

Intra-articular platelet-rich plasma (PRP) injections for treating knee pain associated with osteoarthritis of the knee in the Japanese population: a phase I and IIa clinical trial

Yu Taniguchi¹, Tomokazu Yoshioka^{1,2}, Akihiro Kanamori¹, Katsuya Aoto¹,
Hisashi Sugaya^{1,2}, and Masashi Yamazaki¹

¹*Department of Orthopedic Surgery, Faculty of Medicine, University of Tsukuba, Tsukuba, Japan*
²*Division of Regenerative Medicine for Musculoskeletal System, Faculty of Medicine, University of Tsukuba, Tsukuba, Japan*

ABSTRACT

Intra-articular platelet-rich plasma (PRP) injection has been found to be effective for treating osteoarthritis in patients from Western countries; however, the safety and efficacy of PRP have not been sufficiently investigated in Japanese patients. The present study aimed to evaluate the safety and feasibility of intra-articular PRP injection in Japanese patients with knee osteoarthritis. PRP without white blood cells was prepared using a single-spin centrifuge (PRGF-Endoret; BTI Biotechnology Institute, Vitoria, Spain). A 6-mL PRP volume was injected in the knee joint three times at 1 week intervals. All patients were prospectively evaluated before intervention and at 1, 3, and 6 months after the treatment. Adverse events, the Visual Analog Scale (VAS) pain score, Japanese Knee Osteoarthritis Measure (JKOM) score and Japanese Orthopedic Association score were evaluated. Ten patients (all women; average age, 60.6 years) were treated. Only minor adverse events after injection were noted, and symptoms resolved within 48 hours after the injection. The average VAS pain scores were 71.6 mm and 18.4 mm at baseline and the 6-month follow-up, respectively ($P < 0.05$). At the 6-month follow-up, 80% of patients had a decrease in the VAS pain score of 50% or more. The average JKOM scores were 35.2 and 14.3 at baseline and at the 1-month follow-up, respectively ($P < 0.05$). Intra-articular PRP injection likely represents a safe treatment option for Japanese patients with mild-to-moderate knee osteoarthritis, and has the potential to relieve pain for up to 6 months, but further study is needed to verify the efficacy.

Keywords: platelet-rich plasma, knee osteoarthritis, treatment safety, intra-articular injection

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INTRODUCTION

Osteoarthritis of the knee is one of the main causes of musculoskeletal disability, and is characterized by gradual abrasion of the articular cartilage, osteophyte formation, subchondral bone sclerosis, and inflammation of the joint. The number of patients with osteoarthritis continues to increase as the world population ages. In the USA, knee osteoarthritis affects more than

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Corresponding author: Tomokazu Yoshioka, MD, PhD

Department of Orthopedic Surgery and Division of Regenerative Medicine for Musculoskeletal System, Faculty of Medicine, University of Tsukuba, 1-1-1 Tennodai, Tsukuba 305-8575, Japan

Email: tynd99@tsukuba-seikei.jp

20% of individuals older than 45 years.¹⁾ In Japan, the prevalence of age-associated diseases of the musculoskeletal system has also increased, and it was estimated that, in 2009, radiographic osteoarthritis was present in 25,300,000 individuals (8,600,000 men and 16,700,000 women) aged 40 years or older, while symptomatic osteoarthritis was present in approximately 7,800,000 individuals.²⁾ In addition, the Japanese lifestyle differs from that in Western countries, and activities of Japanese individuals generally require a greater range of motion. Common movements used in daily life in Japan include floor-sitting, squatting, and kneeling (e.g., seiza-sitting).³⁻⁶⁾

Clinical guidelines recommend various non-surgical procedures to treat knee osteoarthritis.⁷⁾ Non-pharmacological treatments include: patient education and self-management with exercise; weight reduction; use of crutches and bracing; shoe and insole modification; and local cooling. Pharmacologic therapies include the use of paracetamol, non-steroidal anti-inflammatory drugs, and opioids. If orally administered drugs are ineffective, intra-articular (IA) injection of corticosteroids and hyaluronic acid (HA) is the next preferred non-operative treatment.⁸⁾ IA-HA is widely used in Japan because it is strongly recommended in the guideline for the management of knee osteoarthritis, published by the Japanese Orthopedic Association (JOA). However, IA-HA is not recommended in the corresponding guideline published by the American Academy of Orthopedic Surgeons.⁹⁾ Currently, the efficacy of IA-HA for osteoarthritis of the knee is considered controversial.

Therapy with platelet-rich plasma (PRP) has emerged as a biological, conservative treatment for knee osteoarthritis. PRP is broadly defined as a plasma fraction containing abundant platelets,¹⁾ although no clear definition is given in terms of platelet counts or platelet concentration. As PRP has high concentrations of autologous growth factors and secretory proteins that may enhance cellular-level healing processes, it has been used in the clinical setting to enhance healing. Several studies have shown that treatment involving IA-PRP is superior to the use of IA-HA in terms of improving the Western Ontario & McMaster Universities (WOMAC) total score and other parameters.¹⁰⁻¹⁵⁾ However, these studies were conducted in Europe and the USA, while the efficacy of IA-PRP therapy has not been sufficiently evaluated in Japanese patients with knee osteoarthritis. Recent research has shown that platelet function differs among ethnicities.¹⁶⁾ Therefore, it is important to evaluate the safety and efficacy of IA-PRP among Japanese patients with knee osteoarthritis.

The primary objective of the present study was to verify the feasibility and safety of IA injections with PRP manufactured in our facility. We hypothesized that the use of IA-PRP represents a safe and feasible biological approach for treating pain due to osteoarthritis of the knee.

MATERIALS AND METHODS

Patient selection

The study protocol was approved by the appropriate institutional review board (application no.: H25-040). We recruited patients with early-phase knee osteoarthritis to confirm the safety of IA-PRP. All patients provided written informed consent to participate. The inclusion criteria were as follows: age between 50 and 75 years; a history of chronic (for at least 3 months) knee joint pain defined as a Visual Analog Scale (VAS) score >35 mm on a 0–100-mm scale; radiographically documented knee osteoarthritis of grades 1 to 3, according to the Kellgren-Lawrence (K-L) radiographic classification scale; body mass index ranging between 20 and 32 kg/m², so that the study sample reflected the average physique in the Japanese population; need for infiltration of the knee on one side. The exclusion criteria were as follows: polyarticular disease; knee arthroscopy within the previous year; HA or steroid IA infiltration within the previous 3 months; a history

of infectious disease and systemic disorders such as diabetes, rheumatoid arthritis, hematological diseases (coagulopathy), severe cardiovascular diseases, infections, or immunodepression; therapy with anticoagulants or an anti-aggregating agent; use of non-steroidal anti-inflammatory drugs 2 weeks before blood sampling; and hemoglobin levels <10 g/dL.

PRP preparation

The PRP required for injection was prepared in compliance with good manufacturing practices at the Cell Processing Factory at our hospital (Fig. 1). To avoid the effect of food intake on purified PRP, the patients were instructed to fast for 4 hours before blood collection on the day of injection. Water intake was not restricted. Using an aseptic technique, approximately 36 mL of venous blood were drawn from the antecubital vein in an effort to avoid irritation and trauma to the platelets. The blood was collected in four extraction tubes containing 3.8% sodium citrate as an anticoagulant. Subsequently, the tubes were centrifuged at 2100 rpm for 8 minutes at room temperature (PRGF System IV centrifuge; BTI Biotechnology Institute, Vitoria, Spain) to separate the blood in each tube into the plasma, the buffy coat, and residual red blood cells (RBCs).

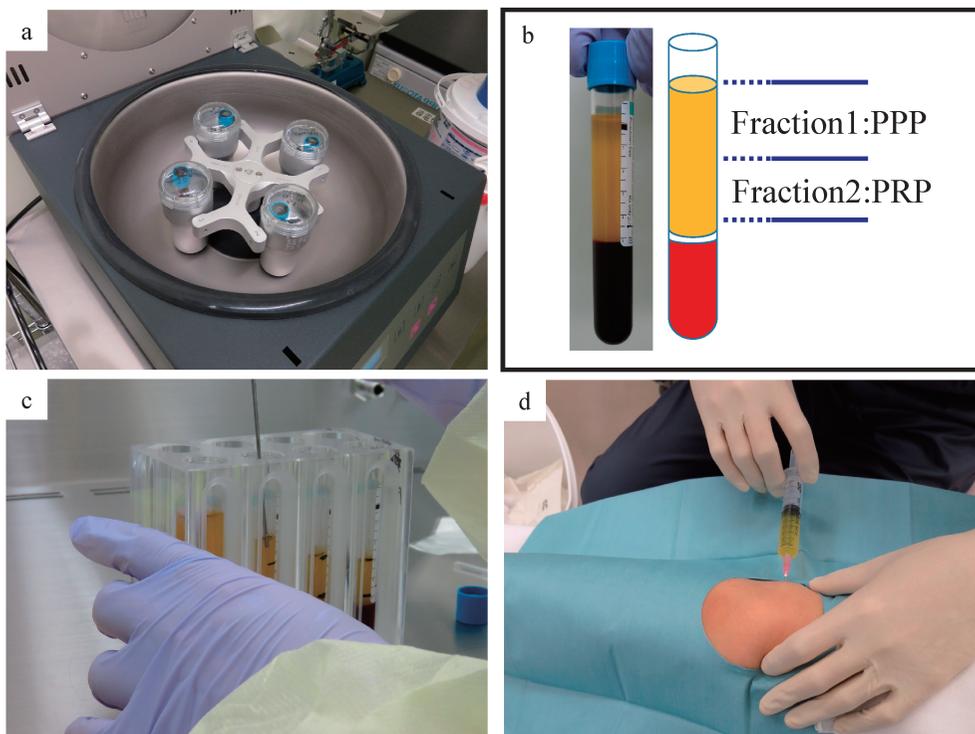


Fig. 1 Platelet-rich plasma (PRP) preparation workflow

- (a) Centrifugation at 2100 rpm for 8 minutes using the single-spin system (PRGF System IV; BTI Biotechnology Institute, Vitoria, Spain).
- (b) Photograph of the blood-collecting tube after centrifugation and scheme for fraction separation. The safety margin was set to exclude red blood cells and white blood cells. The lower half of the supernatant was defined as PRP, while the upper half was defined as platelet-poor plasma (PPP).
- (c) Aspirating PPP and PRP using an exclusive pipette in a clean bench.
- (d) Under aseptic conditions, 6 mL of PRP were injected into the suprapatellar pouch using the superolateral approach.

Hereafter, the procedure was performed entirely inside the biosafety cabinet. The platelet-poor plasma (PPP), which represented the uppermost 2-mL fraction in each tube, was aspirated with a pipette and sent for hematological analysis. The PRP, which corresponded to the lower 2-mL fraction from each tube, located just above the selectively precipitated RBCs but not including the buffy coat, was meticulously aspirated from each tube using a pipette. For each patient, four 2-mL PRP samples were collected (from the four blood-collecting tubes) and mixed into an 8-mL preparation, from which 6 mL were used for the IA injection^{14,15}. The remaining 2 mL of PRP preparation were divided into two small units of 1-mL each; one unit was sent to the laboratory for hematological analysis, while the other unit was stored in Eppendorf tubes at -80°C for subsequent determination of growth factor concentrations.

Procedures

The patient was placed in supine position with the knee in 20° flexion. Under aseptic conditions, 6 mL of PRP were injected into the suprapatellar pouch through the superolateral approach, using a 21-gauge needle. No local anesthetic was used. Blood pressure, heart rate, and body temperature were measured before and at 30 minutes after the injection. After the injection, the patients were instructed to refrain from physical exercise for at least 24 hours, but no restriction was specified regarding activities of daily living. Three IA-PRP injections were administered at 1-week intervals. The interval between injections, as well as the number of injections, was determined based on previous studies.^{11,14} The injections were performed by the same physician who was involved in recruitment and assessment of participants.

Hematological analysis

The white blood cell (WBC), RBC, and platelet counts of the whole-blood samples, as well as those of the PPP and PRP fractions, were determined using an automated cell count analyzer (Sysmex KX-21N; Sysmex Corp., Kobe, Japan).

Measurement of growth factor concentrations

A single freeze-thaw cycle was used to induce platelet activation and the release of growth factors. The samples were thawed and centrifuged for 10 minutes at 10,000 rpm, and the supernatants were tested. Quantitative determination of platelet-derived growth factor BB (PDGF-BB), tissue growth factor (TGF)- β 1, and vascular endothelial growth factor (VEGF) levels in the PRP fraction was performed using a commercially available enzyme-linked immunosorbent assay kit (R&D Systems, Minneapolis, MN, USA), according to the manufacturer's instructions. All evaluations were conducted in multi-well plates, and all measurements were performed in duplicate. The color intensity of each well was measured using a spectrophotometer (Varioskan; Thermo Fisher Scientific, Yokohama, Japan) at 450 nm and a wavelength correction of 570 nm.

Outcome measures

To ascertain the safety of the prepared PRP, part of the prepared PRP was subjected to a bacterial culture test. For assessing the safety of IA-PRP, adverse events occurring during the treatment and follow-up period were documented at each visit. The onset, duration, and severity of events such as knee pain, stiffness, swelling, and burning sensation near the injection site were recorded in detail. The only permitted medication throughout the clinical trial was acetaminophen.

For assessing the feasibility of IA-PRP in managing knee osteoarthritis, all patients were evaluated at baseline (before the infiltration procedure) and at 1, 3, and 6 months after the last injection. The following measures were evaluated: VAS pain score, Japanese Knee Osteoarthritis Measure (JKOM) score,¹⁷⁾ and Japanese Orthopedics Association (JOA) score¹⁸⁻²⁰⁾, which represent

standard tools for assessing knee function in Japan. The reliability and validity of both the JOA score and the JKOM score have been demonstrated in the past by comparison against other measures such as the WOMAC score and the 36-Item Short Form Health Survey (SF-36) score. With respect to the VAS pain score, we recorded the percentage of patients with a decrease of at least 50% in the total score between the baseline and the 6-month follow-up measurements. Radiographs were obtained at baseline (before injection) and at the 1-month, 3-month, and 6-month follow-up visit. Radiographs in the supine anteroposterior view, lateral view, and skyline view were obtained during the same visits. Two authors (YT and TY) reviewed all radiographs and graded each case using the K-L radiographic classification scale. Serum hyaluronic acid (sHA) was measured as a known biomarker of knee osteoarthritis.^{21,22)}

Statistical analysis

Differences between outcome measures at various time points (within-group differences) were evaluated using analysis of variance with Bonferroni correction. Between-group differences were evaluated using the Student t-test. A *P*-value of <0.05 was considered to indicate statistical significance. All statistical analyses were performed using SPSS Statistics 21.0 (International Business Machines Co., New York, NY, USA).

RESULTS

Patients

Eleven patients participated in this study, but one patient was excluded during the follow-up period because of fever (attributed to upper airway inflammation) and the presence of cloudy PRP due to a high-fat diet; thus, 10 patients were included in the analysis. The demographic and clinical characteristics of the patients are shown in Table 1. All patients were women, with a mean age of 60.6 years and a mean body mass index of 23.4 kg/m². Osteoarthritis affected the right knee in 6 patients and the left knee in 4 patients. Knee osteoarthritis was graded according to the K-L classification as grade I in 4 patients, grade II in another 4 patients, and grade III in the remaining 2 patients. Regarding the affected compartment, 8 knees had medial osteoarthritis,

Table 1 Demographic and clinical characteristics of 10 patients with knee osteoarthritis

Patient	Age (years)	Sex	Laterality	BMI (kg/m ²)	K-L grade	Compartment
1	59	F	R	27.6	3	Medial, PF
2	61	F	R	21.2	2	Lateral
3	55	F	L	20.7	3	Medial, PF
4	70	F	L	21.1	1	Medial
5	52	F	R	22.9	1	Medial
6	64	F	R	22.2	3	Medial, Lateral, PF
7	70	F	L	23.9	2	Medial
8	57	F	R	25.5	2	Lateral
9	67	F	R	23.4	3	Medial, Lateral, PF
10	51	F	L	25.0	2	Medial

BMI, body mass index; F, female; K-L, Kellgren-Lawrence radiographic classification scale; L, left; PF, patello-femoral joint; R, right.

4 had lateral osteoarthritis, and 4 knees showed osteoarthritic changes in the patella-femoral joint. Physical examination did not reveal hydrarthrosis of the knee in any patient, and no patient underwent synovial fluid aspiration. Over the course of the study (i.e., from baseline until the end of follow-up), no patient gained or lost more than 2 kg in weight.

Blood and growth factor findings

The findings of the hematological analysis and growth factor concentrations are presented in Table 2. The average platelet count in the PRP ($39.4 \pm 13.1 \times 10^4/\mu\text{L}$) was significantly higher than those in peripheral blood ($24.0 \pm 3.7 \times 10^4/\mu\text{L}$) and PPP ($27.1 \pm 5.8 \times 10^4/\mu\text{L}$). The platelet count in the PRP was 1.7-fold higher than that in peripheral blood. Based on the platelet count and the presence of WBC and neutrophils, the PRP preparation was classified as P2-B β according to the Platelets, Activation, and White cells classification system.²³⁾ The mean PDGF-BB, VEGF, and TGF- β 1 concentrations were 6.8 ± 3.4 ng/mL, 213 ± 207 pg/mL, and 33.3 ± 10.8 ng/mL, respectively.

Safety of IA-PRP

Bacterial cultures of the prepared PRP were negative for all specimens. The adverse events noted during the study period are described in Table 3. Twenty-two adverse events were reported in relationship to 30 injections in 10 patients. All events represented generally mild reactions, and no patients withdrew from the study because of a serious adverse event due to IA-PRP injection. All adverse events resolved spontaneously within 48 hours after the injection.

Feasibility of IA-PRP

The mean VAS pain score decreased from 71.6 mm at baseline to 12.5 mm at 1 month, 15.1 mm at 3 months, and 18.4 mm at 6 months after the last injection (Fig. 2). The benefit in pain reduction, as measured in terms of the VAS score, was significant ($P < 0.001$). Moreover, the improvement in VAS pain score was maintained from the last injection until the last follow-up visit (at 6 months after treatment). Specifically, at the 6-month follow-up, 80% of patients had a decrease in the VAS pain score of 50% or more. In addition, the mean JKOM total score decreased from 36.2 at baseline to 14.3 at 1 month, 13.3 at 3 months, and 15.5 at 6 months after the last injection (Fig. 3). The JKOM score decreased significantly from baseline to 1 month after the end of the treatment ($P = 0.044$). The mean overall JOA scores at the 6-month follow-up were significantly higher than the baseline values (Fig. 4; 70 at baseline versus 89.5 at 6 months after treatment; $P = 0.002$). Regarding JOA domains, there was significant improvement

Table 2 Properties of the autologous blood product of 10 patients with knee osteoarthritis

	PB	PPP	PRP
WBC (μL)	$5,803 \pm 1,564$	0	79 ± 162
RBC ($\times 10^4/\mu\text{L}$)	437 ± 38	1.1 ± 3.3	1.8 ± 0.6
PLT ($\times 10^4/\mu\text{L}$)	24.0 ± 3.7	27.1 ± 5.8	39.4 ± 13.1
PDGF-BB (ng/mL)			6.8 ± 3.4
VEGF (pg/mL)			213 ± 207
TGF- β 1 (ng/mL)			33.3 ± 10.8

PB, peripheral blood; PLT, platelet count; PRP, platelet-rich plasma; RBC, red blood cell; TGF, tissue growth factor; PDGF-BB, platelet-derived growth factor BB; VEGF, vascular endothelial growth factor; WBC, white blood cells

regarding pain while walking and pain while ascending or descending stairs ($P = 0.034$ and $P < 0.001$, respectively). Radiographic findings showed that there was no progression in the K-L grade from baseline to 6 months after the end of the treatment. The sHA levels were 48.2 ± 23 ng/mL before treatment, 69.0 ± 58 ng/mL at 1 month after injection, 98.2 ± 64 ng/mL at 3 months after injection, and 97.5 ± 58 ng/mL at the end of follow-up.

Table 3 Adverse events in 10 patients with knee osteoarthritis who received intra-articular injections with platelet-rich plasma

Patient	Injection	Adverse event	Duration
1	1st	Acute local pain, stiffness	Immediately
	2nd	None	
	3rd	None	
2	1st	Uncomfortable feeling	Immediately
	2nd	Subcutaneous bleeding	≤48 h
	3rd	None	
3	1st	Cold chill, pain during walking, itching in the knee	≤48 h
	2nd	Expanding acute pain at injection site	≤48 h
	3rd	Pain during walking, itching in the knee	≤48 h
4	1st	Tingling sensation in the knee	Immediately
	2nd	None	
	3rd	Pain during walking, itching in the knee	≤24 h
5	1st	None	
	2nd	None	
	3rd	None	
6	1st	Pain during walking	≤24 h
	2nd	Pain during walking	≤24 h
	3rd	Acute knee pain, stiffness	≤24 h
7	1st	Tingling sensation in the knee	≤24 h
	2nd	Stiffness	Immediately
	3rd	Acute pain in the area behind the knee joint	1 h
8	1st	Itching in the knee	Immediately
	2nd	Pain during walking, itching in the knee	≤24 h
	3rd	None	
9	1st	Pain during walking, itching in the knee	1 h
	2nd	Sharp acute pain in the knee	1 h
	3rd	Feeling of pressure in the knee	Immediately
10	1st	Acute pain in the knee	≤48 h
	2nd	Acute pain in the knee	≤48 h
	3rd	Stiffness	≤48 h

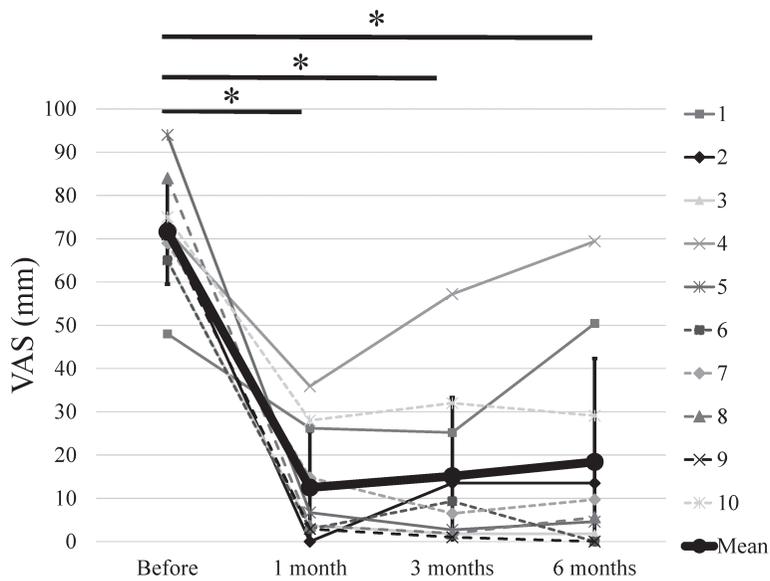


Fig. 2 Trends in the mean Visual Analog Scale (VAS) scores for pain recorded over a follow-up of six months. Ten patients with knee osteoarthritis received intra-articular injections with platelet-rich plasma, administered once per week for three weeks. VAS scores were assessed at baseline (before injection) and during subsequent follow-up visits. * $P < 0.05$.

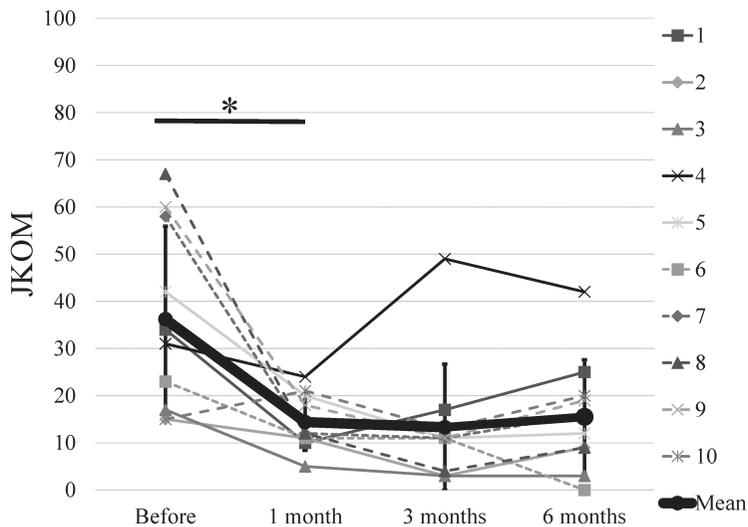


Fig. 3 Trends in the mean Japanese Knee Osteoarthritis Measure (JKOM) scores over a 6-month follow-up. Ten patients with knee osteoarthritis received intra-articular injections with platelet-rich plasma, administered once per week for three weeks. JKOM scores were assessed at baseline (before injection) and during subsequent follow-up visits. * $P < 0.05$.

PRP injections for knee OA

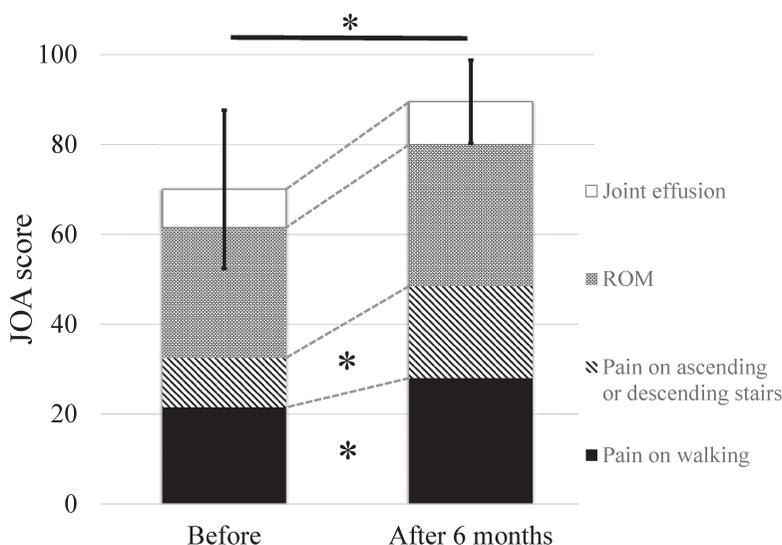


Fig. 4 Mean Japanese Orthopedic Association (JOA) scores. The patients with knee osteoarthritis received intra-articular injections with platelet-rich plasma, administered once per week for three weeks. JOA scores were assessed at baseline (before injection) and at the 6-month follow-up visit. Different categories are represented by different filling patterns. * $P < 0.05$. ROM, range of motion.

DISCUSSION

The most important finding of the present study is that, in Japanese patients with knee osteoarthritis, IA-PRP did not cause any serious adverse events during the first 6 months after the treatment ended. All adverse events observed were minor and included acute knee pain, stiffness, tingling sensation, and walking pain just after the injection. These adverse events were observed for 22 of the 30 injections administered (73%), but all symptoms resolved spontaneously within 48 hours. Sánchez et al.¹⁴ evaluated 176 patients (PRP group, 89 patients vs. HA group, 87 patients) with symptomatic knee osteoarthritis. In their study, adverse events due to IA injections were generally mild and evenly distributed between the two groups, and all events such as local pain around the infiltration site disappeared within 48 hours after injection. Patel et al.¹² reported various adverse events such as syncope, dizziness, headache, nausea, gastritis, sweating, and tachycardia at the time of injection; however, none was severe enough to cause concern, and all subsided within half an hour when the patients were under observation. In a study by Filardo et al.,¹¹ no major complications related to the PRP injections were observed, but the incidence of post-injection pain reaction was higher in the PRP group than in the HA group. Filardo et al.¹¹ considered the deleterious effects of PRP as being related to proteases and reactive oxygen released from WBCs. The absence of serious adverse events, noted in previous studies and in the present investigation, suggests that IA-PRP represents a safe treatment option for patients with knee osteoarthritis.

Regarding the efficacy of IA-PRP for knee osteoarthritis, Meheux et al.²⁴ conducted a systematic review of six randomized, controlled trials, and found that PRP injection resulted in significant clinical improvements for up to 12 months post-injection; moreover, the clinical outcomes and WOMAC scores were significantly better after treatment with leukocyte-poor PRP than after treatment with HA at 3–12 months post-injection. Despite the favorable outcomes reported

with PRP treatment, the evaluated studies only included Caucasian patients, and their findings are not applicable to the Japanese population. As recent studies have claimed that platelet function differs with ethnicity,^{16,25)} a study of the safety and efficacy of PRP in the Japanese population was warranted. Our present study represents the first report regarding the outcome of IA-PRP in Japanese patients with knee osteoarthritis, and showed that 8 of 10 patients had a decrease in the VAS pain score of $\geq 50\%$ (compared to VAS score at baseline), with the beneficial effects persisting for 6 months. In this study, the JOA and JKOM scores, both of which are specific to Japanese patients, were used for evaluation. Regarding the JOA score, improvements in pain brought about improvements in total score, as well as in the scores for walking ability and for ascending and descending stairs. On the other hand, the JKOM score improved significantly after 1 month, but the improvements seen after 3 and 6 months were not statistically significant. The JKOM was specifically developed as a tool for evaluating quality of life in patients with knee osteoarthritis, and its reliability and validity were previously demonstrated by comparisons with other measures such as the SF-36 and WOMAC.^{17,19)} The improvement in JKOM score from baseline (i.e., before treatment) to 1 month after PRP injection may indicate that PRP is effective to the extent that it improves the patients' quality of life.

The pathogenesis of knee osteoarthritis is complex and driven by inflammatory mediators within the affected joint.²⁶⁻²⁸⁾ Joint changes such as synovitis, subchondral bone remodeling (thickening, bone collapse, bone cysts), degeneration of ligaments and menisci, and hypertrophy of the joint capsule are involved in the pathogenesis of osteoarthritis.^{29,30)} PRP represents an autologous biological therapy option in which the patient's own plasma and platelet-derived growth factors are used during the wound-healing process for regenerative purposes, to stimulate processes such as angiogenesis, mitogenesis, cell proliferation, and cell migration to chemo-attractants. Several studies have reported that PRP affects joint formation and promotes restoration of joint homeostasis. Akeda *et al.*³¹⁾ compared the response of adult porcine chondrocytes cultured in the presence of 10% PRP, 10% PPP, or 10% fetal bovine serum, and reported that PRP treatment was associated with significantly higher DNA and proteoglycan content, as well as enhanced collagen synthesis. Spreafico *et al.*³²⁾ argued that, compared to human serum, PRP had a stronger effect on cell proliferation and chondrocyte activity, which suggests that this preparation may be useful for the safe and economic engineering of cartilage constructs. Anitua *et al.*³³⁾ contended that IA administration of plasma rich in growth factors may be beneficial in restoring the HA concentration and balancing angiogenesis, but it does not counteract the effects of interleukin-1 β on synovial cells. Bendinelli *et al.*³⁴⁾ suggested that expression of the chemokine receptor CXCR4 was reduced in chondrocytes exposed to activated PRP, which may have clinical significance in the management of inflamed synovia (for example, in terms of reducing the expression of matrix-metalloproteinases and other cartilage-matrix degrading enzymes). In the present study, we measured the change in sHA levels as a biomarker for knee osteoarthritis, and found that sHA levels increased from baseline (i.e., before treatment) to 3 months after treatment. It was previously reported that sHA is a biomarker of radiographic knee osteoarthritis²¹⁾ and a burden-of-disease marker in severe knee osteoarthritis (K-L grade 4), but is not a useful marker in mild-to-moderate knee osteoarthritis.²²⁾ In this study, we did not observe any changes over time regarding plain X-ray findings, which did not correspond to the evolution of sHA levels. As our patients had mild-to-moderate knee arthritis of K-L grade 1–3, sHA may not have been an appropriate biomarker in such cases. The use of alternative biomarkers should be considered in subsequent clinical comparative trials. While the *in vitro* study by Bendinelli *et al.*³⁴⁾ showed that PRP had a beneficial effect on joint formation, the mechanism through which IA-PRP may improve VAS pain scores in patients with knee osteoarthritis remains uncertain. It can be presumed that PRP promotes the restoration of joint homeostasis by inhibiting synoviocyte production of

inflammatory mediators. Nevertheless, further investigation is needed to prove this hypothesis.

The present study has several limitations. First, this was an open-label study, so no comparison with a control group was performed. However, this study was a I/IIa clinical trial that verified safety and feasibility; furthermore, it did not verify the efficacy as there was no control group. Second, the follow-up period was relatively short as a clinical trial to verify the efficacy. According to the systematic review by Campbell et al.³⁵, IA-PRP is a viable treatment for knee osteoarthritis, and can induce symptomatic relief for up to 12 months. However, this clinical study was not designed to verify effectiveness, but because it was a I/IIa test to verify safety and feasibility, the 6-month period was appropriate¹⁴. Third, our imaging evaluation was insufficient for verification of efficacy because only X-ray imaging was used, while magnetic resonance imaging (MRI) is recommended to evaluate the severity of intra-articular osteoarthritis and the extent of synovitis. MRI is useful for determining the pathophysiology when osteoarthritis is considered a whole joint disease³⁶.

Subsequent trials following on from the present phase I/IIa clinical trial will include evaluation of intra-articular findings on MRI before and after the injections. Fourth, sHA is inadequate as a biomarker of knee osteoarthritis, as it is not specific for changes in the knee, but is affected by changes in joints throughout the body. Based on the experience of the present study findings; however, we intend to conduct a clinical trial to verify the efficacy of using IA-PRP for relief of knee pain for osteoarthritis. This future study will be a multi-center, double-blind, controlled clinical trial; the control group will receive hyaluronic acid injection.

CONCLUSIONS

This study was I/IIa clinical trial and was done to verify safety and feasibility. Our findings suggest that IA-PRP is safe for use in Japanese patients with knee osteoarthritis, and, while local and minor adverse events related to PRP injection occurred, all symptoms disappeared within 48 hours. This therapy has the potential to induce pain relief that is maintained for up to 6 months, but further study is needed to verify the efficacy.

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CONFLICTS OF INTEREST

The authors have no conflicts of interest to declare.

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