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Consecutive injections of leukocyte-rich platelet-rich plasma are effective in not only mild but also severe knee degeneration

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ABSTRACT

Introduction: How can non-cultured platelet-rich plasma (PRP) therapy be the ultimate intervention in the treatment of total knee arthroplasty (TKA) -adaptive levels of knee osteoarthritis, as opposed to stem cell therapy that requires culture?
Methods: An intra-articular injection of leukocyte-rich PRP (LR-PRP) was administered to 260 patients every 4 weeks for over four times (mean 5.8 times); they were followed up for a maximum of 24 months. The clinical evaluation used the Knee Injury and Osteoarthritis Outcome Score, visual analogue scale, and magnetic resonance imaging osteoarthritis knee score-body mass lesions to determine the therapeutic effect using the Outcome Measures in Rheumatology-Osteoarthritis Research Society International responder criteria for osteoarthritis.
Results: Among those administered with LR-PRP, the responder rate was 72.0%, 78.1%, 78.1%, and 77.1% at 3, 6, 12, and 24 months, respectively.
Conclusions: Our manually prepared LR-PRP was effective following multiple consecutive injections, despite severe degeneration.

1. Introduction

The last-line intervention for patients with knee osteoarthritis (KOA), who are refractory to conservative management, including weight loss, rehabilitation, hyaluronic acid (HA) injections, corticosteroid (CS) injections, and/or nonsteroidal anti-inflammatory drugs, is total knee arthroplasty (TKA). Therefore, effective treatment is required to relieve pain before TKA, with regenerative medicine presenting a potential treatment option. Furthermore, it has been reported that platelet-rich plasma (PRP) therapy relieves pain resulting from KOA; however, this therapy is effective only for mild-to-moderate degeneration. Leukocyte-poor PRP (LP-PRP) has relatively fewer catabolic effects and is suitable for intra-articular injection in KOA patients.[1] PRP appears to have no therapeutic effect when the degree of degeneration is severe.[2–7] However, consecutive leukocyte-rich PRP (LR-PRP) injections have been shown to have therapeutic effects, regardless of the degree of degeneration,[8,9] suggesting the benefits of consecutive LR-PRP injections for patients with severe knee degeneration.

We started conducting PRP therapy in 2015; this comprised manually prepared PRP, created using an open technique, because the domestically approved PRP product kit was not yet available. By the time PRP product kit was approved and marketed in Japan, we had already started evaluating our hypothesis. Our manually prepared PRP has shown satisfactory results despite severe degeneration, for which LP-PRP is considered to be ineffective, even after 2 years of follow-up. Accordingly, we report the results of our original PRP study here.

The dose, properties of PRP, administration period, and methods of obtaining the optimum effects vary between PRP therapies.[10–14] Moreover, PRP therapy has not been shown to be effective for severely degenerated KOA. Furthermore, changes in cartilage damage, especially reductions in inflammation of the subchondral bone and continuous cartilage surface, observed upon magnetic resonance imaging (MRI) osteoarthritis knee score (MOAKS), suggest that PRP is effective for cartilaginous tissue. The paracrine effect of PRP may stimulate stem cells causing them to promote regeneration of cartilage or cartilage-like tissue.[15] Therefore, we hypothesized that consecutive PRP administration would be effective for all KOA patients, including those with severe degeneration, and assessed the therapeutic efficacy of monthly LR-PRP

; Leukocyte-rich platelet-rich plasma, *LR-PRP*; Outcome Measures in rheumatology-osteoarthritis research society international, *OMERACT-OARSI*; Magnetic resonance imaging, *MRI*; Bone marrow lesion, *BML*; total knee arthroplasty, *TKA*.

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injections.

This study aimed to compare dynamic time-series changes in the visual analog scale (VAS) score, Knee Injury and Osteoarthritis Outcome Score (KOOS), and MOAKS for bone marrow lesions (BMLs). It also aimed to determine whether LR-PRP is effective for severe degeneration, and if it provides a therapeutic effect associated with changes observed upon MRI. Comparisons of the time required to achieve 50% improvement in the VAS score, administration period, and number of injections were also performed.

2. Material and methods

This prospective cohort study was approved by the organizational ethics review committee (details blinded for peer review) and was performed in accordance with the Regenerative Medicine Safety Law[16] following specific evaluation by a committee. The Ministry of Health, Labor, and Welfare had licensed the use of regenerative medicine. This study has been registered with the clinical trial register of the Japan Medical Association Center for Clinical Trials (details blinded for peer review). All participants underwent this study in accordance with the Declaration of Helsinki and provided written informed consent for participation.

Patients who received LR-PRP treatment between June 2016 and May 2021 were enrolled. Overall, 1248 patients (95 men and 165 women; age, 67.1 ± 11.1 years; age range, 34–90 years; body mass index, 25.5 ± 4.2 kg/m2) who underwent 295 injections of LR-PRP were prospectively assessed.

2.1. Study description and enrollment

Limiting the study only to cases that could be investigated could exert some confounding, to a certain degree. Therefore, a scheme was implemented for the involved study period (Fig. 1). Clinical findings and MRI results at 3, 6, 12, and 24 months of patients who were administered LR-PRP therapy three or more times were evaluated. Upon MRI, the MOAKS was used to evaluate BMLs. This treatment is not covered by insurance in Japan, with each injection costing 50,000 yen. Because of the burden to the patient in terms of cost, one injection was administered per month, for 6 consecutive months, and a prospective study was conducted based on this method (Fig. 2).

The 260 subjects in this study were selected according to the following criteria: difficulties in performing activities of daily living despite continuous conservative treatment, involving weight loss, rehabilitation, HA or CS injections, and/or nonsteroidal anti-inflammatory drugs to avoid surgery, and no treatment with HA or CS injections, or any other knee injections during the LR-PRP therapy period. The exclusion criteria were as follows: history of a systemic disorder, such as rheumatoid arthritis, malignant cancer, hematological disease, infection, or immunodeficiency; recent intra-articular CS or HA injections during the past 4 or 2 weeks, respectively; recent administration of anti-cancer drugs or immunosuppressive drugs; and children and adolescents without closure of the epiphyseal plane. All patients provided written informed consent after being counselled about the potential benefits of LR-PRP, treatment procedures, and follow-up period.

2.2. Study procedures

We referred to reports by Boyer[17] and Bannuru et al.,[18] which indicate that HA injections are effective from 4 weeks after intra-articular injection into the knee. They reach their maximum benefit at 8 weeks, and have effects that last up to 6 months. After considering the effect of HA injections, PRP was injected monthly for 6 months to determine the peak effect. This protocol was based on our previous finding of sufficient fibrocartilage coverage in the cartilage defect approximately 7 months after meniscal repair with PRP and platelet-rich fibrin.8 We considered

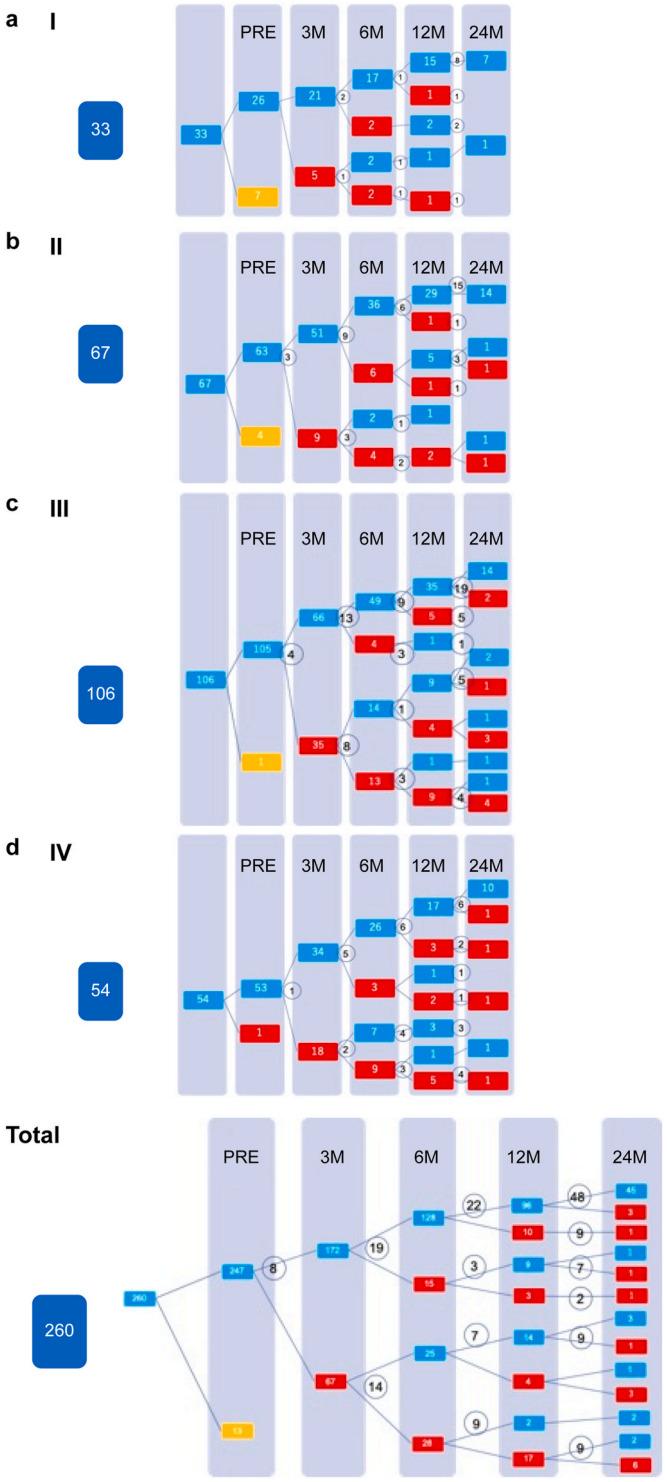

Fig. 1. Flow chart of all cases evaluated during the study period. There were 172 responders (of 239; 72.0%) at 3 months, 153 (of 196; 78.1%) at 6 months, 121 (of 155; 78.1%) at 12 months, and 54 (of 70; 77.1%) at 24 months. The blue squares indicate responders; the red squares indicate non-responders; and the circles indicate patients who did not meet the evaluation criteria. A circled number indicates an excluded case. (A) Grade I: 3 months, 80.8%; 6 months, 82.6%; 12 months, 90%; and 24 months, 100%. (B) Grade II: 3 months, 85%; 6 months, 79.2%; 12 months, 89.7%; and 24 months, 88.9%. (C) Grade III: 3 months, 65.3%; 6 months, 78.8%; 12 months, 71.9%; and 24 months 65.5%. (D) Grade IV: 3 months, 65.4%; 6 months, 73.3%; 12 months, 68.8%; and 24 months, 73.3%.

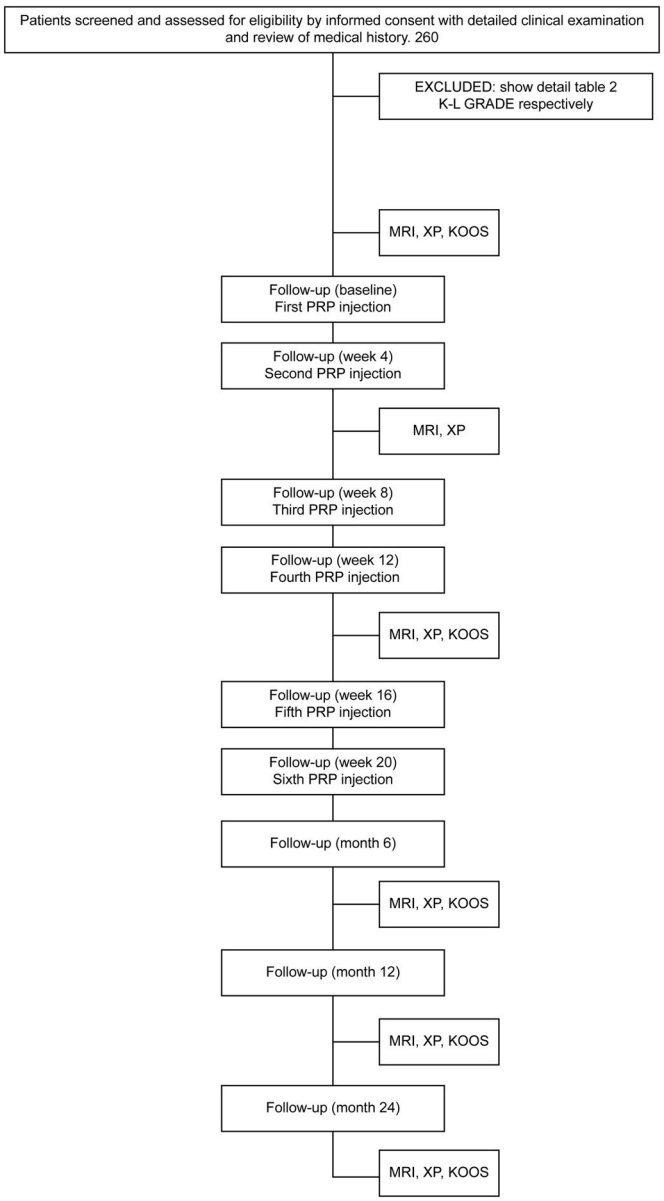

Fig. 2. Study protocol.

continuous administration of LR-PRP for ≥6 months.

During the six-injection protocol, PRP was discontinued upon achievement of subjectively satisfactory outcomes as qualified by each patient. However, clinical and imaging follow-ups were continued despite discontinuation of treatment.

Self-funded PRP therapy for adults who did not consent to surgical treatment and showed no improvement despite conservative management as provided by insurance was performed. Hence, the patients' baseline data before treatment was used as control data. Their progress was closely monitored following the commencement of therapy.

2.3. Processing LR-PRP

2.3.1. Preparation

The duration of venous blood extraction for participants was within 1 min. Blood was immediately transported to the operating room and subsequently centrifuged. Two-time centrifugation with different centrifugal forces was required to create the LR-PRP. First, 1 mL of anticoagulant (sodium citrate solution) was added to a 20-mL blood sample collected from the patient. Then, the mixture was immediately centrifuged for 5 min at 1000 g (Desktop Centrifuge 2420; Kubota Corporation, Tokyo Japan). The upper and middle buffy coats formed by the first centrifugation were collected in a single dry glass, and the tubes were subsequently centrifuged for 15 min at 1500 g. This second centrifugation separated the blood into three different layers. The upper layer was removed, and 2.4 mL of LR-PRP was obtained. Detailed information regarding the standard protocol for obtaining LR-PRP is provided in a previous study.8.

2.3.2. Injections

One intra-articular injection was administered under ultrasound guidance (portable type echo, SonoSite iViz; Fuji Film Medical Co. Ltd., Tokyo Japan) every 4 weeks, for 6 months, by a well-trained, senior orthopedic surgeon with 25 years of experience. The PRP produced was injected and administered within approximately 7 min. The LR-PRP injection technique with ultrasound guidance was performed with the patient's knee slightly bent at an angle of approximately 20°. The injection was administered under sterile conditions using a 21-gauge needle and a suprapatellar approach from the outside of the knee. The intra-articular space in the supra-patellar pouch and the approximate depth were assessed using an inspection probe and a ruler, respectively. Using an ultrasound screen for visualization, a 21-gauge needle was inserted into the patellar capsule in a parallel manner and LR-PRP was injected following confirmation that there was no resistance. If fluid was present in the joint, PRP injection was implemented following aspiration of as much of the fluid as possible. Aspiration was performed before injections in patients with joint effusion. Following injection, patients were instructed to unrestrictedly perform their activities of daily living.

2.4. Clinical assessment

Patients were clinically evaluated using subjective and objective assessments before injections and at 3, 6, 12, and 24 months following treatment to determine the primary clinical outcomes of LR-PRP therapy. Radiographic and MRI examinations were also performed at 3, 6, 12, and 24 months.

Furthermore, we examined the average number of injections required to reduce pain by 50% or more. Pain was assessed using the VAS.[19] Clinical assessments included the KOOS, KOOS-total, KOOS--symptoms, KOOS-pain, KOOS-activity, KOOS-sports, and KOOS-quality of life (KOOS-Q)[20]; radiographic and MRI examinations were also conducted (0.3-T open-type instrument, Medico Airis Bent; Hitachi, Tokyo, Japan).

Therapeutic efficacy was determined by the Outcome Measures in Rheumatology-Osteoarthritis Research Society International (OMERACT-OARSI) responder criteria for osteoarthritis.[21]

BMLs were assessed using the MOAKS.[22] Furthermore, the severity, nature, and duration of adverse events associated with the study protocol were evaluated. It was recommended that rehabilitation should be performed as much as possible, with the main focus on quadriceps femoris training, improvement of knee flexion contracture, and improvement of the stiffness around the patella.

3. Theory/calculation

Continuous data are reported as means±standard deviations, and Welch's *t*-test was used for comparisons. A linear mixed model analysis was conducted to examine whether there were differences in the time series trends among the Kellgren-Lawrence (K-L) classifications for pain assessment (VAS score), clinical assessment (KOOS), and MOAKS for BMLs. The analytical model was constructed with subjects as a random factor and group (K-L classification), time (before injection and 3, 6, 12, and 24 months after injection), and their interaction term (group × time) as fixed factors. Least-square means and their 95% confidence intervals (CIs) were calculated. Pairwise comparisons were made between the K-L classifications at each time point.

The duration of treatment required to achieve 50% improvement in terms of the VAS score was compared using log-rank test. Kaplan-Meier curve was used to show the time course of the improvement rate.

A linear mixed model (with subjects as a random factor) was used to compare the durations of treatment until peak improvement in terms of the VAS score was achieved. The peak was defined as the time at which the VAS score showed the lowest value at 12 months. Therefore, only patients who completed 12 months of follow-up were included in the analysis. Furthermore, a comparison of the frequency of peaks occurring within 12 months was performed using a generalized linear mixed model with the link function as the logit.

Statistical significance was set at $P < 0.05$. All statistical analyses were performed using SPSS version 24.0 for Windows (IBM Japan, Tokyo, Japan).

4. Results

The demographic and clinical characteristics of the patients are shown in Table 1. The PRP used in this study was defined as LR-PRP.

Fig. 1 shows the follow-up and OMERACT-OARSI results for all 260 patients treated with PRP. According to the K-L classification, 33 patients had grade-I KOA (Figs. 1A), 67 had grade-II KOA (Figs. 1B), 106 had grade-III KOA (Figs. 1C), and 54 had grade-IV KOA (Fig. 1D). There were 172 responders (out of 239; 72.0%) at 3 months, 153 (out of 196; 78.1%) at 6 months, 121 (out of 155; 78.1%) at 12 months, and 54 (out of 70; 77.1%) at 24 months. In total, 16.9% of participants were excluded because they chose to leave the study or did not conform to the protocol (36.4% with K-L grade I; 14.9% with K-L grade II; 12.3% with K-L grade III; and 16.7% with K-L grade IV). The details of the excluded participants are shown in Table 2. There was a significant difference between the average age of participants with each K-L classification ($P < 0.0002$); however, there was no significant difference between participants with K-L grades I and II ($P = 0.07$) and those with K-L grades III and IV ($P = 0.19$). The average improvement in the VAS score was 58.1 ± 29.4%. There were no correlations between age and VAS score improvement ($r = -0.05$; $P < 0.01$) or between body mass index and VAS score improvement ($r = -0.04$; $P < 0.01$). Similarly, there were no correlations between the white blood cell concentrations ($r = -0.12$; $P < 0.01$) and platelet concentrations ($r = -0.17$; $P < 0.01$) and the rate of improvement in the VAS score. There were no obvious changes observed on radiographs and no obvious adverse events.

4.1. Comparison of time series transitions

An examination of the time and group interactions indicated a significant difference in the time series transitions between the K-L classifications, VAS scores, and KOOS-Q (VAS, $P = 0.015$; KOOS-Q, $P < 0.001$). Comparisons of the VAS score with each K-L grade before and

Table 1
Demographic data.

Characteristics	Total	K-L grade I	K-L grade II	K-L grade III	K-L grade IV
Cases, n	260	33	67	106	54
Injections, n	1295	129	307	554	305
Age, years	67.1 ± 11.1	59.2 ± 10.9	63.4 ± 9.8	69.6 ± 11.3	71.6 ± 7.6
BMI	25.5 ± 4.2	23.7	25.2	25.3	27.1
Mean FTA	180.9	178	178.3	181.0	185.5
PLT CR	5.6	5.1	5.3	5.4	5.5
WBC CR	2.9	2.6	2.9	2.9	2.9

Abbreviations: K-L, Kellgren-Lawrence; BMI, body mass index; FTA, femorotibial angle; PLT, platelet; PRP, platelet-rich plasma; WBC, white blood cell; WB, whole blood; PLT CR, PLT concentration ratio (PRP/WB); WBC CR, WBC concentration ratio (PRP/WB).

Table 2
Patients lost to follow-up or those who dropped out of the study.

	I				II				III				IV				TOTAL			
	PRE	3 M	6 M	12 M	24 M	PRE	3 M	6 M	12 M	24 M	PRE	3 M	6 M	12 M	24 M	PRE	3 M	6 M	12 M	24 M
TOTAL	7	0	3	3	12	4	3	12	9	20	1	4	21	16	34	1	1	7	13	17	13	8	43	41	84
P Non-conform	3					2					1										6				
SI	2		1	3	3		2	1	4	1		1	6	3	2	1		1	4	3	3	3	9	14	9
AD	1		1			2						1	1				1	1			3	2	3		
HTO								1						1									1	1	
TKA					1			1		1					1				4				1	4	3
FOR COVID	1								1				1								1		1	1	
LOD							1					2										3			4
Death															1					3					
In progress			1		8			9	4	18			13	12	30			5	5	11			28	21	67

This table provides details about cases excluded per patient flow scheme presented in Fig. 1. P Non-conform; Protocol Non-conformation, SI; Self-interruption, AD; Another disease, HTO; High Tibial Osteotomy, TKA; Total Knee Arthroplasty, For COVID; Interruption due to COVID-19, LOD; Lack of data.

after treatment showed that the VAS score was significantly lower before the injection (per group) in all groups (all $P < 0.001$). Furthermore, the pretreatment scores were low for those with K-L grade I and high for those with K-L grade IV; however, a large decrease in time until improvement was observed for those with K-L grades I and II (VAS score with grade I: 51.9 [95% confidence interval: 43.6–60.2] to 15.0 [95% CI: 2.727.4] at 2 years, difference = −36.9; VAS score with grade II: 62.7 [95% CI: 57.5–68.0] to 27.5 [95% CI: 19.0–35.9], difference = −35.2). For those with K-L grades III and IV, the reduction tended to decrease (VAS score with grade III: 59.9 [95% CI: 55.8–64.1] to 43.7 [95% CI: 37.1–50.4], difference = −16.2; VAS score with grade IV: 69.8 [95% CI: 63.9–75.6] to 44.8 [95% CI: 34.7–54.8], difference = −25.0). Therefore, it was found that the VAS score at 2 years was significantly lower for those with K-L grades I and II than for those with K-L grades III and IV.

Comparison of the KOOS-Q before and after treatment with each K-L grade indicated that the KOOS-Q was significantly lower before injections in all groups (all $P < 0.001$). A comparison of the K-L classifications revealed no statistically significant difference among the values before treatment. The magnitude of the increase in the KOOS-Q score tended to decrease in those with K-L grades II to IV compared with those with K-L grade I (KOOS-Q score with grade I: 32.0 [95% CI: 24.2–39.8] to 63.2 [95% CI: 52.2–74.2], difference = 31.2; KOOS-Q score with grade II: 34.5 [95% CI: 29.6–39.4] to 53.5 [95% CI: 46.0–60.9], difference = 19.0; KOOS-Q score with grade III: 30.6 [95% CI: 26.7–34.6] to 43.4 [95% CI: 37.5–49.4], difference = 12.8; KOOS-Q score with grade IV: 30.5 [95% CI: 25.1–36.0] to 39.7 [95% CI: 31.3–48.0], difference = 9.1). Therefore, it was determined that the KOOS-Q score at 2 years was significantly higher for those with K-L grades I and II than for those with K-L grades III and IV.

Regarding the KOOS-pain and KOOS-activity, a significant increase was observed with time for each K-L classification, although no significant difference was observed in the time-series transition between these classifications (KOOS-pain: $P = 0.230$; KOOS-activity: $P = 0.280$). However, we also observed higher scores for those with K-L grades I and II compared with those with K-L grades III and IV at 2 years.

Regarding the MOAKS for BMLs, no significant difference was found in the time-series transition between K-L classifications ($P = 0.542$). A statistically significant decrease was observed at 2 years only for those with K-L grades III and IV; however, it was confirmed that the score at 2 years was not lower than the score before treatment for those with K-L grades I and II (grade III: 8.54 [95% CI: 7.72–9.37] to 6.68 [95% CI: 5.28–8.09], $P = 0.007$; grade IV: 12.34 [95% CI: 11.22–13.47] to 8.94 [95% CI: 6.97–10.90], $P < 0.001$) (Table 3).

4.2. Comparisons of time to 50% improvement in the VAS score

The period required for a minimum improvement of 50% in terms of the VAS score was reckoned from the start of therapy. An improvement in the VAS score of 50% or more has a significant impact on the OMERACT-OARSI responder criteria. The average time to achieve this improvement was significantly longer for those with K-L grades III and IV than for those with K-L grade II, and it was also significantly longer for those with K-L grade IV than for those with K-L grade I (grade I: 7.57 [95% CI: 3.97–11.18] months; grade II: 4.03 [95% CI: 3.14–4.92] months; grade III: 10.68 [95% CI: 8.65–12.72] months; grade IV: 12.95 [95% CI: 10.04–15.86] months; grade III vs. grade II, $P < 0.001$; grade IV vs. grade II, $P < 0.001$; grade IV vs. grade I, $P = 0.006$).

4.3. Comparison of treatment periods until the peak of VAS improvement was achieved

There were no statistically significant differences between K-L classifications during the treatment period, peak VAS score, and peak improvement within 12 months. An improvement of up to 75% within 12 months occurred in patients with K-L grade IV, and an improvement of up to 60% within 12 months occurred in patients with K-L grade I; however, no significant difference was detected ($P = 0.283$).

5. Discussion

Studies reporting that PRP is only effective for treating mild degeneration2-7 included a small number of cases, had a short treatment duration, and used a low dose of administration. In contrast, our method effectively treated severe degeneration, showing improvements in the VAS score and KOOS-Q. Although there were no statistically significant differences in the magnitude of improvement in pain or activities of daily living, the therapeutic effect was considered to be sufficiently successful. Moreover, in proportion to the K-L classification, the more severe the degeneration, the longer it had taken for the therapeutic effect to appear.

The MOAKS decreased with higher K-L classifications while it increased with higher initial K-L classifications before treatment; however, it did not decrease below the pretreatment values for those with K-L grades I and II, despite observed improvements. In other words, a therapeutic effect cannot be expected for patients with K-L grades III and IV until they have achieved the same MOAKS as in patients with K-L grade I before treatment. Therefore, this treatment does not guarantee cartilage regeneration.

Although not statistically significant, patients with K-L grade IV tended to achieve peak values at 1 year after treatment, and these values then tended to taper compared with those of patients with K-L grade I, suggesting that more severe degeneration requires longer-term treatment and additional doses. Further investigations are needed to determine whether there were histological changes on the cartilage surface and if the associations with improved quality of life were accidental findings.

Currently, LP-PRP, which has fewer catabolic effects, is recommended for PRP treatment; however, less inflammatory cytokines may be better for PRP injections in the knee.[23] Our findings showed a slight improvement in the MOAKS for BMLs; therefore, it is necessary to investigate how continuous administration of LR-PRP affects the repair of damaged cartilage. Furthermore, histopathological examinations of the cartilage surface following LR-PRP administration are also necessary.

5.1. Limitations

A limitation of this study was that there was no distinct control group. Furthermore, only one sequence of injections was administered. Because only LR-PRP was studied, there was likely a significant placebo effect. Furthermore, patients who received injections had to pay for them. Additionally, some patients were unable to visit the hospital because of the coronavirus disease 2019 (COVID-19) pandemic; some stopped attending their hospital appointments because their treatment was ineffective; and some patients died. COVID-19 has had a great impact on daily life in Japan. Therefore, it was difficult to collect and manage case data, and accurate data may not have been obtained because of decreased activities and daily life restrictions. Finally, there were several demographic variables, such as age, body mass index, and sex. Our protocol was planned before the minimum information for studies evaluating biologics in orthopedics was established for PRP[24]; therefore, patients with conditions, such as diabetes, and those who were smokers were included. In particular, a 71.6-year-old in the K-L grade-IV group was significantly older than a 59.2-year-old in the K-L grade I. However, we followed our treatment protocol and randomly intervened in patients with their consent. Therefore, this analysis shows that the effect of the intervention is genuine and unrelated to the variability of the subject groups.

6. Conclusions

Manually prepared LR-PRP could reduce pain and improve quality of

Table 3
Comparison of time series transitions for VAS scores, KOOS, and MOAKS for BML (linear mixed model).

K-L	I				II				III				IV					P-value (comparison)					
Time	mean	95% CI		P-value vs. Pre	mean	95% CI		P-value vs. Pre	mean	95% CI		P-value vs. Pre	mean	95% CI		P-value vs. Pre	P-value Time * group	I vs II	I vs III	I vs IV	II vs III	II vs IV	III vs IV
VAS																	0.015						
Pre	51.9	43.6	60.2		62.7	57.5	68.0		59.9	55.8	64.1		69.8	63.9	75.6			0.026	0.089	0.001	0.386	0.074	0.004
3 M	23.7	15.2	32.1	0.000	31.4	26.1	36.7	0.000	38.4	34.1	42.6	0.000	50.1	44.2	56.0	0.000		0.114	0.002	0.000	0.032	0.000	0.001
6 M	24.3	15.6	33.0	0.000	33.0	27.3	38.8	0.000	35.3	30.7	39.9	0.000	44.3	38.1	50.5	0.000		0.091	0.028	0.000	0.521	0.008	0.015
1Y	21.3	12.2	30.4	0.000	29.0	22.7	35.2	0.000	34.8	29.9	39.7	0.000	49.4	42.2	56.6	0.000		0.155	0.010	0.000	0.134	0.000	0.001
2Y	15.0	2.7	27.4	0.000	27.5	19.0	35.9	0.000	43.7	37.1	50.4	0.000	44.8	34.7	54.8	0.000		0.100	0.000	0.000	0.002	0.009	0.862
KOOS (pain)																	0.230						
Pre	64.9	58.2	71.6		57.0	52.8	61.2		54.0	50.6	57.3		44.4	39.7	49.1			0.040	0.004	0.000	0.238	0.000	0.000
3 M	81.0	74.3	87.7	0.000	71.3	67.0	75.5	0.000	64.3	60.9	67.7	0.000	57.6	52.9	62.3	0.000		0.012	0.000	0.000	0.007	0.000	0.015
6 M	80.1	73.1	87.0	0.000	70.7	66.2	75.3	0.000	68.4	64.7	72.1	0.000	62.4	57.4	67.3	0.000		0.022	0.003	0.000	0.404	0.013	0.039
1Y	82.4	75.2	89.6	0.000	75.8	70.8	80.7	0.000	67.8	63.9	71.7	0.000	60.3	54.6	66.0	0.000		0.118	0.000	0.000	0.009	0.000	0.024
2Y	86.2	76.5	95.9	0.000	76.9	70.3	83.5	0.000	66.3	61.1	71.5	0.000	53.9	46.5	61.3	0.012		0.115	0.000	0.000	0.010	0.000	0.005
KOOS (ADL)																	0.280						
Pre	75.4	69.7	81.2		70.6	67.0	74.2		67.0	64.0	69.9		63.4	59.3	67.4			0.129	0.008	0.001	0.071	0.005	0.099
3 M	84.0	78.3	89.8	0.001	80.3	76.7	83.9	0.000	74.1	71.1	77.0	0.000	71.5	67.4	75.5	0.000		0.238	0.002	0.000	0.002	0.001	0.243
6 M	85.6	79.7	91.5	0.000	80.5	76.7	84.4	0.000	76.7	73.5	79.8	0.000	76.1	71.9	80.3	0.000		0.126	0.008	0.010	0.079	0.109	0.807
1Y	85.8	79.7	91.9	0.000	82.9	78.8	86.9	0.000	76.7	73.4	80.0	0.000	71.7	67.0	76.4	0.000		0.393	0.009	0.000	0.010	0.000	0.054
2Y	87.1	79.3	94.8	0.001	85.1	79.9	90.3	0.000	73.9	69.7	78.0	0.000	69.7	63.8	75.5	0.023		0.662	0.003	0.000	0.000	0.000	0.216
KOOS (QOL)																	0.000						
Pre	32.0	24.2	39.8		34.5	29.6	39.4		30.6	26.7	34.6		30.5	25.1	36.0			0.567	0.759	0.764	0.181	0.274	0.974
3 M	55.1	47.3	62.8	0.000	47.4	42.5	52.4	0.000	41.6	37.7	45.6	0.000	39.1	33.6	44.6	0.001		0.086	0.002	0.001	0.048	0.023	0.419
6 M	59.2	51.1	67.2	0.000	51.2	46.0	56.5	0.000	47.5	43.3	51.8	0.000	42.9	37.2	48.6	0.000		0.087	0.011	0.001	0.241	0.032	0.165
1Y	65.3	57.0	73.7	0.000	59.3	53.6	64.9	0.000	46.7	42.2	51.1	0.000	37.4	30.9	43.9	0.032		0.212	0.000	0.000	0.000	0.000	0.014
2Y	63.2	52.2	74.2	0.000	53.5	46.0	60.9	0.000	43.4	37.5	49.4	0.000	39.7	31.3	48.0	0.029		0.143	0.002	0.001	0.030	0.015	0.452
MOAKS BML																	0.542						
Pre	4.20	2.53	5.87		5.15	4.13	6.16		8.54	7.72	9.37		12.3	11.2	13.5			0.307	0.000	0.000	0.000	0.000	0.000
3 M	4.00	2.34	5.65	0.785	5.50	4.48	6.52	0.451	7.94	7.11	8.77	0.093	11.9	10.8	13.0	0.360		0.101	0.000	0.000	0.000	0.000	0.000
6 M	3.58	1.89	5.26	0.419	4.56	3.48	5.63	0.239	7.56	6.68	8.43	0.012	10.9	9.69	12.0	0.005		0.302	0.000	0.000	0.000	0.000	0.000
1Y	2.32	0.41	4.23	0.032	4.53	3.27	5.80	0.316	6.39	5.40	7.38	0.000	11.1	9.78	12.5	0.054		0.045	0.000	0.000	0.014	0.000	0.000
2Y	2.62	0.07	5.18	0.207	3.93	2.32	5.55	0.125	6.68	5.28	8.09	0.007	8.94	6.97	10.9	0.000		0.384	0.006	0.000	0.008	0.000	0.059

Results of a comparative study of K-L classifications and pain evaluations (VAS scores), clinical evaluation (KOOS), and MOAKS for BML transition over time (time series transition). A linear mixed model analysis including the interaction term (group × time) of the subjects as a variable factor and group (KL classification) and time (before treatment and 3 months, 6 months, 12 months, and 24 months after treatment) as fixed factors. n = 247 cases that received treatment. Abbreviations: ADL, activities of daily living; CI, confidence interval; K-I, Kellgren-Lawrence; QOL, quality of life; VAS, visual analogue scale; KOOS, Knee Injury and Osteoarthritis Outcome Score; MOAKS, magnetic resonance imaging osteoarthritis knee score; BML, bone marrow lesion.

life, even in the case of severe degeneration. Continuous LR-PRP injection may be useful in improving pain and quality of life even in cases with severe knee degeneration. Furthermore, the use of repeated doses of LR-PRP over time helped to determine new conservative treatment strategies for patients with advanced KOA and mild degeneration. We verified LR-PRP, and by administering it multiple times every 4 weeks, we were able to show the effectiveness of the treatment method regardless of the degree of deformation.

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Unblinded study registration

This study has been registered with the clinical trial register of the Japan Medical Association Center for Clinical Trials (JMA-IIA00351).

Declarations of interest

None.

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