Regenerative Therapy 29 (2025) 227–236

Contents lists available at ScienceDirect

Regenerative Therapy

journal homepage: http://www.elsevier.com/locate/reth

Original Article

Optimal frequency of platelet-rich plasma injections for managing osteoarthritis: A longitudinal study

Masahiko Kemmochi*

Kemmochi Orthopedic Surgery Sports Clinic, 42-1 Higashi-honcho, Ota, Gunma Prefecture 373-0026, Japan

ARTICLE INFO

Article history:
Received 21 August 2024
Received in revised form
27 January 2025
Accepted 12 February 2025

Keywords:
Optimal frequency
Platelet-rich plasma
Multiple injections
Knee osteoarthritis
Longitudinal study

ABSTRACT

Introduction: Recent reviews suggest that PRP injections can improve pain and function more effectively than other treatments; however, consensus on the optimal number of injections is lacking. We aimed to determine the optimal administration frequency and number of PRP injections for management of osteoarthritis (OA) symptoms, to examine long-term effects and structural improvements with PRP, and to determine correlations between clinical outcomes and imaging findings.

Methods: This longitudinal study included 167 patients with knee OA, categorized using the Kellgren−Lawrence (KL) grading system. Participants received up to six PRP injections and were followed-up for 24 months. Pain levels were assessed using the visual analog scale (VAS); functional recovery was measured using the Knee Injury and Osteoarthritis Outcome Score (KOOS). To determine whether PRP can induce sustained structural improvements, we used the MRI Osteoarthritis Knee Score (MOAKS) to monitor changes in bone-marrow lesions (BMLs). Data were analyzed using repeated-measures analysis of variance to identify significant changes in pain and functional outcomes.

Results: VAS and KOOS scores significantly improved after PRP treatment. Patients with KL grades 1 and 2 exhibited maximum pain relief after the fourth injection; those with KL grades 3 and 4 showed optimal results after the fifth injection. Improvements were maintained or enhanced at the 24-month follow-up. The effect size increased as the number of treatments progressed, and especially after the fourth treatment, with a Cohen's d values of −1.22, −1.28, and −0.99 ($p < 0.0001$).

Conclusions: PRP injections administered at specific intervals can significantly reduce pain and improve function in patients with OA, with the required frequency depending on disease severity. These findings support the customization of PRP-treatment protocols based on individual patient profiles to maximize therapeutic benefits.

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

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

Level of evidence: II.

1. Introduction

Osteoarthritis (OA) is a prevalent degenerative disease, leading to joint pain, stiffness, and reduced function and significantly impacting quality of life and healthcare costs. Traditional treatments such as non-steroidal anti-inflammatory drugs (NSAIDs) and corticosteroids offer only temporary relief, with notable long-term side effects reported [1,2]. Autologous platelet-rich plasma (PRP) therapy has emerged as a promising treatment, believed to enhance joint healing by delivering high concentrations of growth

Abbreviations: OA, osteoarthritis; NSAIDs, non-steroidal anti-inflammatory drugs; PRP, platelet-rich plasma; KOA, knee OA; MOAKS, MRI Osteoarthritis Knee Score; BML, bone marrow lesions; JMACCT, Japan Medical Association Center for Clinical Research; KL, Kellgren−Lawrence; ADL, activity of daily living; QoL, quality of life; JF, joint fluid; LMM, linear mixed-effects model.

* Kemmochi Orthopedic Surgery Sports Clinic, 42-1 Higashi-honcho, Ota, Gunma Prefecture 373-0026, Japan.

E-mail address: kossmos@rainbow.plala.or.jp.

Peer review under responsibility of the Japanese Society for Regenerative Medicine.

https://doi.org/10.1016/j.reth.2025.02.006

factors [3,4]. However, the optimal injection frequency for best outcomes remains unclear.

The role of PRP in treating knee OA (KOA) needs further investigation. Existing studies suggest potential benefits but often lack focus on KOA's chronic nature, involving cartilage degradation and subchondral bone changes observable on magnetic resonance imaging (MRI).

PRP treatment is based on the principle of delivering growth factors to stimulate joint repair. However, evidence of consistent long-term clinical improvements is lacking, highlighting the need for methodically designed trials to clarify PRP's dose-response relationship [5].

Preliminary data suggest that PRP may alleviate symptoms of KOA, such as pain and stiffness, but the variability in outcomes indicates a lack of standardized protocols. Recent reviews suggest that PRP injections can improve pain and function more effectively than other treatments; however, consensus on the optimal number of injections is lacking [6,7]. Therefore, this study aimed to address this gap by rigorously evaluating the dose-response relationship of PRP in OA management. This study aimed to evaluate symptomatic relief and MRI-detected changes in joint structure over 24 months following PRP injections with the goal of defining an optimal PRP treatment regimen.

1.1. Study objectives

This study aimed to determine the optimal number and frequency of PRP injections required to maximize therapeutic outcomes, taking into account patient compliance and clinical feasibility. Additionally, it sought to evaluate the long-term effects of PRP injections on joint structure and function, with a particular focus on assessing bone marrow lesions (BMLs) using the MRI Osteoarthritis Knee Score (MOAKS). Finally, this study aimed to correlate clinical improvements, measured by the visual analogue scale (VAS) score and Knee Injury and Osteoarthritis Outcome Score (KOOS), with MRI findings to substantiate the impact of PRP on joint health.

2. Methods

2.1. Study design and participants

This study adhered to Japan's strict legal framework for regenerative medicine. Before treatment initiation, a detailed treatment plan was approved by a Certified Regenerative Medicine Review Committee and registered with the Ministry of Health, Labour and Welfare, as required under the Act on Ensuring the Safety of Regenerative Medicine. Patients were provided with detailed explanatory documents, signed comprehensive informed consent forms, and were covered by mandatory insurance for potential adverse events. The informed consent also included provisions for using treatment data for research purposes.

The treatments were prospectively planned and conducted, while the data analysis for this manuscript was performed retrospectively, two years after treatment completion, to evaluate long-term outcomes. Thus, this study is best described as a retrospective analysis of prospectively planned treatments.

This prospective, interventional, nonrandomized clinical trial was approved by the Institutional Review Committee of the Japanese Association for the Promotion of State of the Art in Medicine [approval numbers: PB3180007(June 1, 2018) and PB3210070(August 23, 2021)]and registered with the Japan Medical Association Center for Clinical Research (JMACCT) (JMA-IIA00351). The study complied with the Declaration of Helsinki and relevant US Health Insurance Portability and Accountability Act regulations. All patients diagnosed with KOA were treated at our clinic between June 2018 and November 2023 after providing comprehensive informed consent.

2.2. Participant recruitment and selection

A total of 320 patients with chronic KOA who did not respond to conventional treatments, such as hyaluronic acid or steroid injections, were recruited for this study through various channels, including hospital bulletins, social media, radio, and health magazines. Detailed data were consistently maintained over two years for 167 participants (Fig. 1). Patients receiving PRP treatment were categorized according to the Kellgren-Lawrence (KL) grading system [8], with treatment response evaluated at multiple follow-up points using the Outcome Measures in Rheumatology and Osteoarthritis Research Society International (OMERACT-OARSI) criteria [9]. According to the OMERACT-OARSI criteria, a “responder” was defined by one of the following outcomes:

An improvement of at least 50 % in pain or function and an absolute change of 10 points on a 0–100 scale.

Alternatively, an improvement of at least 20 % and an absolute change of 10 points in at least two of the following three outcomes: pain, function, and overall patient assessment.

These criteria allowed for a broad evaluation of treatment efficacy, covering multiple dimensions of health and quality of life. Logistic regression analysis was conducted to assess the effect of the number of PRP injections on achieving responder status while adjusting for age, sex, and baseline disease severity. The study adhered to the Minimum Information for the Study of Biologics in Orthopedics (MIBO) standards [10], ensuring the consistent collection of demographic data for treatments involving PRP and mesenchymal stem cells.

2.3. Eligibility criteria

Eligibility was restricted to patients with a confirmed diagnosis of KOA, who experienced insufficient relief from standard therapies.

2.4. PRP preparation and administration protocol

PRP was prepared using a standardized dual-spin centrifugation method, achieving high concentrations of white blood cells and platelets [11]. Blood samples were collected in two 10 mL sterile glass tubes containing sodium citrate as an anticoagulant. The first centrifugation (1000 g for 5 min at room temperature) separated the blood components, and the platelet-rich fraction was then combined and subjected to a second centrifugation (1500 g for 15 min) to yield approximately 2.4 mL of PRP, ready for clinical application. The prepared PRP was immediately placed in a buffer case and transported in a cold bag maintained at approximately 20 °C, with administration to the patient initiated within 10 min. Injections were administered intra-articularly under ultrasound, the SonoSite iViz portable ultrasound system (Fujifilm Medical Co. Ltd., Tokyo, Japan), using a sterile technique in an outpatient setting, with each patient receiving up to six monthly PRP injections. Treatment could be discontinued after the fourth injection if significant clinical improvement was observed, based on a mutual decision between the patient and physician. The data shown in Table 1 are based on the prepared PRP and reflect the concentration and composition within the PRP, not the original whole blood. Specifically, “Average Leukocyte (fold)" and “Average Lymphocyte (fold)" represent concentrations within the PRP, while “Lymphocyte Fraction (%)" and “Granulocyte Fraction (%)" show the cellular composition in the PRP.

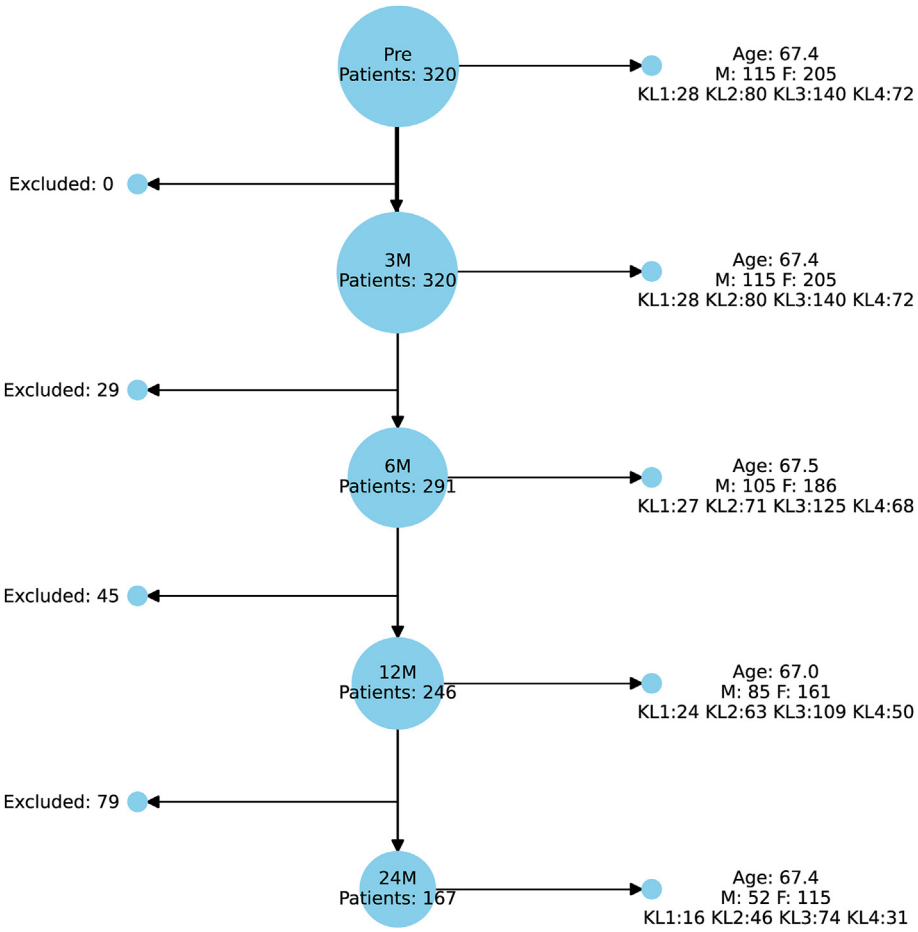

Fig. 1. Patient flow and demographic information over time. This flowchart illustrates the progression and retention of patients throughout the study period, from baseline (Pre) to follow-ups at 3, 6, 12, and 24 months. Each circle represents the number of patients assessed at each time point, with detailed demographic information shown alongside. The arrows indicate the continuity between each phase, emphasizing the reduction in patient numbers over time due to exclusions. Abbreviations: KL: Kellgren–Lawrence classification, M: Male, F: Female.

2.5. Rehabilitation protocol

A structured rehabilitation protocol focusing on enhancing muscle strength and mobility while minimizing knee stress was implemented. Emphasis was placed on maintaining full knee extension and optimizing gait mechanics, particularly to avoid deep knee flexion.

2.6. Follow-up and outcome assessment

Evaluations of pain (VAS score) [12], function (KOOS-pain, ADL, and QoL) [13], and joint structure (MOAKS-BML score) [14] were conducted 3, 6, 12, and 24 months after the initial injection. MRI (0.3T open-type; Fuji Film, Airis Bento, Tokyo, Japan) imaging assessments were performed preoperatively and at each follow-up interval. Treatment efficacy was further assessed using the OMERACT-OARSI criteria to determine responder status, providing a comprehensive measure of therapeutic impact.

2.7. Joint effusion

In this study, nonparametric statistical techniques were used to evaluate changes in joint fluid (JF) levels in patients undergoing treatment for OA. Data were collected at baseline and at 3, 6, 12, and 24 months post-treatment. In cases where joint effusion was observed during a routine examination, joint aspiration was performed, and the extracted synovial fluid was visually assessed in a syringe. The volume of joint effusion was measured and recorded in units of 1 mL. As the Shapiro–Wilk test confirmed a non-normal distribution of JF levels, the Wilcoxon signed-rank test was applied to compare baseline JF levels with those at each follow-up time point. Additionally, a linear mixed-effects model (LMM) was employed to account for individual variations and repeated measures across the study period. This approach provided a robust framework for analyzing longitudinal changes in JF, capturing both between-patient variability and within-patient fluctuations over time.

2.8. Statistical analyses

2.8.1. Statistical framework

The statistical framework for this study included the following components:

Data Preprocessing: Missing data were handled using multiple imputation, and outliers were identified and assessed using SD and interquartile range methods. Normality was verified with the Shapiro-Wilk and D'Agostino-Pearson tests, with non-normal variables appropriately transformed.

Comparative Analyses: For normally distributed variables (e.g., VAS, KOOS-pain, ADL, QoL, MRI MOAKS-BML), ANOVA was used; for

Table 1
Demographic and baseline characteristics of study participants.

KL Grade	Avg Age	Avg FTA	Avg BMI	Avg Platelet (fold)	Avg Leukocyte (fold)	Avg Lymphocyte (fold)	Lymphocyte Fraction (%)	Granulocyte Fraction (%)	Avg Pre JF (ml)	Smoking (n, %)	DM (n, %)	ATDs (n, %)	HA (n, %)	Steroids (n, %)	NSAIDs (n, %)
Overall	67.4	180.9	25.4	5.3	2.8	4.0	52.1	37.8	6.3	14 (4.4 %)	31 (9.7 %)	22 (6.9 %)	209 (65.3 %)	28 (8.8 %)	213 (66.6 %)
1	59.0	177.9	23.9	5.0	2.6	3.9	50.3	36.5	5.8	4 (14.3 %)	0 (0.0 %)	0 (0.0 %)	17 (60.7 %)	1 (3.6 %)	15 (53.6 %)
2	63.0	178.4	25.0	5.3	2.8	4.0	48.7	35.2	5.8	6 (7.5 %)	6 (7.5 %)	6 (7.5 %)	49 (61.3 %)	5 (6.3 %)	52 (65.0 %)
3	69.2	180.8	25.1	5.4	2.8	4.0	47.2	34.8	5.4	1 (0.7 %)	16 (11.4 %)	10 (7.1 %)	89 (63.6 %)	14 (10.0 %)	98 (70.0 %)
4	72.1	185.2	26.8	5.4	2.8	4.1	46.5	33.1	8.7	3 (4.2 %)	9 (12.5 %)	6 (8.3 %)	54 (75.0 %)	8 (11.1 %)	48 (66.7 %)

KL, Kellgren–Lawrence; Avg, average; FTA, femoro tibial angle; BMI, body mass index; Avg pre JF, Average Pre-joint fluid; DM, Diabetes Mellitus; ATDs, antithrombotic drugs; HA, hyaluronic acid; NSAIDs, non-steroidal anti-inflammatory drugs.

non-normal variables, the Kruskal-Wallis test was applied to analyze the effects of demographic factors, disease severity, and treatment regimens.

Correlation and Association Analyses: Spearman's rank correlation was employed to explore relationships between clinical and demographic factors and treatment outcomes, providing insights into PRP treatment efficacy.

Multivariate Regression: Multivariate regression analysis was conducted to assess the influence of KL classification on 12-month VAS scores, adjusting for the independent effects of osteoarthritis severity.

Dose-Response Relationship: Segmented regression within linear mixed-effects models was used to identify the optimal number of PRP administrations, determining the dosage regimen where additional injections provided no further significant improvement.

Longitudinal Analysis: Longitudinal changes in clinical and structural outcomes were analyzed using linear mixed-effects models, providing a comprehensive view of the long-term effects of PRP treatment.

2.8.2. Software and tools

All analyses were conducted using R statistical software (version 4.1.2), with “lme4” [15] for linear mixed-effects models and “ggplot2” [16] for data visualization, ensuring flexibility and robustness for complex datasets.

3. Results

3.1. Demographic and baseline characteristics

The demographic and baseline characteristics of the study participants are summarized in Table 1. The average age of participants increased in correlation with the severity of OA, reaching a peak of 72.1 years in patients classified as grade 4. Femorotibial angle (FTA) measurements showed an increase with OA severity, which indicates more pronounced joint deformities in patients with advanced OA. Body mass index (BMI) also increased with OA severity, suggesting a potential association between greater body weight and more severe joint degradation. Additionally, platelet, leukocyte, and lymphocyte counts showed a slight increase with advancing OA grades, which may reflect an inflammatory response in more severe cases.

In terms of PRP characteristics, the PRP used was leukocyte-rich, containing platelets at a concentration five times higher than baseline blood values and leukocytes at 2.5 times the baseline, primarily composed of lymphocytes. The prevalence of smoking was lower, while the incidence of diabetes was higher among patients in the advanced grades, likely reflecting common comorbidities with OA severity. Antithrombotic medication use was slightly higher in these advanced stages, indicating an increased prevalence of cardiovascular comorbidities. Patients in higher KL grades also exhibited increased usage of pre-treatment injections, such as hyaluronic acid and steroids, reflecting a more aggressive approach to symptom management. NSAIDs usage remained consistently high across all grades, with the highest use observed in grade 3 patients.

3.2. Trends in medication use

Over the 24-month follow-up period, usage patterns of adjunctive medications showed diverse trends. Hyaluronic acid usage remained stable at 65.3 % from baseline to 24 months. In contrast, steroid use decreased from 8.8 % to 1.2 %, indicating a potential reduction in steroid dependency. However, NSAIDs usage

increased from 66.6 % to 92.8 %, possibly due to the inclusion of topical forms, such as patches, in the NSAIDs category.

3.3. Dose-response relationship analysis

The dose-response analysis revealed a substantial reduction in VAS scores across multiple PRP injections, as detailed in Table 2. Following the first injection, there was a mean improvement of −7.7 (SD: 13.49), and the effect size was −0.57, with a 95 % confidence interval (CI) ranging from −9.63 to −5.78. This trend of increasing pain reduction continued with each subsequent injection, with the fourth injection yielding a mean improvement of −22.25 (SD: 18.21) and an effect size of −1.22 (95 % CI: −24.85 to −19.65). The fifth injection reached the highest effect size of −1.28, corresponding to a mean improvement of −25.26 (SD: 19.72), with diminishing returns observed in the sixth injection. All improvements were statistically significant ($p < 0.0001$), with the greatest effects seen up to the fifth injection, suggesting a robust dose-response relationship that plateaued after the fifth administration. Further research may be required to determine the optimal number of injections for sustained pain relief.

3.4. Statistical analysis results

Table 3 provides a comprehensive summary of the statistical analysis results for pain reduction, functional improvement, structural changes, and KOOS-ADL. For each analysis, Table 3 presents the mean reduction or improvement, p-values indicating statistical significance, effect sizes, and 95 % confidence intervals. These results highlight the therapeutic effects observed in different KL grades and at various follow-up intervals, providing insights into the effectiveness of PRP treatment across multiple clinical outcomes. Fig. 2 illustrates the overall trend of VAS score reductions over time. VAS scores decreased significantly from pre-treatment to 24 months post-treatment across all KL grades. Higher KL grades had higher initial VAS scores, but significant reductions were observed over time, particularly up to the fifth PRP injection. For KL grade 1 patients, the VAS score decreased from a pre-treatment level of 47.7 to 10.9 at 24 months. Fig. 3 shows the reduction in VAS scores was particularly significant after each PRP administration, with KL grade 1 patients showing the largest pain reduction (−39.4 points) following the fourth injection ($p < 0.0001$). For KL grade 2, the fourth injection was also the most effective, showing a reduction of −36 points ($p < 0.0001$), whereas KL grades 3 and 4 showed optimal responses after the fifth injection, with reductions of −26.8 points ($p < 0.0001$) for grade 3 and -20.7 points ($p = 0.0001$) for grade 4.

3.5. Overall trend in VAS scores

There was a general trend of decreasing VAS scores with an increasing number of PRP injections across all KL grades. Notably,

Table 2
Dose-response VAS scores.

Injection number	Mean improvement	Standard deviation	P-value	Effect size	95 % confidence interval
1	−7.7	13.49	$p < 0.0001$	−0.57	(−9.63, −5.78)
2	−12.4	15.18	$p < 0.0001$	−0.82	(−14.57, −10.24)
3	−17.6	17.07	$p < 0.0001$	−1.03	(−20.04, −15.17)
4	−22.25	18.21	$p < 0.0001$	−1.22	(−24.85, −19.65)
5	−25.26	19.72	$p < 0.0001$	−1.28	(−28.07, −22.45)
6	−23.06	23.4	$p < 0.0001$	−0.99	(−26.39, −19.72)

VAS, visual analogue scale.

Table 3
Statistical analysis results.

Pain reduction analysis results				
KL Grade	Mean Reduction	p-Value	Effect Size	95 % Confidence Interval
1	39.44	$p < 0.0001$	1.72	(28.19, 50.69)
2	36	$p < 0.0001$	1.33	(28.17, 43.83)
3	26.78	$p < 0.0001$	1.02	(20.82, 32.74)
4	20.71	$p = 0.0001$	0.79	(11.49, 29.93)
Functional improvement analysis results				
Time point	**Mean improvement**	**p-Value**	**Effect size**	**95 % confidence interval**
3 M	15.77	$p < 0.0001$	0.59	(12.8, 18.73)
6 M	18.87	$p < 0.0001$	0.67	(15.62, 22.13)
12 M	19.03	$p < 0.0001$	0.59	(14.99, 23.07)
24 M	17.4	$p < 0.0001$	0.55	(12.6, 22.21)
Structural change analysis results				
Time point	**Mean improvement**	**p-Value**	**Effect size**	**95 % confidence interval**
3 M	−0.54	$P = 0.0165$	−0.22	(-0.97, −0.10)
6 M	−1.3	$p < 0.0001$	−0.41	(-1.86, −0.78)
12 M	−1.77	$p < 0.0001$	−0.53	(-2.37, −1.17)
24 M	−2.24	$p < 0.0001$	−0.53	(-3.00, −1.48)
KOOS ADL analysis with 95 % confidence intervals				
Time point	**Mean improvement rate (%)**	**p-value**	**Effect size (Cohen's d)**	**95 % confidence interval**
3 M	15.77	$p < 0.0001$	0.59	(12.8, 18.73)
6 M	18.87	$p < 0.0001$	0.67	(15.62, 22.13)
12 M	19.03	$p < 0.0001$	0.59	(14.99, 23.07)
24 M	17.4	$p < 0.0001$	0.55	(12.6, 22.21)

KL, Kellgren–Lawrence; M, Months; KOOS, Knee Injury and Osteoarthritis Outcome Score; ADL, activity of daily living.

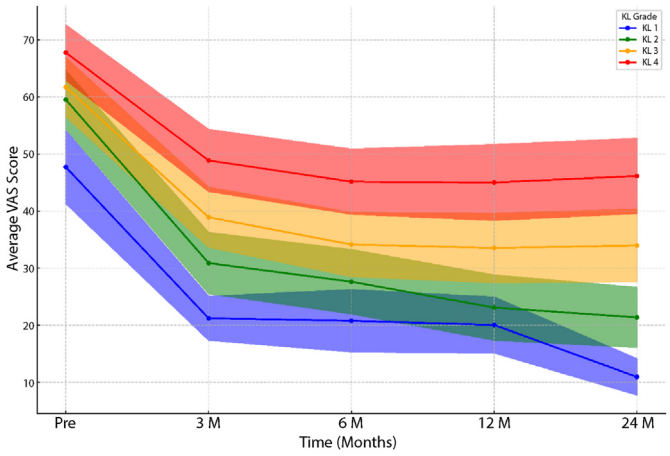

Fig. 2. Changes in VAS scores over time by KL grade. This figure depicts the longitudinal changes in the VAS scores, categorized by KL grades, throughout the study period. Each line corresponds to a specific KL grade, illustrating a marked reduction in perceived pain levels across all categories. These results underscore the overall effectiveness of PRP treatment in alleviating symptomatic knee pain associated with osteoarthritis.
Abbreviations: M: Months, VAS: Visual analog scale, KL: Kellgren–Lawrence classification, PRP: Platelet-rich plasma.

VAS scores were significantly reduced after the first PRP injection compared to the pre-treatment scores.

Changes by KL Grade: Across all KL grades (1–4), VAS scores decreased following PRP injections. Higher KL grades (from 1 to 4) showed higher initial VAS scores pre-treatment, but each subsequent PRP injection resulted in a notable reduction in scores.

Effect of the Number of PRP Administrations: The pain-alleviating effect of PRP injections was sustained from the first to sixth administrations, with more injections leading to more significant reductions in VAS scores. This effect was particularly prominent in KL grades 1 and 2, where the VAS scores were the lowest after the fifth and sixth PRP injections.

These findings indicate the efficacy of repeated PRP administrations in reducing pain levels across various severities of osteoarthritis, with the most pronounced effects observed after multiple injections.

3.6. Functional improvement and structural outcomes

Functional improvement was significant across various time points, as illustrated in Fig. 4. KOOS-ADL scores showed marked improvements, with an increase of 15.77 % at 3 months, 18.87 % at 6 months, 19.03 % at 12 months, and 17.4 % at 24 months, all with p-values <0.001. The effect sizes for these improvements were moderate, ranging from 0.55 to 0.67 (Cohen's d).

In terms of structural outcomes, Fig. 5 illustrates that BML scores were significantly reduced over the 24-month period. For KL grade 1 patients, BML scores decreased from 3.3 to 1.7, while KL grade 2 patients showed a reduction from 5.1 to 4.0. KL grade 3 patients experienced a decrease from 8.9 to 6.4, and KL grade 4 patients showed a reduction from 12.9 to 10.8. These findings suggest that PRP therapy provides substantial pain relief and functional improvement, particularly in early to moderate OA cases, with these effects sustained over time.

3.7. Reduction in synovial fluid production

An analysis of JF levels demonstrated significant reductions across various KL grades over time (Table 4). For KL grade 1, significant reductions were observed starting at 12 months and sustained through to 24 months ($p = 0.0004$ at 12 months, $p = 0.0002$ at 24 months). KL grade 2 patients showed consistent reductions from 12 months onward ($p < 0.0001$ at 12 months, $p = 0.0011$ at 24 months). In KL grade 3, reductions were observed at 6 and 12 months ($p = 0.0211$ at 6 months, $p = 0.0012$ at 12 months), but significance was not maintained at 24 months ($p = 0.1010$). KL grade 4 exhibited significant reductions at each time point, with notable significance at both 12 and 24 months ($p < 0.0001$ for both).

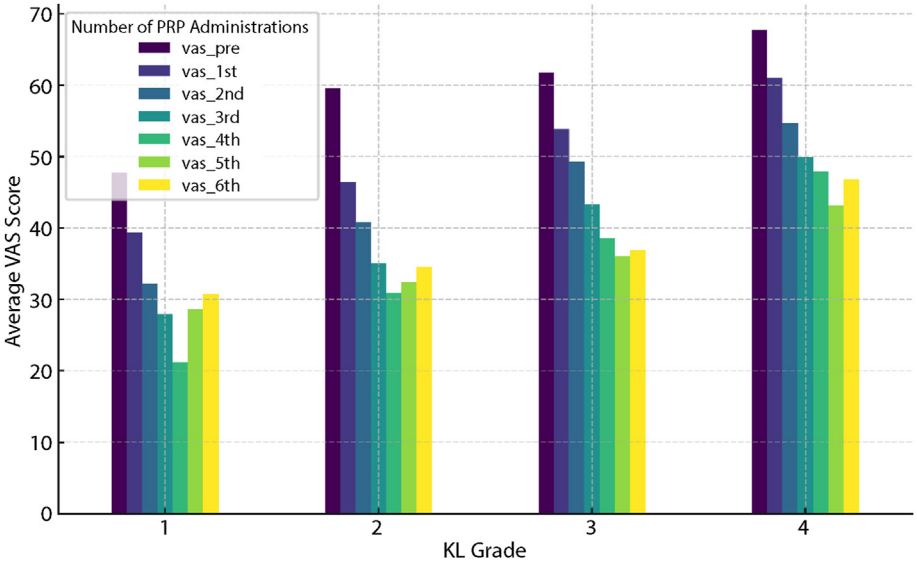

Fig. 3. Subgroup analysis of VAS improvements by KL grade and number of PRP administrations. This figure presents a detailed subgroup analysis showing the progression of VAS scores across different KL grades in relation to the number of PRP administrations. Each bar represents the average VAS score, with different colors corresponding to the number of PRP treatments received (from the initial to the sixth administration). The decreasing height of the bars across successive PRP administrations reflects the positive reduction in symptoms, indicating the cumulative effectiveness of repeated PRP injections. Abbreviations: VAS: Visual analog scale, KL: Kellgren–Lawrence classification, PRP: Platelet-rich plasma.

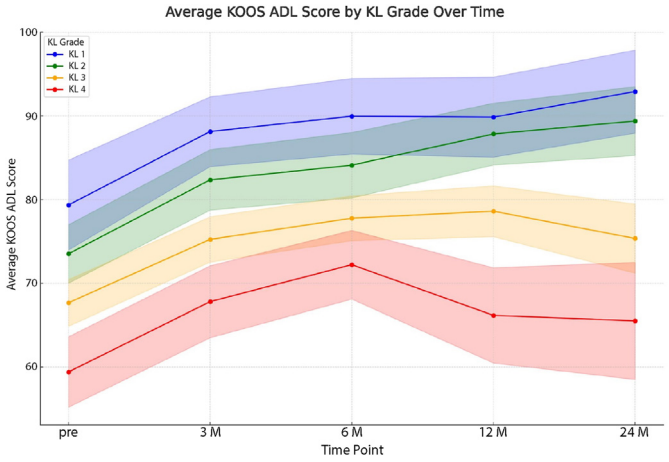

Fig. 4. Changes in KOOS ADL scores over time by KL grade. This graph shows the changes in KOOS for activities of daily living across different KL grades during the study period. This highlights the improvements in functional outcomes, particularly illustrating a dose–response relationship in which lower KL grades exhibit significant improvements, suggesting the adaptability and efficacy of PRP treatment in managing more severe cases of osteoarthritis. Abbreviations: KOOS: Knee Injury and Osteoarthritis Outcome Score, KL: Kellgren–Lawrence classification, PRP: Platelet-rich plasma.

3.8. Responder analysis

At the 24-month follow-up, 73 % (122 of 167) of participants met the OMERACT-OARSI threshold for responder status, indicating significant improvements in pain and functional scores. The highest response rate was observed among KL grade 1 patients, with 93.8 % (15 of 16) meeting the response criteria. For KL grade 2, 80.4 % (37 of 46) were classified as responders, while KL grade 3 saw a 68.9 % (51 of 74) response rate. The lowest response was in KL grade 4, with 61.3 % (19 of 31) responding to PRP therapy.

Further analysis indicated that response rates increased with the number of PRP injections. Patients who completed a full course of six injections exhibited the highest overall response rates, suggesting a dose-dependent relationship between the number of injections and outcomes. However, a notable plateau in the response rate was observed after the fifth injection, indicating limited additional benefit from further treatments. The optimal number of injections was found to be four, as this yielded the highest overall response rate (85.42 %). While early-stage OA patients (KL grades 1 and 2) benefited from fewer injections, those with advanced OA (KL grades 3 and 4) required additional injections for significant improvement, although the improvement rates were lower.

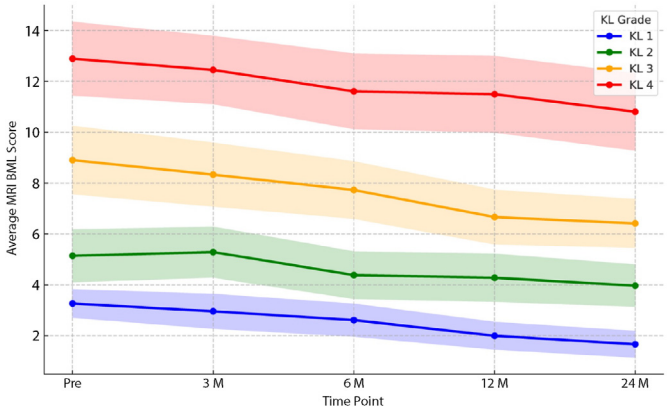

Fig. 5. Changes in MRI BML scores over time by KL grade. This figure illustrates the changes in MRI-detected BML scores, categorized by KL grades, over the course of the study. Each line represents a specific KL grade, showing a downward trend in BML scores over time. This reduction indicates structural improvements within the joint, providing objective evidence of the regenerative effects of PRP treatment, particularly in patients with higher grades of osteoarthritis. Abbreviations: BML: Bone marrow lesion, KL: Kellgren–Lawrence classification, MRI: Magnetic Resonance Imaging, PRP: Platelet-Rich Plasma.

3.9. Multivariate regression analysis

The multivariate regression analysis, as illustrated in Fig. 6, showed that several factors significantly influenced VAS changes over the 3-, 6-, 12-, and 24-month follow-ups. Female patients experienced smaller VAS changes compared to male patients, indicating less pain relief. Higher BMI was associated with larger VAS changes, suggesting greater pain relief with PRP therapy in individuals with higher BMI. Moreover, an increased number of PRP administrations correlated with larger VAS changes, highlighting a positive relationship between the frequency of PRP administration and pain relief. These findings underscore the considerable influence of gender, BMI, and PRP administration frequency on treatment outcomes for knee osteoarthritis.

4. Discussion

4.1. Novel protocol development

The paracrine and harmony theories emphasize the crucial role of growth factors and coordinated cellular actions in maintaining joint health [17]. The therapeutic effects of PRP peak within weeks of injection but tend to diminish over time without repeated doses [18]. To sustain these benefits, we have developed a protocol involving monthly PRP injections over 6 months, designed to maintain the intra-articular environment and support cartilage repair. By implementing this novel protocol, we aimed to optimize the intra-articular environment for cartilage regeneration and enhance therapeutic outcomes for patients with KOA.

This study seeks to fill a crucial gap in understanding PRP therapy, providing a clear, actionable framework for clinical settings and enhancing treatment efficacy for osteoarthritis.

4.2. Summary of the study

This clinical trial was conducted prospectively to investigate the optimal frequency of PRP injections for managing KOA. While categorized under treatment guidelines rather than pure research,

Table 4
Changes in average joint fluid volume following treatment at various time points across different KL grades.

Timepoint	Overall change (ml, p-value)	KL grade 1 (ml, p-value)	KL grade 2 (ml, p-value)	KL grade 3 (ml, p-value)	KL grade 4 (ml, p-value)
3 months	5.5 (0.0913)	2.8 (0.4899)	5.2 (0.9715)	5.4 (0.5249)	7.2 (0.1647)
6 months	4.6 (0.0026)	0.1 (0.1655)	3.8 (0.8757)	5.4 (0.0211)	5.5 (0.0066)
12 months	1.7 (0.0409)	0.8 (0.0004)	1.2 (<0.0001)	2.0 (0.0012)	2.1 (<0.0001)
24 months	1.4 (0.0903)	0.0 (0.0002)	0.2 (0.0011)	2.0 (0.1010)	2.4 (<0.0001)

KL, Kellgren–Lawrence.

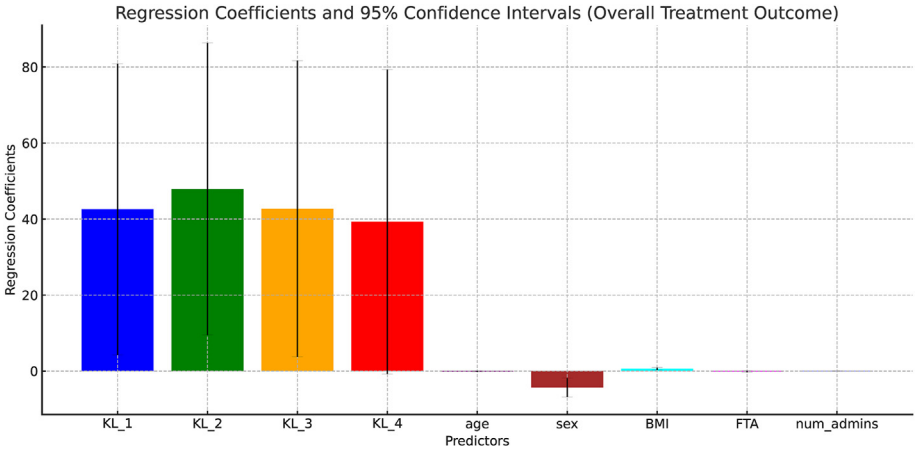

Fig. 6. Multivariate regression analysis of clinical predictor impact on PRP treatment efficacy for OA. This figure presents a comprehensive multivariate regression analysis assessing the impact of various clinical predictors on the efficacy of PRP treatments for osteoarthritis. The predictors include KL grades, age, sex, BMI, FTA, and the number of PRP administrations. Each bar represents the regression coefficient associated with a specific predictor, with error bars indicating the 95 % confidence intervals. Positive coefficients suggest that the predictor enhances treatment efficacy, while negative coefficients indicate a potential reduction in efficacy. This analysis helps quantify the influence of each factor on the overall treatment outcome. Abbreviations: OA: Osteoarthritis, PRP: Platelet-rich plasma, KL: Kellgren–Lawrence classification, BMI: Body mass index, FTA: Femorotibial angle.

it also incorporates a research-focused methodology. The study tracked 167 participants over a two-year period within a structured treatment protocol, gathering and analyzing data on PRP injection frequency, pain reduction, and functional improvement. Long-term effects on joint structure were evaluated through regular, protocol-defined MRI follow-ups, providing a robust framework supported by evidence for the clinical application of PRP in KOA management.

In contrast to certain unstandardized PRP treatments, this trial underscores the importance of a systematic, evidence-based approach. By offering clear guidelines on treatment frequency and administration, we aim to mitigate risks associated with arbitrary protocols, thereby enhancing both safety and efficacy for osteoarthritis patients.

4.3. Main findings

The study revealed that the optimal number of PRP treatments varies with the severity of osteoarthritis, as classified by the KL grading system. Patients with early-stage OA (KL grade 1) achieved the best outcomes with four PRP treatments, while those with more advanced OA (KL grades 3 and 4) required up to five treatments [19,20]. These findings were supported by statistical analyses, including linear mixed-effects models, which validated the optimal treatment thresholds across different KL grades.

4.4. Clinical implications of treatment frequency

The findings suggest that the therapeutic benefits of PRP, including significant pain reduction and functional improvement, plateau beyond the optimal number of treatments. This highlights the importance of a customized treatment plan that enhances efficacy while avoiding overtreatment and its associated complications. Clinicians can utilize these insights to maximize the regenerative potential of PRP and minimize unnecessary interventions, thereby improving patient outcomes and optimizing the use of resources. In Japanese outpatient settings, there is a general preference for prescribing NSAIDs patches while minimizing steroid use, particularly among regenerative medicine specialists. This reflects a treatment philosophy that aims to manage symptoms locally without systemic side effects. The PRP protocol examined in this study aligns with this approach, providing a tailored treatment regimen that optimizes the regenerative environment within the joint.

4.5. Statistical validation of optimal treatment numbers

The study employed robust statistical methods, including non-parametric techniques and linear mixed-effects models, to validate the optimal number of PRP treatments [21]. The LMM analysis, particularly, highlighted significant decreases in JF levels across treatment intervals, demonstrating the sustained impact of PRP on synovial fluid production. Significant improvements in joint function and pain reduction were observed at specific treatment thresholds, with the greatest effects seen up to the fifth injection [22]. These statistically significant findings ($p < 0.05$) provide a solid basis for recommending a maximum of four to five treatments, depending on the patient's baseline condition.

4.6. Integration into clinical practice

The optimized treatment frequencies derived from this study can standardize PRP therapy protocols, ensuring consistent and predictable patient outcomes [23]. Further research, particularly randomized controlled trials (RCTs), is essential to establish a definitive treatment regimen that can be widely adopted in orthopedic and regenerative medicine settings. Given the characteristics of Japan's insurance-based medical system and the common approach in outpatient care to favor NSAIDs patches and limit steroid use, this protocol offers a practical framework adaptable to clinical environments with similar treatment preferences. Further studies, especially RCTs, will help establish a widely applicable regimen that aligns with both global and local clinical standards.

4.7. Study limitations and future directions

This study's non-randomized design and focus on patients unresponsive to conventional treatments may limit the generalizability of the findings. Future studies should incorporate RCTs with more diverse patient demographics to better assess PRP's efficacy. Additionally, improvements in PRP preparation techniques and a deeper understanding of the biological mechanisms underlying PRP's effects are critical areas for future research [24].

Our recommendations include:

1. RCTs: Future studies should include randomized controlled trials comparing PRP treatments with standard care practices to provide higher-level evidence of efficacy and safety.
2. Longer follow-up periods: Extending the follow-up period beyond 24 months would help determine the long-term effects and sustainability of PRP treatments.
3. Broader patient populations: Including more diverse patient demographics in terms of age, sex, and comorbid conditions would help generalize the findings.
4. Mechanistic studies: Investigating the biological mechanisms by which PRP exerts its effects on knee joint tissues could lead to more targeted and effective treatment approaches.
5. Cost-effectiveness analysis: Given the increasing use of PRP, studies evaluating its cost-effectiveness compared to other treatment modalities are essential to support its broader adoption in clinical practice.

Our findings substantiate the efficacy of PRP therapy as a non-surgical intervention in the management of KOA, offering significant contributions to the development of treatment strategies in the field of regenerative medicine [25]. Further exploration and refinement of PRP applications are expected to improve clinical outcomes and advance orthopedic practice.

5. Conclusion

This study confirms the efficacy of autologous PRP therapy in managing KOA, demonstrating significant pain reduction, functional improvement, and structural benefits in the knee. The findings support a customized treatment regimen involving up to four to five PRP administrations based on individual patient profiles and disease severity. Our study contributes to the development of optimized treatment strategies in regenerative medicine, paving the way for improved clinical outcomes and advancements in orthopedic practice.

Author contributions

All aspects of this manuscript, from conception to the finished article, were the sole responsibility of Masahiko Kemmochi (kossmos@rainbow.plala.or.jp). The contributions include the following: Conception and design, Analysis and interpretation of the data, Drafting of the article, Critical revision of the article for important intellectual content, Final approval of the article, Provision of study materials or patients, Statistical expertise, Acquisition of funding, Administrative, technical, and logistic support, and Collection and assembly of data. Masahiko Kemmochi takes full responsibility for the integrity of the work as a whole.

Disclosures

The number of clinical trials is currently abolished, so it cannot be searched, but this number was active until March 31, 2022. At the 28th Standing Board Meeting held on January 11, the Japan Medical Association agreed to reorganize the “Drug Affairs Office” and organize the clinical trial promotion center project as a reorganization of the secretariat after April this year. Specifically, (1) the Research Division of the Clinical Trial Promotion Center will be abolished on March 31 this year, and the “Drug Affairs Countermeasures Office” will be reorganized into the “Medical Technology Division” on April 1, and (2) the Clinical Trial Promotion Center Promotion Division and General Affairs Department will be abolished on March 31 next year. As of April 1, the “Clinical Trial Promotion Office” will be newly established under the “Medical Technology Division”. The “Medical Affairs Countermeasures Office” was established under the Regional Medical Division in 2013 to address a wide range of pharmaceutical affairs and pharmaceutical affairs issues in a centralized manner.

Data Statement

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Declaration of Generative AI and AI-assisted technologies in the writing process

To prepare this manuscript, we employed generative artificial intelligence (AI) (ChatGPT 4o) technology to improve readability and language quality. The contributions of the AI were overseen and controlled by the human author to ensure accuracy and integrity in presenting the research findings. The AI output was carefully reviewed, edited, and verified to avoid incorrect, incomplete, or biased information. In line with Elsevier's AI policy for authors, AI technology was not listed as an author or co-author nor cited as an author. All intellectual responsibilities and tasks associated with research and manuscript preparation were performed solely by the listed human author.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

We thank the clinical staff and study participants for their dedication and contribution to this research. We would like to thank Editage (www.editage.jp) for English language editing.

References

[1] Selig DJ, Kress AT, Horton IM, Livezey JR, Sadik EJ, DeLuca JP. Pharmacokinetics, safety and efficacy of intra-articular non-steroidal anti-inflammatory drug injections for the treatment of osteoarthritis: a narrative review. J Clin Pharm Therapeut 2022;47:1122–33. https://doi.org/10.1111/jcpt.13669.
[2] Qi Y, Tang R, Shi Z, Feng G, Zhang W. Wnt5a/platelet-rich plasma synergistically inhibits IL-1β-induced inflammatory activity through NF-κB signaling pathway and prevents cartilage damage and promotes meniscus regeneration. J Tissue Eng Regen Med 2021;15:612–24. https://doi.org/10.1002/term.3198.
[3] Lee JS, Guo P, Klett K, Hall M, Sinha K, Ravuri S, et al. VEGF-attenuated platelet-rich plasma improves therapeutic effect on cartilage repair. Biomater Sci 2022;10:2172–81. https://doi.org/10.1039/d1bm01873f.
[4] Kobayashi Y, Saita Y, Nishio H, Ikeda H, Takazawa Y, Nagao M, et al. Leukocyte concentration and composition in platelet-rich plasma (PRP) influences the growth factor and protease concentrations. J Orthop Sci 2016;21:683–9. https://doi.org/10.1016/j.jos.2016.07.009.
[5] Kenmochi M. Clinical outcomes following injections of leukocyte-rich platelet-rich plasma in osteoarthritis patients. J Orthop 2020;18:143–9. https://doi.org/10.1016/j.jor.2019.11.041.
[6] Filardo G, Previtali D, Napoli F, Candrian C, Zaffagnini S, Grassi A. PRP injections for the treatment of knee osteoarthritis: a meta-analysis of randomized controlled trials. Cartilage 2021;13:364S. https://doi.org/10.1177/1947603520931170. 75S.
[7] Oeding JF, Varady NH, Fearington FW, Pareek A, Strickland SM, Nwachukwu BU, et al. Platelet-rich plasma versus alternative injections for osteoarthritis of the knee: a systematic review and statistical fragility index-

based meta-analysis of randomized controlled trials. Am J Sports Med 2024;29:3147–60. https://doi.org/10.1177/03635465231224463.
[8] Kellgren JH, Lawrence JS. Radiological assessment of osteo-arthrosis. Ann Rheum Dis 1957;16:494–502. https://doi.org/10.1136/ard.16.4.494.
[9] Pham T, van der Heijde D, Altman RD, Anderson JJ, Bellamy N, Hochberg M, et al. OMERACT-OARSI initiative: osteoarthritis Research Society International set of responder criteria for osteoarthritis clinical trials revisited. Osteoarthr Cartil 2004;12:389–99. https://doi.org/10.1016/j.joca.2004.02.001.
[10] Murray IR, Geeslin AG, Goudie EB, Petrigliano FA, LaPrade RF. Minimum information for studies evaluating biologics in orthopaedics (MIBO): platelet-rich plasma and mesenchymal stem cells. J Bone Joint Surg Am 2017;99:809–19. https://doi.org/10.2106/JBJS.16.00793.
[11] Shin HS, Woo HM, Kang BJ. Optimisation of a double-centrifugation method for preparation of canine platelet-rich plasma. BMC Vet Res 2017;13:198. https://doi.org/10.1186/s12917-017-1123-3.
[12] Breivik H, Borchgrevink PC, Allen SM, Rosseland LA, Romundstad L, Hals EK, et al. Assessment of pain. Br J Anaesth 2008;101:17–24. https://doi.org/10.1093/bja/aen103.
[13] Roos EM, Roos HP, Lohmander LS, Ekdahl C, Beynnon BD. Knee injury and osteoarthritis outcome score (KOOS) – development of a self-administered outcome measure. J Orthop Sports Phys Ther 1998;28:88–96. https://doi.org/10.2519/jospt.1998.28.2.88.
[14] Hunter DJ, Guermazi A, Lo GH, Grainger AJ, Conaghan PG, Boudreau RM, et al. Evolution of semi-quantitative whole joint assessment of knee OA: MOAKS (MRI Osteoarthritis Knee Score). Osteoarthr Cartil 2011;19:990–1002. https://doi.org/10.1016/j.joca.2011.05.004.
[15] Bates D, Mächler M, Bolker B, Walker S. Fitting linear mixed-effects models using lme4. J Stat Software 2015;67:1–48.
[16] Wickham H. ggplot2: elegant graphics for data analysis. New York: Springer-Verlag; 2016.
[17] Ratajczak MZ, Kucia M, Jadczyk T, Greco NJ, Wojakowski W, Tendera M, et al. Pivotal role of paracrine effects in stem cell therapies in regenerative medicine: can we translate stem cell-secreted paracrine factors and microvesicles into better therapeutic strategies? Leukemia 2012;26:1166–73. https://doi.org/10.1038/leu.2011.389.
[18] Kobayashi E, Flückiger L, Fujioka-Kobayashi M, Sawada K, Sculean A, Schaller B, et al. Comparative release of growth factors from PRP, PRF, and advanced-PRF. Clin Oral Invest 2016;20:2353–60. https://doi.org/10.1007/s00784-016-1719-1.
[19] Yurtbay A, Say F, Çinka H, Ersoy A. Multiple platelet-rich plasma injections are superior to single PRP injections or saline in osteoarthritis of the knee: the 2-year results of a randomized, double-blind, placebo-controlled clinical trial. Arch Orthop Trauma Surg 2022;142:2755–68. https://doi.org/10.1007/s00402-021-04230-2.
[20] Zhuang W, Li T, Li Y, Zhang Y, Gao J, Wang X, et al. The varying clinical effectiveness of single, three and five intraarticular injections of platelet-rich plasma in knee osteoarthritis. J Orthop Surg Res 2024;19:284. https://doi.org/10.1186/s13018-024-04736-6.
[21] Khoury MA, Chamari K, Tabben M, Alkhelaifi K, Papacostas E, Marín Fermín T, et al. Knee osteoarthritis: clinical and MRI outcomes after multiple intra-articular injections with expanded autologous adipose-derived stromal cells or platelet-rich plasma. Cartilage 2023;14:433–44. https://doi.org/10.1177/19476035231166127.
[22] Patel S, Mishra NP, Chouhan DK, Nahar U, Dhillon MS. Chondroprotective effects of multiple PRP injections in osteoarthritis by apoptosis regulation and increased aggrecan synthesis- immunohistochemistry based Guinea pig study. J Clin Orthop Trauma 2022;25:101762. https://doi.org/10.1016/j.jcot.2022.101762.
[23] Lewis E, Merghani K, Robertson I, Mulford J, Prentice B, Mathew R, et al. The effectiveness of leucocyte-poor platelet-rich plasma injections on symptomatic early osteoarthritis of the knee: the PEAK randomized controlled trial. Bone Joint Lett J 2022;104–B:663–71. https://doi.org/10.1302/0301-620X.104B6.BJJ-2021-1109.R2.
[24] Kon E, Di Matteo B, Delgado D, Cole BJ, Dorotei A, Dragoo JL, et al. Platelet-rich plasma for the treatment of knee osteoarthritis: an expert opinion and proposal for a novel classification and coding system. Expet Opin Biol Ther 2020;20:1447–60. https://doi.org/10.1080/14712598.2020.1798925.
[25] Saita Y, Kobayashi Y, Nishio H, Wakayama T, Fukusato S, Uchino S, et al. Predictors of effectiveness of platelet-rich plasma therapy for knee osteoarthritis: a retrospective cohort study. J Clin Med 2021;10:4514. https://doi.org/10.3390/jcm10194514.