Clinical Response After Treatment of Knee Osteoarthritis With a Standardized, Closed-System, Low-Cost Platelet-Rich Plasma Product

1-Year Outcomes

Judit Fernández-Fuertes,*†‡ MD, PhD, Tamara Arias-Fernández,†§ MD, Andrea Acebes-Huerta,† PhD, Marlene Álvarez-Rico,‡ MD, PhD, and Laura Gutiérrez,†|| PhD

Investigation performed at Hospital Universitario de Cabueñes (CAHU), Gijón, Asturias, Spain

Background: Intra-articular infiltration of platelet-rich plasma (PRP) is an alternative therapeutic option to classic hyaluronic acid for the treatment of symptomatic knee osteoarthritis (KOA). However, variation in preparation methods and quality assessment of PRP makes the study of its real clinical efficacy difficult.

Purpose: To (1) evaluate the clinical efficacy of a characterized PRP product prepared in a standardized manner and in a closed-system for the treatment of KOA and to (2) evaluate the association of the clinical response to PRP-related variables.

Study Design: Case series; Level of evidence, 4.

Methods: We recruited 130 patients with nonoperative KOA and evaluated them for 1 year. PRP was prepared from a donation of autologous blood, obtaining 3 aliquots of approximately 10mL of product, which were frozen, allowing platelet disruption, platelet factor release, and long-term storage, until administration. Patients were treated 3 consecutive times every 4 weeks with an intra-articular PRP knee injection under sterile conditions. Complete blood count was performed on the whole-blood sample and the processed PRP before freezing it, for product quality assessment. Patients were assessed using the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) and basic satisfaction scale at 3 months, 6 months, and 1 year after intervention.

Results: Quality assessment confirmed a leukocyte-poor PRP product (white blood cell count, 0.09 ± 0.09 × 10^9/L) with a high platelet purity (platelet count, 630.86 ± 191.75 × 10^9/L). WOMAC scores improved, and basic satisfaction was achieved in 70% of patients. No adverse events were reported. No correlations were observed between PRP quality parameters and clinical results. PRP complete treatment production costs were €108/US$125 (€36/US$41.6 per injection).

Conclusion: This standardized PRP production method resulted in improved WOMAC scores at 1 year postoperatively in 70% of patients with KOA. This technique was safe and affordable and ensured consecutive infiltrations with the same product to each patient.

Keywords: infiltration; knee; osteoarthritis; platelet-rich plasma (PRP); regenerative medicine

The lifetime risk of developing knee osteoarthritis (KOA) is estimated to be around 14% in the United States, with prevalence of at least some degree of KOA in almost 40% of patients older than 45 years. In Europe, the KOA prevalence in the general population ranges depending on the study, and in Spain it is around 14% (range of 95% CI, 12.66%-15.11%). Platelet-rich plasma (PRP) injection is a well-known nonsurgical therapeutic option indicated for the management of symptomatic KOA. PRP contains a cocktail of platelet-derived growth factors, which are crucial in tissue regeneration. They contribute to the regulation of cartilage anabolism and articular homeostasis, including menisci and synovia. Furthermore, according to in vitro studies, PRP appears to stimulate endogenous hyaluronic acid (HA) production, inducing chondrocyte and mesenchymal stem cell proliferation, and proteoglycan and type 2 collagen deposition.

There is an important effort worldwide to establish therapeutic strategies to modify the natural history of KOA, increasing the interest in orthobiologic products. PRP has
demonstrated its supremacy in comparison with placebo and tends to be considered superior to HA in several clinical trials, being considered by Dhillon et al as “the best option available that could modify the disease process in early KOA.” However, most studies addressing the efficacy of PRP in orthobiologic medicine highlight the same conclusion: evidence is limited by the substantial heterogeneity among PRP preparation methods, processing, administration form, storage, or reporting of quality parameters. In fact, a 2020 evidence-based-guideline published by the American College of Rheumatology and the Arthritis Foundation strongly discourages PRP treatment in patients with KOA, based on “the heterogeneity and lack of standardization in available preparations of PRP, as well as techniques used, making it difficult to identify exactly what is being injected.” It is calculated that only 5% to 6% of reports specify the type and cellular composition of the PRP used. In addition, the high fee burden to patients is recognized, as there is no consensus or regulation on production costs, and many private clinics use expensive kits for its production.

There have been several attempts to categorize PRP according to various variables (the preparation method; fibrin content; the presence of white blood cells [WBCs] or red blood cells [RBCs]; final volume; platelet concentration or enrichment; activation method, including freezing and delivery method), with more or less complexity. Gradually, this field is improving thanks to the crosstalk of various disciplines (traumatologists and hematologists, among others), and the need for standardization has been recognized by the scientific community. We have recently reviewed this issue and have proposed a tentative nomenclature system to make possible comparison among groups. In addition, an expert consensus with specifications for the Minimum Information for Studies Evaluating Biologics in Orthopedics (MIBO) for PRP has been published that should be reported by clinical studies involved in this field.

In Spain, PRP has been considered “medicine for human use” by the Spanish Agency of Medicines and Medical Devices since 2013 (V1/23052013). Since 2018, our certified local blood bank has implemented a closed-system PRP standardized preparation method, which allows the production of autologous PRP at low cost (€36/US$41.6 per injection). This product ensures safety (closed system and leuko reduced) and an optimal platelet enrichment (approximately 2- to 3-fold). Furthermore, it is stored frozen in aliquots, which allows the release of platelet factors in the plasma fraction (through freezing-induced platelet lysis) and the autologous use of the same product in consecutive infiltrations per patient (reproducibility). In the present study, we evaluated the clinical efficacy of a characterized PRP product prepared in a standardized manner and in a closed system as KOA treatment, and the association of the clinical response to PRP-related variables in 130 patients, during a 1-year follow-up period.

**METHODS**

**Patients**

Patients with symptomatic KOA without surgery indication were recruited during their orthopaedic clinic visit at our hospital from August 2018 to January 2020. The study was approved by our local hospital ethical committee, and all patients participated upon informed consent. The protocol is registered in a specific website to assess (https://www.precis-2.org), with its tool, the real grade of pragmatism of this study (Figure 1).

Study inclusion criteria were symptomatic KOA (femorotibial and/or femoropatellar), regardless of the Kellgren-Lawrence (KL) radiological grade, without response to conservative management (nonsteroidal anti-inflammatory drugs [NSAIDs], physical rehabilitation, weight loss, or other infiltrations such as corticoids or HA) during at least 6 months and with no indication of knee surgery (based on medical criteria or patient rejection), and signed informed consent. Exclusion criteria were corticosteroid infiltrations 6 weeks before or HA less than 1 year before PRP infiltration, loss of follow-up during the study, severe effusion of the knee, knee surgery candidates, serious heart disease, hemostasis disorders, severe autoimmune disease, history of neoplasia at the site of PRP application, hematological alteration in recent blood analysis, positivity to serological tests, or genomic detection of hepatitis B and C viruses, human immunodeficiency virus, or syphilis.

**Study Design and Intervention**

This was a single-center and prospective interventional case series with pretest-posttest design, conducted in accordance with the CONSORT (Consolidated Standards of Reporting Trials) guidelines (extension for pragmatic...
trials). It was performed at our hospital’s orthopaedics department outpatient clinic, and all patients were recruited during the daily clinical practice of 2 orthopaedic consultants (J.F.F., M.A.R.), after clinical and radiological evaluation. Full weightbearing knee radiographs were obtained the day of recruitment (around 3 months before the first injection). Patients were considered for the study after assessment at the hematology department outpatient clinic, where the autologous blood harvesting (150mL) took place.

Blood was processed at the local blood bank to obtain the 3 PRP aliquots of around 10mL for autologous use as described previously. In brief, donated blood underwent 2 rounds of differential centrifugation in order to separate the PRP fraction, using the infrastructure of a certified blood bank, which allows the processing to be completed within a closed system. Aliquots were stored frozen at −40°C until use. Complete blood counts (CBCs) were performed on the donated blood and the PRP prior to freezing. All quality-control parameters were displayed (including labeled information about patient identification, as it is for autologous use, blood group, platelet count, serological test results, and blood screening by nucleic acid testing) under local guidelines, which accomplished Good Manufacturing Practices. Patients were treated in the same orthopaedic outpatient clinic, proceeding knee intra-articular injection under sterile conditions, through superolateral approach, of a thawed 10mL aliquot each. The infiltration scheme consisted of 3 consecutive infiltrations, every 4 weeks. The study design and workflow are shown in Figure 2. A specific rehabilitation protocol was not applied to patients. Patients were allowed to continue with mild analgesics such as acetaminophen (not NSAIDs).

Data and Outcomes Assessed

The following information was registered from each patient: sex, age, body mass index (BMI), knee side, labor disputes, and comorbidities such as diabetes mellitus, dyslipidemia, thyroid disease, rheumatologic conditions, and mental disease.

CBCs from each blood harvesting and processed PRP were obtained using a Sysmex XS-1000i hematocounter. From the harvested whole-blood CBC we used the platelet and WBC counts, and from the PRP CBC we used the platelet, WBC, and RBC counts.

The following PRP quality assessment parameters were calculated as previously described: platelet enrichment, dose of injected platelets, platelet and leukocyte efficiency, purity (percentage of total cells) in platelets, WBC, and RBC.

Patients were prospectively assessed for clinical response at baseline, and at 3-month, 6-month, and 1-year follow-up after the first injection. The primary outcome measure was the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) score (through its validated Spanish version), and cellular parameters were measured for PRP characterization. We also stratified patients according to baseline WOMAC scores (low, <35.3; medium, 35.4-51.4; and high, >51.5), as suggested by Tubach et al.

As a secondary outcome measure, patient satisfaction was surveyed by a simple categorical scale (none/low/ enough/high). During the coronavirus disease 2019 (COVID-19) alarm state, these data were collected by telephone interview. The KL classification system was used to assess radiographic KOA. In addition, we noted any adverse events, such as knee septic arthritis, knee pain...
not controlled with usual analgesic, and deep venous thrombosis.

Statistical Analysis

The sample size for this study was calculated based on the mean prevalence of KOA in Spain (around 10%) and our city population (300,000), using a 95% CI, a 6% precision, and an expected proportion of loss to follow-up of 10%. We calculated the final sample size needed to avoid sampling errors (adjusted for losses) was 109 patients. The Kolmogorov-Smirnov or chi-square test were used to assess normality. Paired-samples or independent Student t test, Mann-Whitney U test, paired-samples Wilcoxon test, analysis of variance, and Kruskal Wallis H test were employed to determine statistical significance. Correlation for quantitative data was calculated by Pearson or Spearman coefficient. We stratified in subgroups related with age and sex. All data were analyzed using SPSS (Version 16.0). Statistical significance was considered at $P < .05$.

RESULTS

Demographic Data

From the 130 patients included in the study, 60 were men (46.2%) and 70 were women (53.8%), with a mean age of 63.04 ± 10.7 years (range, 40-90 years) and median BMI of 28.37 ± 3.98 (range, 21.2-41.6). The right knee was treated in 73 cases (56.2%) and the left knee in 57 cases (43.8%). Regarding comorbidities, 13 (10%) had diabetes mellitus, 30 (23.1%) had dyslipidemia, 26 (20%) had thyroid pathologies, 58 (44.6%) had history of mental disease (depression or anxiety), and 25 (19.2%) had other rheumatologic conditions (rheumatoid arthritis, gout, polymyalgia rheumatica); 28 patients (21.5%) were involved in labor disputes.

The most frequent radiographic osteoarthritis grade at study enrollment was KL grade 2 (53 cases; 40.8%), followed by grade 3 (39 cases; 30%), grade 1 (22 cases; 17%), and grade 4 (16 cases; 12.2%).

Product Characterization

Our PRP production method is summarized in Table 1.43 CBC analysis of whole-blood samples showed a mean platelet count of $232.97 ± 59.51 \times 10^9/L$ (range, 101-434 $\times 10^9/L$) and a mean WBC count of $6.05 ± 1.50 \times 10^9/L$ (range, 3.72-12.19 $\times 10^9/L$).

PRP cytological and quality characteristics are shown in Table 2 and Supplemental Table S1.
baseline WBC (based on the PAW [Platelets, Activation, White Cells] classification\textsuperscript{12}), ACA (very high dose of platelets, >90\% efficiency of platelet recovery rate, very pure PRP, based on the DEPA (Dose Efficiency Purity Activation) classification\textsuperscript{36}, frozen-thawed preparation, platelet count less than 900 × 10^9/L obtained by gravitational centrifugation techniques based on the International Society on Thrombosis and Haemostasis classification\textsuperscript{24}, and PRP obtained from whole-blood donation that has been frozen-thawed once before application based on the classification by Acebes-Huerta et al\textsuperscript{1}.

Clinical Results

The baseline and follow-up scores for the WOMAC global, WOMAC subscales, and baseline WOMAC categories are shown in Table 3 and Supplemental Table S1.

Differences in WOMAC scores between baseline and 3-month, 6-month, and 1-year follow-ups are shown in Table 4. Statistical analysis revealed significant improvement at all time points studied versus baseline. Improvement was observed already at 3 months postoperatively and was maintained throughout the complete follow-up period (Table 4 and Figure 3). Similar results were seen across all WOMAC subscales, with only 1 particularity: WOMAC Stiffness improved further between 3- and 6-month follow-up ($P = .03$).

When considering the clinical significance of the results, the difference in WOMAC scores between baseline and 1-year follow-up represented a reduction of 25.72\% ± 46.04\%, which was considered clinically relevant according to Hmamouchi et al\textsuperscript{25} (minimal clinically importance difference [MCID] >16\%). The mean percentage reduction on the WOMAC subscales was 28.42\% ± 47.24\% for WOMAC Pain and 24.66\% ± 48.36\% for WOMAC Function, with a median (±SD) percentage reduction of 33.33\% ± 87.4\% for WOMAC Stiffness.

When stratified by WOMAC category, improvement from baseline was clinically significant according to Tubach et al\textsuperscript{55} at 1-year follow-up in all 3 categories (mean difference, 5.81 ± 14.29, $P = .01$ for low WOMAC; 14.25 ± 19.04, $P = .0001$ for medium WOMAC; and 17.11 ± 18.61, $P = .0001$ for high WOMAC). Furthermore, the MCID was achieved in the low WOMAC and medium WOMAC categories, according to data and ranges reported by Tubach et al. Patients in the high WOMAC group did not reach the proposed MCID (20.4). Nevertheless, MCID was achieved on the global WOMAC (12.32 ± 17.95), according to the same authors (MCID, 9.1) (Table 4 and Figure 3)\textsuperscript{55}.

Patient satisfaction at 1-year follow-up was “none” in 3.8\% of patients, “low” in 25.4\%, “enough” in 27.7\%, and “high” in 43.1\%. No adverse events were reported except, occasionally, local pain due to the infiltration procedure, which was treated with usual analgesics (eg, acetaminophen).

Correlation of Age, Sex, and PRP Quality With Clinical Response

We did not find any correlation between age and clinical results (WOMAC pre- vs 1-year posttreatment, $P = .16$; satisfaction at 1 year, $P = .26$) or PRP quality parameters

\begin{table}
\centering
\caption{PRP Complete Blood Count and Calculated Quality Variables\textsuperscript{a}}
\begin{tabular}{lll}
\hline
PRP Cell Type & Mean ± SD & Median (range) \\
\hline
Platelets (×10^9/L) & 630.86 ± 191.75 & 603.00 (280-1155) \\
WBC (×10^9/L) & 0.09 ± 0.09 & 0.06 (0.01-0.51) \\
RBC (×10^9/L) & 22.48 ± 14.49 & 20.00 (0.00-70.00) \\
Platelet enrichment factor, fold increase & 2.75 ± 0.65 & 2.72 (1.09-4.64) \\
Platelet dose (×10^9) & 6.31 ± 1.92 & 6.03 (2.80-11.55) \\
Platelet capture efficiency (%) & 54.94 ± 12.99 & 54.49 (21.81-92.87) \\
Leukocyte-reducing efficiency (%) & 99.69 ± 0.35 & 99.80 (97.51-99.97) \\
Purity (% relative to platelets) & 96.64 ± 1.68 & 96.87 (91.92-100) \\
Relative composition in WBC (%) & 0.01 ± 0.01 & 0.01 (0.002-0.10) \\
Relative composition in RBC (%) & 3.35 ± 1.68 & 3.11 (0.00-8.05) \\
\hline
\end{tabular}
\textsuperscript{a}PRP, platelet-rich plasma; RBC, red blood cell; WBC, white blood cell.
\end{table}

\begin{table}
\centering
\caption{WOMAC Scores at Baseline and 3-Month, 6-Month, and 1-Year Follow-up\textsuperscript{a}}
\begin{tabular}{lccccc}
\hline
Variable & Baseline & 3-Month Follow-up & 6-Month Follow-up & 1-y Follow-up \\
\hline
WOMAC global & 44.2 ± 17.2 & 31.8 ± 20.6 & 31.7 ± 21.2 & 31.9 ± 20.8 \\
WOMAC subscale & & & & \\
Pain & 9.1 ± 3.5 & 6 ± 4.2 & 6 ± 4.1 & 6.1 ± 4.3 \\
Stiffness & 3 ± 1.7 & 3 ± 1.9 & 2 ± 2.9 & 2 ± 2.9 \\
Function & 31.4 ± 12.9 & 22.9 ± 14.9 & 22.9 ± 15.4 & 22.8 ± 14.9 \\
WOMAC baseline score\textsuperscript{b} & & & & \\
Low & 26.6 ± 7.4 & 21.1 ± 14.1 & 21 ± 14.6 & 20.8 ± 13.8 \\
Medium & 42.4 ± 4.4 & 27.6 ± 17.5 & 27.8 ± 19.6 & 28.2 ± 18.8 \\
High & 64.4 ± 10.2 & 47.2 ± 20.3 & 47.1 ± 20.1 & 47.3 ± 20.1 \\
\hline
\end{tabular}
\textsuperscript{a}Data are presented as mean ± SD. WOMAC, Western Ontario and McMaster Universities Osteoarthritis Index. \\
\textsuperscript{b}Low, <35.3; medium, 35.4-51.4; high, >51.5.\textsuperscript{55}
\end{table}
TABLE 4
Difference in WOMAC Scores Between Follow-up Time Points\textsuperscript{a}

| WOMAC Global and Subscales | Mean Difference | \textit{P} | WOMAC Category | Mean Difference | \textit{P} |
|-----------------------------|-----------------|----------|----------------|-----------------|----------|
| WOMAC Baseline–3 months     | 12.45 ± 17.16   | <.0001  | Low            | 5.34 ± 13.55    | .01      |
| WOMAC Baseline–6 months     | 12.43 ± 17.84   | <.0001  | Baseline–3 months | 5.61 ± 14.56    | .01      |
| WOMAC Baseline–1 y          | 12.32 ± 17.95   | <.0001  | Baseline–6 months | 5.81 ± 14.29    | .01      |
| WOMAC 3 months–6 months     | -0.08 ± 8.39    | .9      | 3 months–6 months | -0.07 ± 6.44    | .94      |
| WOMAC 3 months–1 y          | -0.2 ± 10.28    | .81     | 3 months–1 y    | 0.09 ± 5.78     | .91      |
| WOMAC 6 months–1 y          | -0.1 ± 7.01     | .86     | 6 months–1 y    | 0.2 ± 4.64      | .77      |
| WOMAC Pain Baseline–3 months | 3.03 ± 3.89   | <.0001  | Baseline–3 months | 14.84 ± 17.73   | <.0001  |
| WOMAC Pain Baseline–6 months | 3.01 ± 4.01   | <.0001  | Baseline–6 months | 14.61 ± 19.62   | <.0001  |
| WOMAC Pain Baseline–1 y     | 2.91 ± 4.15     | <.0001  | Baseline–1 y    | 14.25 ± 19.04   | <.0001  |
| WOMAC Pain 3 months–6 months | -0.02 ± 2.44   | .91     | 3 months–6 months | -0.22 ± 8.43    | .85      |
| WOMAC Pain 3 months–1 y     | -0.12 ± 2.84    | .62     | 3 months–1 y    | -0.59 ± 8.92    | .66      |
| WOMAC Pain 6 months–1 y     | -0.1 ± 1.57     | .47     | 6 months–1 y    | -0.36 ± 5.4     | .65      |
| WOMAC Stiffness Baseline–3 months | Z = -4.38 | .0001  | Baseline–3 months | 17.23 ± 18.75   | <.0001  |
| WOMAC Stiffness Baseline–6 months | Z = -5.36 | <.0001 | Baseline–6 months | 17.28 ± 17.2    | <.0001  |
| WOMAC Stiffness Baseline–1 y | Z = -4.95     | <.0001  | Baseline–1 y    | 17.11 ± 18.61   | <.0001  |
| WOMAC Stiffness 3 months–6 months | Z = -2.14 | .03     | 3 months–6 months | 0.04 ± 10.13    | .97      |
| WOMAC Stiffness 3 months–1 y | Z = -1.31     | .18     | 3 months–1 y    | -0.11 ± 14.57   | .95      |
| WOMAC Stiffness 6 months–1 y | Z = -0.97     | .32     | 6 months–1 y    | -0.16 ± 10.06   | .91      |
| WOMAC Function Baseline–3 months | 8.63 ± 12.28  | <.0001  | Baseline–3 months | 17.23 ± 18.75   | <.0001  |
| WOMAC Function Baseline–6 months | 8.52 ± 12.73  | <.0001  | Baseline–6 months | 17.28 ± 17.2    | <.0001  |
| WOMAC Function Baseline–1 y | 8.6 ± 12.82    | <.0001  | Baseline–1 y    | 17.11 ± 18.61   | <.0001  |
| WOMAC Function 3 months–6 months | -0.14 ± 6.01  | .79     | 3 months–6 months | -0.22 ± 8.43    | .85      |
| WOMAC Function 3 months–1 y | -0.06 ± 7.5    | .92     | 3 months–1 y    | -0.59 ± 8.92    | .66      |
| WOMAC Function 6 months–1 y | 0.07 ± 5.25    | .86     | 6 months–1 y    | -0.36 ± 5.4     | .65      |

\textsuperscript{a}Data are presented as mean ± SD unless otherwise indicated. Bold \textit{P} values indicate statistically significant difference between time points (\textit{P} < .05). WOMAC, Western Ontario and McMaster Universities Osteoarthritis Index.

\textsuperscript{b}Wilcoxon test (Z statistic) was used for WOMAC Stiffness (nonparametric value).

(dose, \textit{P} = .13; enrichment, \textit{P} = .36; efficiency, \textit{P} = .26; purity, \textit{P} = .53), even when age was stratified into <65 and ≥65 years (clinical results: WOMAC pre- vs 1-year post-treatment, \textit{P} = .64; satisfaction at 1-year, \textit{P} = .06; PRP quality:dose, \textit{P} = .39; enrichment, \textit{P} = .39; efficiency, \textit{P} = .41; purity, \textit{P} = .58). Sex was also not correlated with clinical results (WOMAC score pre- vs 1-year posttreatment, \textit{P} = .62; satisfaction at 1 year, \textit{P} = .31), or PRP quality parameters (dose, \textit{P} = .07; enrichment, \textit{P} = .53; efficiency, \textit{P} = .40; purity, \textit{P} = .07). Finally, no significant correlations were found between PRP quality parameters (dose, efficiency, enrichment, or purity) and initial WOMAC score (\textit{P} = .38, \textit{P} = .78, \textit{P} = .20, and \textit{P} = .36, respectively), WOMAC at 1-year follow-up (\textit{P} = .66, \textit{P} = .90, \textit{P} = .62, and \textit{P} = .76, respectively), WOMAC pre- versus 1-year posttreatment (\textit{P} = .72, \textit{P} = .69, \textit{P} = .52, and \textit{P} = .60, respectively) or satisfaction at 1-year follow-up (\textit{P} = .96, \textit{P} = .21, \textit{P} = .21, and \textit{P} = .19, respectively) (Figure 4).

DISCUSSION

This pragmatic and prospective interventional pretest-posttest study evaluates the clinical efficacy in the treatment of KOA of a PRP product prepared using a standardized method, which is safe (closed system), affordable, and reproducible. Of the total patients recruited, 130 completed 1-year follow-up. We considered a treatment regime of 3 infiltrations every 4 weeks, as previous studies support that only multiple injections sustain anti-inflammatory effects in the long term compared with single injections in KOA.\textsuperscript{9,21,56}

Clinical Response

We found statistically significant improvement in WOMAC scores at all time points of observation (3-month, 6-month, and 1-year follow-up) compared with baseline (Figure 2 and Table 3). In addition, our data revealed that the improvement is achieved already at 3 months and is maintained, at least, through the 1 year follow-up, coinciding with published outcomes in recent level 1 evidence randomized controlled trials.\textsuperscript{31,44,47} Clinical response is not influenced by age or sex in our study; however, there are discrepancies regarding this issue.\textsuperscript{7,18,31,45,48} When analyzing the results of WOMAC subscales, we noted the same results, besides an improvement in stiffness from the 3-month to 6-month follow-up. We considered a treatment regime of 3 infiltrations every 4 weeks, as previous studies support that only multiple injections sustain anti-inflammatory effects in the long term compared with single injections in KOA.\textsuperscript{9,21,56}
Furthermore, although patient satisfaction is not reported in many published studies, in our study, satisfaction at 1-year follow-up was rated as “enough” or “high” by 70% of the patients.

MCID is also an important aspect to consider regarding the assessment of clinical response and significance. MCID for global WOMAC score data was achieved, according to both Hmamouchi et al.25 (21% vs 16%) and Tubach et al.55 (12.32 vs 9.1). Besides, and according to the latter author, WOMAC categories may associate with the clinical response to treatment because, as hypothesized, the higher the baseline score, the better the improvement.55 Supporting this notion, WOMAC score differences showed significant improvement in all categories (high, medium, low). Interestingly, MCID was achieved in low and medium WOMAC score group (5.81 vs 5.3 and 12.83 vs 11.8), but not in the high score group (17.11 vs 20.4). Of note, these latter 2 studies calculated these indicators based on treatment of KOA with NSAIDs, not PRP, and the follow-up period was 4 to 6 weeks, rather than 1 year follow-up. Further studies of this indicator involving PRP treatment and longer follow-up should be done to validate the results.

**PRP Quality and Characterization**

Many studies aim to define the appropriate and most efficient “therapeutic platelet dose” when using PRP. While a substantial enrichment of the platelet physiological count is desired, some in vitro studies have pointed out that too much of it could have an inhibitory and detrimental effect on cellular function. To optimize the platelet dose, several studies have been conducted to define the “platelet dose.” Although a consensus has not been established, a common range of platelet dose is 1 million to 50 million platelets per milliliter of PRP. This range is based on the observation that higher platelet doses may result in better clinical outcomes, while lower doses may be associated with fewer side effects. However, further research is needed to establish a definitive range of platelet dosing that balances effectiveness with safety.

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**Figure 3.** Mean Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) scores with subscales and categories at baseline, and at 3-month, 6-month, and 1-year follow-up. Error bars indicate 95% CIs. *P < .05, ***P < .0001.
In a recent systematic review, the platelet count enrichment ranged between 1.3- and 8-fold, although the majority ranged between 2- and 4-fold. In our study, the average PRP enrichment was 2.75 ± 0.65 times, with the reported and expected donor-dependent differences. Interestingly, despite the mean platelet capture efficiency's being 54.9%, classified as “C” (low), the mean platelet purity was 96.64%, which ensures a very safe product (with absence of, or only residual, WBC and RBC, the presence of which may cause undesired side effects of an inflammatory nature). Furthermore, in our previous study, the concentration of 6 growth factors (epidermal growth factor, hepatocyte growth factor, platelet-derived growth factor—BB, vascular endothelial growth factor A and D, and fibroblast growth factor) was measured by multiplex technology in processed PRP samples during the validation period (from 14 different donors), and in the 3 aliquots obtained from each one. While donor-dependent variation was evident, the concentration of analyzed factors remained constant in the 3 frozen aliquots from each single donor, which assures the homogeneity of the PRP composition during the therapeutic regime (3 aliquots from the same donation). Further studies will aim at studying the association of PRP molecular composition with clinical responses in PRP KOA-treated patients.

The amount of growth factors in the PRP product is highly dependent on platelet-enrichment, but also on the postprocessing or activation method (ie, mechanical [through a freeze-thaw process] vs chemical [calcium chloride]) and is also a matter of debate. Several studies show that these methods may be equivalent, highlighting that the freeze-thaw method is a simple and clinically compliant approach to assure the availability of platelet-derived growth factors and bioactive molecules in the final

Figure 4. Correlation of (A) platelet enrichment and (B) platelet dose with clinical results as extrapolated from Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) and satisfaction scores. PLT, platelet; PRP, platelet-rich plasma.
product.\textsuperscript{15} Furthermore, a recent study suggests that frozen-thawed PRP should be preferred for chronic pathologies such as KOA, where injections have to be carried out more regularly.\textsuperscript{27}

The PRP volume used in the treatment of KOA is frequently reported in the literature as less than 6mL,\textsuperscript{30} but other authors suggest that the ideal volume is at least 8mL, to ensure that platelets and plasma can diffuse throughout the joint, reaching all areas.\textsuperscript{45,51} We used aliquots of approximately 10mL, with no adverse events observed. This volume enables us to achieve a “very high dose” of injected platelets, classifying the PRP used in this study as “A,” according to the DEPA classification, with a mean dose of injected platelets of $6.31 \pm 1.92 \times 10^9$ per infiltration. The dose of injected platelets has been described as the most relevant parameter with which to assess clinical efficacy.\textsuperscript{36}

Regarding economic issues, and according to Scientific American, each PRP injection price can range from US$500 to US$2500, usually as out-of-pocket fees.\textsuperscript{46} In Spain, a median cost of €194/US$224.10 per PRP injection (range, €90-€389/US$104-$450) is estimated.\textsuperscript{41} However, final quality assessment of these PRP products, or serological tests or blood screening (nucleic acid testing), is often not performed. These factors were included in the price of the PRP used in this study, and the final production costs per PRP infiltration were €36/US$41.6 (complete treatment €108/US$125). This huge difference is possibly due to the reduction in production costs, as we used a blood bank infrastructure already available and in use. This price is considerably lower than the regular cost of HA in Spain (€200-€400/US$230-$462 per injection), but higher than that of corticosteroid injection (€2-€3 for each triamcinolone ampoule/US$2.3-$3.4). Nevertheless, this product is completely different from PRP (and, therefore, so are its results and effectiveness).

**Relationship Between PRP Quality and Clinical Results**

Of note, no association was found between PRP quality parameter values and clinical response. This fact concurs with the general belief that the larger number of platelets, the better the clinical results, with some caveats: it is possible that, above a certain quality threshold, a satisfactory clinical response is obtained. If producing PRP in this standardized manner ensures a PRP product with sufficient quality to produce positive clinical responses,\textsuperscript{43} the monitoring of the PRP preparation methodology and its quality control should be given first priority in treatments involving PRP products.

The PRP used in this study comprises the characteristics required to classify it as “superdose PRP” and, against the idea that apheresis-based methods are currently the only way to have a reproducible PRP product with highly enriched platelet counts, our study shows that positive clinical responses can be also achieved when standardizing PRP production by differential centrifugation of whole-blood harvesting, using the infrastructure of a blood bank, assuring a leuko-depleted PRP product, with freezing-induced rupture of platelets and release of platelet growth factors.\textsuperscript{13,26,40}

**Strengths and Weakness of the Study**

Our study is nonrandomized and uncontrolled. In pragmatic trials, the question of placebo (as it has shown some improvements by itself as intra-articular injection in patients with KOA) and masking should be raised,\textsuperscript{20} in this case, at the present moment we do not have the possibility of an equivalent control group, as there is no therapeutic alternative used for symptomatic KOA in the daily practice, as most of our patients have tried them before (usually NSAIDs, corticoids, or HA infiltration) without good results. Future studies may address this issue by comparing this PRP product with platelet-lysates, growth factors such as sprifermin (rhFGF18), or other platelet-derived bioproducts on study (such as secretomes). Another limitation of the study is the minimum exclusion criteria in terms of prior nonoperative measures, surgical history, comorbidities, or KOA grade, which results in variability within the patient cohort. We did not assess outcomes as a function of KL severity or BMI. This study did not include assessment of the growth factors in the aliquots after freezing/thawing. Further studies with a more restrictive exclusion criteria and a larger cohort will be necessary to overcome this limitation.

The strengths of this study are mainly its pragmatic point of view, which allowed the recruitment of a high number of patients (N = 130). Another strength is that the post-test results were assessed at 3 different time points (at 3-month, 6-month, and 1-year follow-up), minimizing potential placebo effects. Finally, in this study we report almost all of the MIBO recommended for PRP.\textsuperscript{42}

**CONCLUSION**

In this study, we present a standardized PRP production method for symptomatic KOA with very good clinical results, with statistical (WOMAC) and clinical (MCID) significance and 70\% patient satisfaction at 1-year follow-up. This product is safe, highly reproducible, affordable, and very convenient for the patient. Despite donor-dependent differences, the production method ensured PRP products above a minimum quality threshold, required for positive clinical responses in the treatment of KOA.

**ACKNOWLEDGMENT**

The authors thank all the institutions and personnel involved in this study: Hospital Universitario de Cabueñes (especially Carmen Fernández, chief of the hematology department, and all the workers involved at the Banco de Sangre-Blood Bank, as well as all the orthopaedic clinicians who contributed to this work); Centro Comunitario de Sangre y Tejidos de Asturias (especially María Carmen Muñoz and all the workers involved in the PRP production, and to...
Ana María Ojea for her support); Instituto de Investigación Universitaria del Principado de Asturias and University of Oviedo-Asturias (Spain). Finally, the authors thank Álvaro Cambor and Lorian García for their assistance with data collection.

Supplemental material for this article is available at http://journals.sagepub.com/doi/suppl/10.1177/23259671221076496.

REFERENCES

1. Acebes-Huerta A, Arias-Fernández T, Bernardo Á, et al. Platelet-derived bio-products: classification update, applications, concerns and new perspectives. Transfus Apher Sci. 2020;59(1):102716.
2. Anitua E, de la Fuente M, Riestra A, Merayo-Lloves J, Muruzábal F, Orive G. Preservation of biological activity of plasma and platelet-derived eye drops after their different time and temperature conditions of storage. Cornea. 2015;34(9):1144-1148.
3. Anitua E, Prado R, Orive G. Closing regulatory gaps: new ground rules for platelet-rich plasma. Trends Biotechnol. 2015;33(9):482-485.
4. Belk JW, Kraeutler MJ, Houck DA, Goodrich JA, Dragoo JL, McCarty EC. Platelet-rich plasma versus hyaluronic acid for knee osteoarthritis: a systematic review and meta-analysis of randomized controlled trials. Am J Sports Med. 2021;49(1):249-260.
5. Blanco FJ, Silva-Diaz M, Quevedo Vila V, et al. Prevalence of symptomatic osteoarthritis in Spain: EPISER2016 study. Reumatol Clin. 2021;17(8):461-470.
6. Braun HJ, Kim HJ, Chu CR, Dragoo JL. The effect of platelet-rich plasma formulations and blood products on human synoviocytes: implications for intra-articular injury and therapy. Am J Sports Med. 2014;42(5):1204-1210.
7. Cerza F, Carni S, Carcangi A, et al. Comparison between hyaluronic acid and platelet-rich plasma, intra-articular infiltration in the treatment of gonarthrosis. Am J Sports Med. 2012;40(12):2822-2827.
8. Chahla J, Cinque ME, Piuzzi NS, et al. A call for standardization in platelet-rich plasma preparation protocols and composition reporting: a systematic review of the clinical orthopaedic literature. J Bone Joint Surg Am. 2017;99(20):1769-1779.
9. Chouhan DK, Dhillon MS, Patel S, Bansal T, Bhatia A, Kanwat H. Multiple platelet-rich plasma injections versus single platelet-rich plasma injection in early osteoarthritis of the knee: an experimental study in a guinea pig model of early knee osteoarthritis. Am J Sports Med. 2019;47(10):2300-2307.
10. Cook CS, Smith PA. Clinical update: why PRP should be your first choice for injection therapy in treating osteoarthritis of the knee. Curr Rev Musculoskelet Med. 2018;11(4):583-592.
11. Cui A, Li H, Wang D, Zhong J, Chen Y, Lu H. Global, regional prevalence, incidence and risk factors of knee osteoarthritis in population-based studies. EclinicalMedicine. 2020;29-30:100587.
12. DeLong JM, Russell RP, Mazocca AD. Platelet-rich plasma: the PAW classification system. Arthroscopy. 2012;28(7):998-1009.
13. Dhillon MS, Patel S, Bansal T. Improvising PRP for use in osteoarthrits knee—upcoming trends and futuristic view. J Clin Orthop Trauma. 2019;10(1):32-35.
14. Dohan Ehrenfest DM, Rasmusson L, Albrektsson T. Classification of platelet concentrates: from pure platelet-rich plasma (P-PRP) to leukocyte- and platelet-rich fibrin (L-PRP). Trends Biotechnol. 2009;27(3):158-167.
15. Durante C, Agostini F, Abbruzzese L, et al. Growth factor release from platelet concentrates: analytic quantification and characterization for clinical applications. Vox Sang. 2013;105(2):129-136.
16. Escobar A, Quintana JM, Bilbao A, Azkárate J, Güenaga JL. Validation of the Spanish version of the WOMAC questionnaire for patients with hip or knee osteoarthritis. Clin Rheumatol. 2002;21(6):468-471.
17. Fice MP, Miller JC, Christian R, et al. The role of platelet-rich plasma in cartilage pathology: an updated systematic review of the basic science evidence. Arthroscopy. 2019;35(3):961-976.e3.
18. Filardo G, Kon E, Pereira Ruiz MT, et al. Platelet-rich plasma intra-articular injections for cartilage degeneration and osteoarthritis: single- versus double-spinning approach. Knee Surg Sports Traumatol Arthrosc. 2012;20(10):2082-2091.
19. Filardo G, Kon E, Roffi A, Di Matteo B, Merli ML, Maracci M. Platelet-rich plasma: why intra-articular? A systematic review of preclinical studies and clinical evidence on PRP for joint degeneration. Knee Surg Sports Traumatol Arthrosc. 2015;23(9):2459-2474.
20. Ford I, Norrie J. Pragmatic trials. N Engl J Med. 2016;375(5):454-463.
21. Görmel G, Görmei CA, Ataoglu B, Colak C, Aslantürk O, Ertem K. Multiple PRP injections are more effective than single injections and hyaluronic acid in knees with early osteoarthritis: a randomized, double-blind, placebo-controlled trial. Knee Surg Sports Traumatol Arthrosc. 2017;25(3):959-965.
22. Grambart ST. Sports medicine and platelet-rich plasma: nonsurgical therapy. Clin Podiatr Med Surg. 2015;32(1):99-107.
23. Graziani F, Ivanovski S, Cei S, Ducci F, Tonetti M, Gabriele M. The in vitro effect of different PRP concentrations on osteoblasts and fibroblasts. Clin Oral Implants Res. 2006;17(2):212-219.
24. Harrison P; Subcommittee on Platelet Physiology. The use of platelets in regenerate medicine and proposal for a new classification system: guidance from the SSC of the ISSTH. J Thromb Haemost. 2018; 16(9):1895-1900.
25. Hmaroomi I, Allafi F, Tahiri L, et al. Clinically important improvement in the WOMAC and predictor factors for response to non-specific non-steroidal anti-inflammatory drugs in osteoarthritic patients: a prospective study. BMC Res Notes. 2012;5:58.
26. Kaux J-F, Bouvard M, Lecut C, et al. Reflections about the optimisation of the treatment of tendinopathies with PRP. Muscles Ligaments Tendons J. 2015;5(1):1-4.
27. Kaux J-F, Libertiaux V, Dupont L, et al. Platelet-rich plasma (PRP) and tendon healing: comparison between fresh and frozen-thawed PRP. Platelets. 2020;31(2):221-225.
28. Kelgren JH, Lawrence JS. Radiological assessment of osteo-arthritis. Ann Rheum Dis. 1957;16(4):494-502.
29. Kolaisinski SL, Neogi T, Hochberg MC, et al. 2019 American College of Rheumatology/Arthritis Foundation Guideline for the management of osteoarthritis of the hand, hip, and knee. Arthritis Care Res (Hoboken, NJ). 2020;72(2):149-162.
30. Laver L, Marom N, Dryanesh L, Mei-Dan O, Espregueira-Mendes J, Gobbi A. PRP for degenerative cartilage disease: a systematic review of clinical studies. Cartilage. 2017;8(4):341-364.
31. Lin K-Y, Yang C-C, Hsu C-J, Yeh M-L, Renn J-H. Intra-articular injection of platelet-rich plasma is superior to hyaluronic acid or saline solution in the treatment of mild to moderate knee osteoarthritis: a randomized, double-blind, triple-parallel, placebo-controlled clinical trial. Arthroscopy. 2019;35(1):106-117.
32. Losina E, Paltiel AD, Weinstein AM, et al. Lifetime medical costs of knee osteoarthritis management in the United States: impact of extending indications for total knee arthroplasty. Arthritis Care Res (Hoboken). 2015;67(2):203-215.
33. Loudon K, Treweek S, Sullivan F, Donnan P, Thorpe KE, Zwarenstein M. The PRECIS-2 tool: designing trials that are fit for purpose. BMJ. 2015;350:h2147.
34. Magalon J, Bausset O, Serratrice N, et al. Technical and biological comparison of 5 platelet-rich plasma preparations in a single-donor model. Arthroscopy. 2014;30(5):629-638.
35. Magalon J, Brandin T, Francois P, et al. Technical and biological review of authorized medical devices for platelets-rich plasma preparation in the field of regenerative medicine. Platelets. 2021;32(2):200-208.
36. Magalon J, Chateau AL, Bertrand B, et al. DEPA classification: a proposal for standardising PRP use and a retrospective application of available devices. BMJ Open Sport Exerc Med. 2016;2(1):e000060.
37. Mazzocca AD, McCarthy MBR, Chowaniec DM, et al. Platelet-rich plasma differs according to preparation method and human variability. J Bone Joint Surg Am. 2012;94(4):308-316.
38. Meheux CJ, McCulloch PC, Lintner DM, Varner KE, Harris JD. Efficacy of intra-articular platelet-rich plasma injections in knee osteoarthritis: a systematic review. Arthroscopy. 2016;32(3):495-505.
39. Melchiorre D, Manetti M, Matucci-Cerinic M. Pathophysiology of hemophilic arthropathy. J Clin Med. 2017;6(7):63.
40. Moog R, Zeiler T, Heuft H-G, et al. Collection of WBC-reduced single-donor PLT concentrates with a new blood cell separator: results of a multicenter study. Transfusion. 2003;43(8):1107-1114.
41. Moreno R, Gaspar Carreño M, Alonso Herreros JM, Romero Garrido JA, López-Sánchez P. [Platelet-rich plasma: updating of extraction devices]. Article in Spanish. Farm Hosp. 2016;40(5):385-393.
42. 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(10):809-819.
43. Ojea-Pérez AM, Acebes-Huerta A, Arias-Fernández T, Gutiérrez L, Munoz-Turrillas MC. Implementation of a closed platelet-rich-plasma preparation method using the local blood bank infrastructure at the Principality of Asturias (Spain): back to basic methodology and a demographics perspective after 1 year. Transfus Apher Sci. 2019;58(3):701-704.
44. Park Y-B, Kim J-H, Ha C-W, Lee D-H. Clinical efficacy of platelet-rich plasma injection and its association with growth factors in the treatment of mild to moderate knee osteoarthritis: a randomized double-blind controlled clinical trial as compared with hyaluronic acid. Am J Sports Med. 2021;49(2):487-496.
45. Patel S, Dhillon MS, Aggarwal S, Marwaha N, Jain A. Treatment with platelet-rich plasma is more effective than placebo for knee osteoarthritis: a prospective, double-blind, randomized trial. Am J Sports Med. 2013;41(2):356-364.
46. Piuzzi NS, Ng M, Kantor A, et al. What is the price and claimed efficacy of platelet-rich plasma injections for the treatment of knee osteoarthritis in the United States? J Knee Surg. 2019;32(9):879-885.
47. Raeissadat SA, Gharooee Ahangar A, Rayegani SM, Minator Sajjadi M, Ebrahimpour A, Yavari P. Platelet-rich plasma-derived growth factor vs hyaluronic acid injection in the individuals with knee osteoarthritis: a one year randomized clinical trial. J Pain Res. 2020;13:1699-1711.
48. Raeissadat SA, Rayegani SM, Hassanabadi H, et al. Knee osteoarthritis injection choices: platelet-rich plasma (PRP) versus hyaluronic acid (a one-year randomized clinical trial). Clin Med Insights Arthritis Musculoskelet Disord. 2015;8:1-8.
49. Riboh JC, Saltzman BM, Yanke AB, Fortier L, Cole BJ. Effect of leukocyte concentration on the efficacy of platelet-rich plasma in the treatment of knee osteoarthritis. Am J Sports Med. 2016;44(3):792-800.
50. Roffi A, Filardo G, Assirelli E, et al. Does platelet-rich plasma freeze-thawing influence growth factor release and their effects on chondrocytes and synoviocytes? Biomed Res Int. 2014;2014:692913.
51. Sánchez M, Anitua E, Azofra J, Aguirre JJ, Andia I. Intra-articular injection of an autologous preparation rich in growth factors for the treatment of knee OA: a retrospective cohort study. Clin Exp Rheumatol. 2008;26(5):910-913.
52. Smyth NA, Murawski CD, Fortier LA, Cole BJ, Kennedy JG. Platelet-rich plasma in the pathologic processes of cartilage: review of basic science evidence. Arthroscopy. 2013;29(8):1399-1409.
53. Southworth TM, Naveen NB, Tauro TM, Leong NL, Cole BJ. The use of platelet-rich plasma in symptomatic knee osteoarthritis. J Knee Surg. 2019;32(1):37-45.
54. Sundman EA, Cole BJ, Karas V, et al. The anti-inflammatory and matrix restorative mechanisms of platelet-rich plasma in osteoarthritis. Am J Sports Med. 2014;42(1):35-41.
55. Tubach F, Ravaud P, Baron G, et al. Evaluation of clinically relevant changes in patient reported outcomes in knee and hip osteoarthritis: the minimal clinically important improvement. Ann Rheum Dis. 2005;64(1):29-33.
56. Yung Y-L, Fu S-C, Cheuk Y-C, et al. Optimisation of platelet concentrates therapy: composition, localisation, and duration of action. Asia Pac J Sports Med Arthrosc Rehabil Technol. 2017;7:27-36.