.0	4.3 ± 3.8	
Change from baseline		–0.6 ± 1.1	–0.1 ± 1.0	–0.4 ± 1.9	

[a]Data are presented as mean ± SD or n (%). BMLs, bone marrow lesions; PRP, platelet-rich plasma.
[b]n = 14 at 24 weeks in the placebo group.
[c]One patient in the placebo group had a stroke during follow-up, and that patient was ruled out.

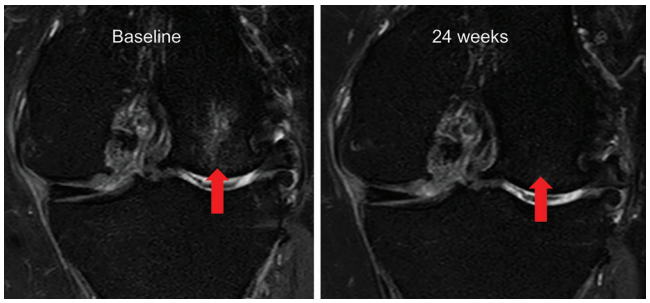

Figure 3. Imaging evaluation. A reduction in bone marrow lesion size was observed (indicated by arrows) at 24 weeks compared with baseline.

(placebo) at 24 weeks of follow-up in symptomatic patients with KL grade 2 or 3 knee OA with effusion or BMLs.

Clinical Outcomes

The primary endpoint, the improvement rate in the WOMAC score, was compared with that in previous studies in which LP-PRP was injected 3 times.[37,45] Here, the PRP group had an equal or higher improvement rate, demonstrating the effectiveness of PRP in improving knee joint function in patients with mild to moderate knee OA. The proportion of patients whose VAS score improved by ≥50% was significantly higher in the PRP group than in the placebo group (11 [73.3%] vs 4 [28.6%] patients, respectively) and approached the 80% value obtained in phase 1 and 2a clinical trials conducted previously,[42] showing a highly reproducible effect on reducing knee joint pain. To assess treatment efficacy in knee OA, OMERACT-OARSI criteria were utilized; patients demonstrating a ≥50% improvement in WOMAC pain or physical function scores, with an absolute improvement of ≥20 points, were classified as "restricted responders." Focusing solely on pain, a VAS was also employed; the proportion of patients with a ≥50% improvement in the pain score was a secondary endpoint. Moreover, considering a previous study utilizing the same protocol[37] in which the proportion of patients experiencing a VAS score reduction of ≥50% was the primary outcome, this variable was established for comparability. Considering the intricate pain expression mechanisms of knee OA,[19,20] it is important to verify PRP's efficacy as a treatment method for patients with knee joint pain alongside discernible abnormalities such as effusion and BMLs, which are predictors of OA progression and conversion to total knee arthroplasty (TKA).[6,10,12,34]

The capability of PRP to affect structural modifications for knee OA has not been elucidated. However, pain reduction and functional improvements after PRP treatment have been suggested in patients with early- to middle-stage OA, with limited efficacy observed in those with advanced KL grade 4 OA.[39] The correlation between the progression of KL grades and coronal lower limb alignment abnormalities, that is, an increase in the HKA angle, has been established.[11] Although KL grade alone can predict lower limb alignment abnormalities, including those caused by extra-articular deformities,[4] an HKA angle of <10° was established to exclude abnormalities resulting from extra-articular deformities. Patients with advanced varus or valgus alignment may require corrective osteotomy or total joint arthroplasty. Thus, the criterion of an HKA angle within <10° aims to identify patients who could potentially benefit from PRP treatment in earlier stages. This criterion was based on previous findings[40] used to determine the sample size for the current study. The results showed a pain-reducing effect of PRP, supported by its anti-inflammatory properties observed in vitro and clinically.[9,41,44] Moreover, PRP was effective in improving BMLs, highlighting the need for further investigation

into its medium- to long-term effects in suppressing arthritis progression. Despite a higher OMERACT-OARSI–restricted responder rate in the PRP group than in the placebo group, this difference did not reach statistical significance.

Imaging Outcomes

Effusion was improved in 4 of 15 patients (27%) in the PRP group from baseline to 24 weeks. In all cases, the improvement was 1 grade. Among cases of grade 2 or 3 before treatment, complete disappearance (grade 0) was not achieved in any case at 24 weeks, with 11 cases showing no change or progression. In the placebo group, no patients experienced improvement, while 13 showed no change, and 1 exhibited progression (from grade 2 to 3). Also, 2 patients, 1 of whom remained at grade 2 with no change, required fine-needle aspiration for joint effusion during the follow-up period. However, there was no significant difference between the groups (P = .100). According to a previous study,[26] the amount of joint effusion and the composition of inflammatory cytokines and catabolic factors at the effusion site may be improved with PRP injections. This showed that clinical outcomes improved even when effusion persisted; therefore, the effect of PRP on joint effusion requires attention to the amount before and after treatment and to the composition of the contents.

The baseline prevalence of BMLs was 87% (13/15) in the PRP group and 80% (12/15) in the placebo group, with a significant improvement observed in the PRP group from baseline to 24 weeks (P = .017). Unlike effusion, the baseline prevalence of BMLs was not 100%. Therefore, changes in the total score were compared between baseline and 24 weeks; the score was significantly improved in the PRP group. Further research is required to determine whether intra-articular PRP directly affects subchondral bone marrow[1,16] and to investigate its underlying mechanisms of action. Several previous studies have reported a reduction in BML size on semiquantitative evaluations using MRI[25] and quantitative image analysis software.[26] However, it is necessary to verify whether the presence of BMLs affects the effectiveness of PRP[10] and to refine research designs accordingly. Nevertheless, as effusion and BMLs are predictors of OA progression, demonstrating the effectiveness of PRP treatment in patient populations with these conditions would enhance its value as a novel nonoperative treatment approach distinct from conventional methods.[5]

Inclusion and Exclusion Criteria

Here, the progression of knee joint deformities was assessed through frontal radiography for KL grades[15] and MRI for joint effusion and BMLs, which served as inclusion criteria. Both imaging modalities focus on the knee joint. The HKA angle, defined as the angle between the functional axes of the femur and tibia, offers a more accurate representation of lower limb alignment on full-length weightbearing radiographs compared with the femorotibial angle. Moreover, it allows for the evaluation of intra-articular and extra-articular deformities.[38] A healthy value is near 0°, but previous research has indicated a mean preoperative HKA angle of 11° for TKA.[4] Therefore, an HKA angle of <10° was chosen to exclude severe lower limb alignment abnormalities necessitating TKA. Correcting lower limb alignment abnormalities using intra-articular PRP injections is challenging under such circumstances. Moreover, considering the mechanical stress implications of severe deformities on knee OA progression, it is necessary to assess intra-articular and extra-articular deformities. Consequently, patients with advanced varus or valgus deformities, for whom correction through osteotomy or total joint replacement is relevant, were excluded based on this criterion. Consistency was ensured by adopting the same criterion as a previous study, which was used to determine the sample size for this investigation.[40]

Cellular Composition Analysis

In our previous study, which was conducted alongside the present study, the production of LP-PRP of equivalent quality to that employed in clinical practice was achieved.[23,24] The utilization of the PRGF-Endoret system, notable for its manual extraction process rather than automation, raises concerns regarding the instability of PRP quality control, owing to its dependence on technical skills.[37,45,46] Our research effectively elucidated that PRP quality remains consistent, regardless of the operator's experience in its preparation process, as long as proper training and expertise are provided[24]; this conclusion is supported by the current study. The debate regarding the efficacy of leukocyte-rich PRP or LP-PRP for knee OA is ongoing.[14,21] However, it is noteworthy that reports have indicated increased adverse events, including pain after injections, associated with LP-PRP. Although our study employed LP-PRP, future clinical endeavors in this field should meticulously classify and utilize PRP variants to further validate its efficacy.

Limitations

This study had limitations, particularly the short observation period of 24 weeks. Knee OA progresses over years, with fluctuations in symptoms such as pain and stiffness, making it uncertain how long observed effects will last or whether further improvements will occur with subsequent PRP treatment.[27] Further validation is required to address this uncertainty.

This short-term study focused on MRI to assess the anti-inflammatory effects of PRP on effusion and BMLs, its most promising mechanisms. However, detailed MRI evaluations over a medium- to long-term period are essential to determine whether PRP can structurally modify and suppress arthritis progression compared with a natural history.

The significant improvement in BMLs observed in the PRP group at the 24-week time point underscores the potential efficacy of PRP; however, determining the minimal clinically important difference remains elusive,

necessitating future research. Additionally, although fine-needle aspiration preceded injections in patients with joint effusion, data on the composition of inflammatory cytokines, anabolic and catabolic growth factors (which act on joint structures), and changes from before to after treatment are lacking, warranting further investigation.

Even though the WOMAC and VAS scores showed some improvement in the placebo group, MRI revealed minimal changes in both effusion and BMLs, indicating a potential placebo effect on subjective evaluations of intra-articular injection treatment.[17,33] This underscores the importance of clarifying the effectiveness of objective evaluation methods.

Another limitation is the difference in obesity definitions between Japan and Western countries. In Japan, patients with a body mass index (BMI) of $\geq$25 and $\geq$30 are classified as obese class 1 and class 2, respectively, whereas the World Health Organization designates a BMI of $\geq$25 as overweight and $\geq$30 as obese class 1. Thus, even though the calculation formula for BMI is universally accepted, the definitions of obesity are discrepant between Japan and Western nations. This variation stems from the prevalence of obesity-related conditions, such as a fatty liver, which persists even at a BMI of $\geq$25.[3] From 2014 to 2015, the rate of obesity (BMI $\geq$30) was 4.4% in men and 3.1% in women in Japan, while it was 35.5% in men and 41.0% in women in the United States.[30] Therefore, generalizing the results of this study, which targets patients with a BMI of <30 in Japan, to countries with higher obesity rates, such as many Western nations, is challenging. Nonetheless, the data could offer valuable insights on regions with a lower obesity prevalence, such as other Asian countries. Additionally, dietary weight management is considered a key treatment method for knee OA by the OARSI.[5] As PRP therapy is not classified among the top-tier core treatment approaches for knee OA, patients with a BMI of $\geq$30 were excluded. Moreover, as this study was conducted at a single institution, a multicenter randomized controlled trial should be conducted to enhance generalizability. Furthermore, a larger sample size and subcategorical analyses may unveil detailed responder predictors.

CONCLUSION

Our findings suggest that 3 LP-PRP injections represent a minimally invasive treatment option for symptomatic patients with mild to moderate knee OA and an HKA angle of <10°. These injections demonstrated clinically significant short-term improvements in symptoms for patients with joint effusion or BMLs on MRI.

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