[Orthopaedic Surgery]

Platelet-Rich Plasma Treatment in Symptomatic Patients With Knee Osteoarthritis: Preliminary Results in a Group of Active Patients

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Background: With increasing frequency, platelet-rich plasma (PRP) preparations have been used to treat cartilage lesions to regenerate tissue homeostasis and retard the progression of knee osteoarthritis (OA).

Purpose: To determine the effectiveness of intra-articular PRP injections in active patients with knee OA and to evaluate clinical outcomes in patients with and without previous surgical treatment for cartilage lesions.

Study Design: Case series.

Materials and Methods: Fifty patients with knee OA were followed for a minimum of 12 months. All were treated with 2 intra-articular injections of autologous PRP. Twenty-five patients had undergone a previous operative intervention for cartilage lesions, whereas 25 had not. Operated patients had undergone either cartilage shaving or microfracture. Multiple evaluative scores were collected at pretreatment and at 6 and 12 months posttreatment. The required sample of patients was determined beforehand by using statistical power analysis; International Knee Documentation Committee (subjective) score was defined as the primary parameter. A P value of less than 0.05 was considered statistically significant. General linear model–repeated measure test evaluated within-time improvement for each variable for all patients. Post hoc test with Bonferroni adjustment for multiple comparisons was performed to investigate the significance in improvement within time evaluations for each variable for the total sample. The differences in improvement between operated and nonoperated patients were also investigated, as were those between sexes.

Results: All patients showed significant improvement in all scores at 6 and 12 months ($P < 0.01$) and returned to previous activities. No significant difference in improvement was found between the evaluated subgroups ($P < 0.01$).

Conclusions: The PRP treatment showed positive effects in patients with knee OA. Operated and nonoperated patients showed significant improvement by means of diminishing pain and improved symptoms and quality of life.

Clinical Relevance: There are only a few studies of PRP treatment for cartilage on osteoarthritic knees. Different PRP products might be more or less appropriate to treat different types of tissues and pathologies. The clinical efficacy of PRP remains under debate, and a standardized protocol has not yet been established.

Keywords: knee; cartilage; osteoarthritis; platelet-rich plasma; microfracture; cartilage shaving

Hyaline cartilage, known for its unique properties, enables almost frictionless joint movement and protects the underlying bone from excessive load and trauma by dissipating the forces produced during movement. However, cartilage has limited intrinsic healing potential because it is avascular and has few specialized cells with a low mitotic activity.[28] Once cartilage is injured, it gradually degenerates, leading to osteoarthritis (OA).[34] The prevalence of chondral defects is frequent in sport injuries (especially in patients older than 40 years), and it often causes persistent pain. OA incidence increases steadily with age, affecting 12.1% of the population from 25 to 74 years old, and it is the leading cause of physical disability in people older than 65 years. Community-based studies have shown that 10% of the population older than 55

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years has troublesome knee pain, and among this community, 25% are severely disabled.[46]

Many conservative treatment options—such as oral and topical nonsteroidal anti-inflammatory drugs, diacerein and intra-articular corticosteroids, and viscosupplementation—have been used for the treatment of OA and have yielded short-term efficacy with local or systemic side effects.[7,24,25,27,64,65] The high cost of bone and cartilage pathologies has influenced the trend toward preventive interventions and therapeutic options that regenerate tissue homeostasis[3,4] and retard progression to OA. Platelet-rich plasma (PRP) is one therapeutic application with promising preliminary clinical results.[30,51,58]

Platelet-Rich Plasma

PRP can be defined as the volume of the plasma fraction from autologous blood with a platelet concentration above baseline count (200 000 platelets/μL).[37] Platelets contain many important bioactive proteins and growth factors (GFs). These factors regulate key processes in tissue repair, including cell proliferation, chemotaxis, migration, cellular differentiation, and extracellular matrix synthesis.[9,44,55] The rationale for the use of PRP is to stimulate the natural healing cascade and tissue regeneration by a "supraphysiologic" release of platelet-derived factors directly at the site of treatment. Autologous PRP can be obtained from simple blood extraction with a commercially available kit. Once the blood is collected into a tube containing anticoagulant, it undergoes a centrifugation process to produce PRP. For PRP gel preparations, platelets are normally activated by thrombin (autologous or animal derived), calcium chloride, or procoagulant enzyme (ie, batroxobin[36]), which works as a fibrinogen-cleaving enzyme inducing rapid fibrin clot formation. When PRP solutions are injected directly for topical treatment, platelets are activated by endogenous thrombin and/or intra-articular collagen.[26] GFs have a half-life from minutes to hours. When compared with collagen activation of platelets, previous thrombin activation could actually decrease their availability.[5,26] In general, the amount of GFs delivered is not necessarily proportional to the platelet count, because of their high variability in platelets among individuals.[11,60] The concentration of platelets and platelet-derived GFs varies among commercially available medical devices to prepare PRP,[37] and the impact on the efficacy of the PRP product is as yet undetermined. Studies have shown that the clinical efficacy of PRP products is expected to increase, at minimum, 2- to 6-fold of platelets count from baseline value.[15,36,37,59]

Growth Factors

Platelets α-granules contain a variety of GFs, including transforming GFs, platelet-derived GFs, hepatocyte GFs, basic fibroblast GFs, epidermal GF, vascular endothelial GFs, and insulin-like GF.[37,38] GFs mediate the biological processes necessary for repair of soft tissues,[18,19] such as muscle, tendon, and ligament, following acute traumatic or overuse injury. Their mode of action is to bind to the extracellular domain of a target GF receptor, which in turn activates the intracellular signal transduction pathways.[32,56] In vitro studies in animal and human chondrocytes[1,45] have demonstrated that PRP-secreted GFs stimulate proliferation and collagen synthesis. Animal studies have demonstrated clear benefits in terms of accelerating healing[6,40] and anti-inflammatory[8] action. More interesting, their positive effect in OA-affected animal joints, by stimulating cartilage matrix metabolism, has been reported.[17,49] Similarly, in clinical studies, therapeutic application of PRP has shown promising results in the treatment of musculoskeletal disorders, including fractures, cartilage defects, and muscle and tendon lesions.[33,40,43,44,52,53] Recent studies[30,51,58] showed promising preliminary clinical results in the treatment of knee OA; however, the clinical efficacy of PRP still remains under debate,[13] and a standardized protocol has not yet been established.

The aim of our study was to investigate the possible positive effects of PRP intra-articular injections in active patients with symptomatic knee OA. Additionally, we studied whether PRP is equally effective in patients who underwent a previous operative intervention for cartilage lesions (cartilage shaving and/or microfracture) and patients who did not undergo any previous operative intervention of the knee.

MATERIALS AND METHODS

We prospectively followed 50 patients with symptomatic knee OA of grade 1-3 per Kellgren-Lawrence classification (Table 1). All patients (31 men and 19 women) were treated with 2 intra-articular injections (once monthly) with autologous PRP (Regen ACR-C, Regen Lab, Switzerland) and followed up for a minimum period of 1 year (range, 12-26 months). The mean age of patients was 47.7 years, ranging from 32 to 60 years, and body mass index was 26.7 ± 2.4. All patients were involved in various sports activities, such as football (14%), skiing (14%), motocross (12%), basketball or volleyball (12%), jogging (10%), and others (tennis, bicycling, walking, trekking, etc) but not at a professional level (Tables 2 and 3).

Twenty-five patients (50%) had undergone a previous operative intervention for cartilage lesions of grade 3 and 4 per International Cartilage Repair Society classification repair (Table 1) on the ipsilateral knee at least 1 year before PRP treatment (S1 group), while 25 patients did not undergo any previous operative intervention for the knee (S2 group). Average time from previous surgery to treatment was 22.4 ± 17.2 months, ranging from 1 to 3 years. Previous operative interventions for cartilage included cartilage shaving (S1a) and microfracture (S1b) for grade 3 and 4 cartilage lesions (International Cartilage Repair Society classification) (Table 2).

The standard radiographic evaluation included a standing anteroposterior long-leg radiograph (including hips and ankles), standing anteroposterior/lateral views of the knees, skyline patellofemoral and standing 45° flexion knee views, and magnetic resonance imaging. Standard blood investigations were done before treatment, including complete blood count,

Table 1. Kellgren-Lawrence and International Cartilage Repair Society classifications.

Grade	Kellgren-Lawrence	International Cartilage Repair Society
0	Normal	Normal
1	Nearly normal (small osteophytes of doubtful clinical significance)	Nearly normal (soft indentation and/or superficial fissures and cracks)
2	Definite osteophytes with unimpaired joint space	Abnormal (lesions extending down to < 50% of cartilage depth)
3	Definite osteophytes with moderate joint space narrowing	Severely abnormal (cartilage lesions > 50% of cartilage depth)
4	Definite osteophytes with severe joint space narrowing and subchondral sclerosis	Severely abnormal (penetrating subchondral bone)

Table 2. Patient demographic data and Kellgren-Lawrence and International Cartilage Repair Society classifications.

Patients	No.	Age, y	Male / Female, No.	Right / Left Knee, No.	Kellgren-Lawrence Grade, No.			Intl Cartilage Repair Society Grade,[a] No.	
					1	2	3	3	4
All	50	47.7 ± 2.52	31 / 19	20 / 30	11	19	20	—	—
S1: previous surgery	25	44.7 ± 2.01	14 / 11	7 / 18	3	11	11	14	11
S1a: cartilage shaving	12	44.4 ± 2.39	4 / 8	2 / 10	3	6	3	9	3
S1b: microfracture	13	45.0 ± 1.68	5 / 8	5 / 8	0	5	8	5	8
S2: no previous surgery	25	50.4 ± 2.77	17 / 8	13 / 12	8	8	9	—	—

[a]At the time of surgery.

coagulation profile, and test for transmittable diseases (Table 3). Visual analog scale for pain (0 = no pain at all, 10 = worst pain), International Knee Documentation Committee (IKDC) subjective and objective score,[29] Knee injury and Osteoarthritis Outcome Score (KOOS),[48] Tegner,[57] and Marx[35] scores were collected at pretreatment evaluation and at 6- and 12-month follow-up.

Technique

All patients were treated with 2 intra-articular injections of autologous PRP (1-month interval between injections). After extraction of 8 mL of peripheral blood, the sample was centrifuged for 9 minutes at 3500 revolutions per minute according to recommendations of the manufacturer. The system that we used did not include a second centrifugation step[36,37] (Figure 1A, 1B). Subsequently, we obtained 4 mL of PRP, and we proceeded to the intra-articular infiltration by a suprapatellar approach under sterile aseptic conditions (Figure 1C, 1D). A topical anesthetic skin refrigerant was applied locally before the injections. We did not activate PRP before injection to induce rapid fibrin clot formation. After treatment, patients were allowed weightbearing, and local ice application was recommended 20 minutes every 2 to 3 hours for 24 hours. We recommended restriction of vigorous activities of the knee for at least 48 hours.

Statistical Analysis

Statistical analysis (SPSS 17.0) was performed by an independent statistician, who was blinded to the sample and subgroups. General linear model–repeated measure

Table 3. Inclusion-exclusion criteria.

Inclusion	Exclusion
Age between 30 and 60 years, body mass index < 30, normal results for complete blood count and coagulation control, minimum follow-up of 1 year	Patients with blood diseases, systemic metabolic, immunodeficiency, hepatitis B or C, HIV-positive status, infection and septicemia, local infection
Patients with symptomatic osteoarthritic knees (Kellgren-Lawrence grade 1-3 based on radiographic findings) and partial- or full-thickness cartilage lesions (International Cartilage Repair Society grade 3-4 based on magnetic resonance imaging findings)	Patients with advanced and tricompartmental osteoarthritis, rheumatoid or polyarticular arthritis, symptomatic hip osteoarthritis, or symptomatic contralateral knee osteoarthritis
Patients with severe pain and under anti-inflammatory treatment without improvement > 3 months	Significant joint swelling or clinical signs of acute inflammation (possible inflammation or infection)
Patients with stable knees, normal tibiofemoral alignment, or patellofemoral tracking	Varus-valgus malalignment above 5°, patellofemoral maltracking or untreated instability, and total or subtotal meniscectomy (> 2/3 excised)
Patients with or without previous cartilage shaving and microfracture (other interventions were excluded)	Pretreatment blood platelets value 25% below the reference value or alcoholism, smoking, drugs
Patients who gave consent for treatment with platelet-rich plasma per our protocol	Treatment with corticosteroids < 3 months or medication < 7 days that could interfere with platelet aggregation

test was performed to investigate time improvement in KOOS, visual analog scale, Tegner, IKDC, and Marx scores from pretreatment to 6 and 12 months. A post hoc test with Bonferroni adjustment for multiple comparisons was performed to investigate the improvement for each variable for the total sample.

A χ^2 test was performed to investigate whether S1 and S2 subgroups were homogeneous regarding Kellgren-Lawrence grade of OA.

Because of the relatively small number of patients in each subgroup (S1 vs S2, S1a vs S1b, and male vs female patients), we used the nonparametric Friedman test to detect time significant improvement of variables. Post hoc tests were performed with the Wilcoxon rank test to evaluate improvement from pretreatment to 6 and 12 months for each subgroup.

To compare patients with and without previous surgery, a *t* test was performed. The comparison of the initial absolute values for each subgroup showed that S1 and S2 subgroups were not homogeneous. Thus, we extracted 2 homogeneous groups, each including 20 patients, to compare posttreatment improvement. There was no difference in starting scores; we then compared the absolute values of scores at 6 and 12 months. The nonparametric Mann-Whitney test was performed to analyze the difference in improvement between S1 and S2 subgroups and between male and female patients. Mann-Whitney test detected any difference in improvement between patients who underwent cartilage shaving (S1a) and microfracture (S1b). Continuous data are described as average mean ± SEM. Reported *P* values are 2-tailed with an α level of 0.05 indicating significance.

Power Analysis

A power analysis determined the number of required patients. IKDC subjective score was defined as the primary parameter. An improvement of 10 points was considered clinically important.[54] A sample size of 43 patients was required for $\alpha = 0.05$ and power = 0.80, considering a standard deviation of 20.[54] Therefore, we included 50 patients in our study.

RESULTS

The χ^2 test revealed no significant association ($P = 0.23$) between the patients in the S1 and S2 subgroups within grade of the Kellgren-Lawrence classification system. Both groups were homogeneous regarding grade of OA.

All patients showed significant improvement in all scores at 6 and 12 months ($P < 0.01$) and returned to previous activities, including recreational sports (Figure 2, Table 4). No adverse reactions (eg, swelling or acute pain) or any major complications (eg, infection) were noted. Each subgroup showed significant improvement from pretreatment to 6 and 12 months ($P < 0.01$). Patients who did not have previous surgery did not show improvement in KOOS symptoms and

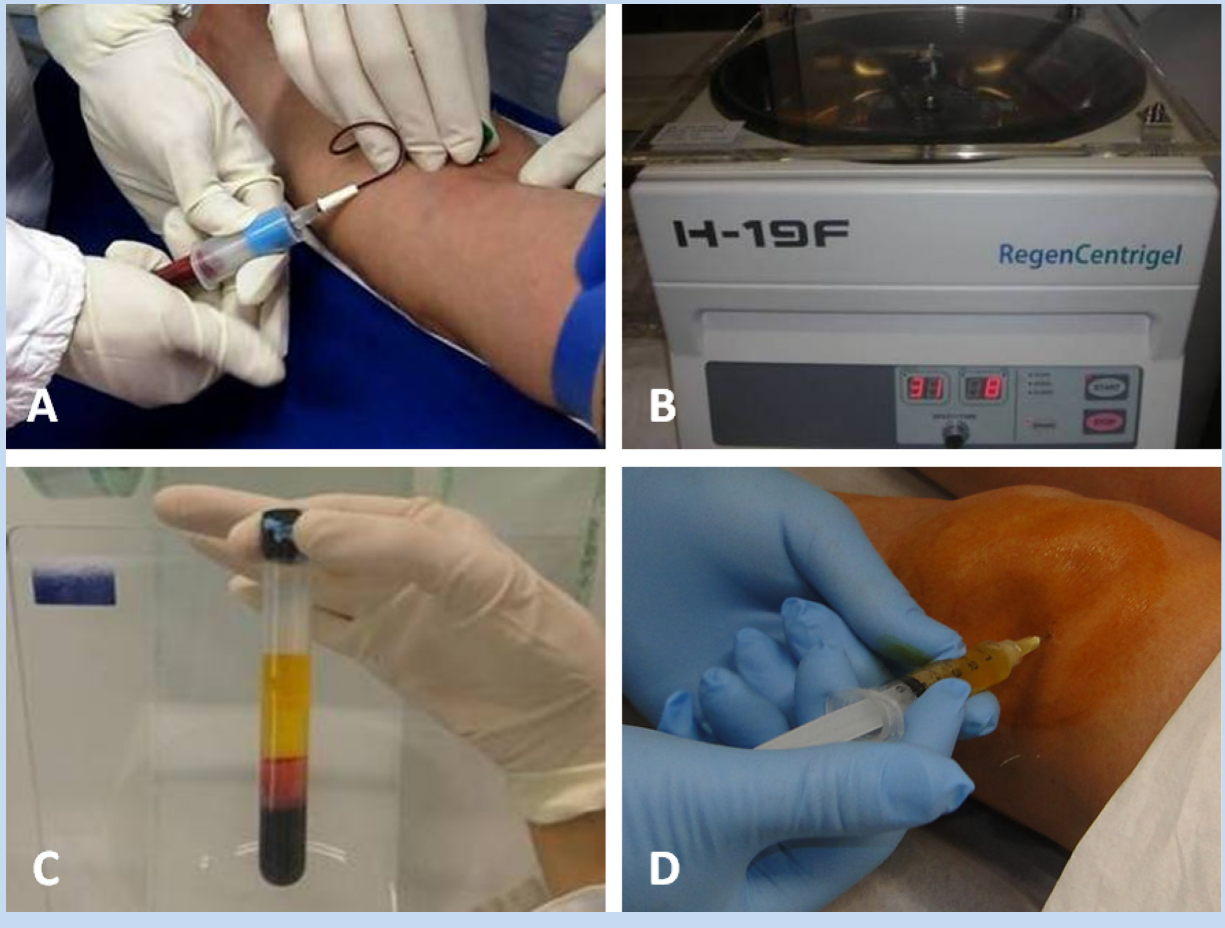

Figure 1. Platelet-rich plasma preparation: (A) blood aspiration, (B) centrifugation of the blood sample for 9 minutes, (C) fraction of platelet-rich plasma after centrifugation (yellow upper part in tube), and (D) knee intra-articular infiltration.

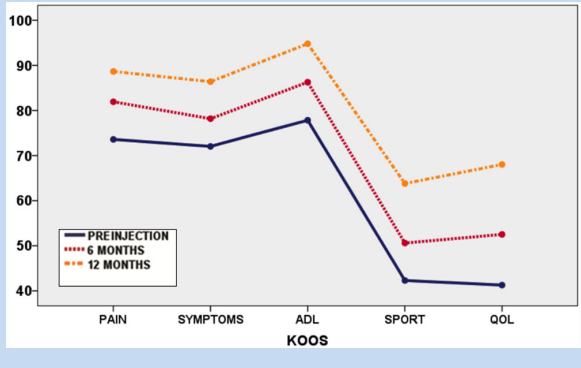

Figure 2. Diagram showing Knee injury and Osteoarthritis Outcome Score improvement from preinjection evaluation to 6- and 12-month follow-up in all patients ($P < 0.01$).

KOOS sports (from pretreatment to 6 months) (Figure 3A-3D and Table 5). However, the Mann-Whitney test did not show any significant difference in improvement between operated (S1) and nonoperated patients (S2) (Table 5). Likewise, patients treated with cartilage shaving (S1a) did not show a significant difference in improvement from patients who were treated with microfracture (S1b) (Figure 4A-4B). There was no significant difference in improvement between men and women.

All patients returned to their previous levels of sporting activity, which varied. Statistical analysis did not reveal any significant difference in Tegner, Marx, and KOOS scores between S1 and S2 subgroups at 6- and 12-month follow-up.

DISCUSSION

The purpose of this study was to investigate the effectiveness of intra-articular PRP injections in active patients with symptomatic knee OA in terms of diminishing pain, improving quality of life, and returning to previous activities. All patients showed significant improvement in all scores at 6 and 12 months ($P < 0.01$), demonstrating that PRP injections can represent a valuable treatment in patients with knee OA. Other studies have demonstrated good results in the treatment of several musculoskeletal problems.[4,43,50]

Table 4. Clinical outcomes.[a]

Variable	Pretreatment	6 mo	12 mo	*F* Test[b]
Visual analog scale	4.1 ± 0.7	2.2 ± 0.4	1.2 ± 0.3	42.155
KOOS				
Pain	73.6 ± 4.3	81.9 ± 4.3	88.7 ± 2.9	32.333
Symptoms	72.0 ± 4.1	78.2 ± 4.2	86.4 ± 3.2	27.674
Activities of daily living	77.8 ± 5.7	86.3 ± 4.7	94.8 ± 2.5	19.163
Sport	42.3 ± 7.3	50.6 ± 7.6	63.8 ± 6.7	22.176
Quality of life	41.3 ± 5.3	52.5 ± 5.2	68.0 ± 5.6	43.305
IKDC				
Subjective	48.2 ± 3.5	65.2 ± 2.6	75.4 ± 3.4	82.900
Objective, No.[c]				
A	0	16	29	
B	16	22	16	
C	23	10	5	
D	11	2	0	
Marx	4.0 ± 0.8	6.9 ± 0.8	9.4 ± 0.8	72.850
Tegner	2.9 ± 0.4	3.9 ± 0.4	4.8 ± 0.5	18.942

[a]Mean ± SEM. KOOS, Knee injury and Osteoarthritis Outcome Score; IKDC, International Knee Documentation Committee. Post hoc test with Bonferroni adjustment for multiple comparisons was performed to investigate the significance in improvement for each variable within time evaluation: 0-6 months, 6-12 months, 0-12 months. All post hoc tests, $P < 0.01$.
[b]General linear model–repeated measure test was performed to investigate within-time improvement. All *F* tests, $P < 0.01$.
[c]$P < 0.001$. IKDC is an ordinary scale, not a continuous data scale; therefore, we performed Freedman test.

Recent studies have documented the effectiveness of GFs in chondrogenesis[1,62] and prevention of joint degeneration[17,49] by controlling the synthesis and degradation of extracellular matrix proteins. Their mode of action is to bind to the extracellular domain of a target GF receptor, which in turn activates the intracellular signal transduction pathways.[32,56] The elucidation of some of the functions of GFs in tissue repair has led to the conclusion that their controlled temporal expression could be important following surgical interventions and in the treatment of musculoskeletal disorders, including bone fractures, cartilage defects, and muscle and tendon lesions. Akeda et al[1] successfully cultured porcine chondrocytes with PRP, showing higher cell proliferation and proteoglycans and collagen synthesis. Moreover, Wu et al,[62] in an experimental animal study, showed the effectiveness of intra-articular injections of PRP with chondrocytes grown in vivo that resulted in the formation of new cartilage tissue. In other animal studies,[17,49] clinical and histologic improvement has been reported in OA-affected joints after treatment with platelet rich plasma. Frisbie et al[17] reported clinical and histologic improvement in OA-affected joints of horses after treatment with PRP. Saito et al[49] reported significantly suppressed progression of OA morphologically and histologically in a rabbit model after administration of intra-articular injections of PRP in gelatin hydrogel microspheres. These preventive effects were attributed to stimulation of cartilage matrix metabolism caused by the GFs contained in PRP.

Anitua et al,[4] in their study on human synovial cells isolated from 10 osteoarthritic patients, showed that an intra-articular injection of PRP could induce an increase in production of hyaluronic acid structure and promote angiogenesis and cell proliferation. Nakagawa et al[45] reported the in vitro efficacy of autologous PRP in stimulating the proliferation and collagen synthesis of human chondrocytes, suggesting the use of this method in the treatment of cartilage defects.

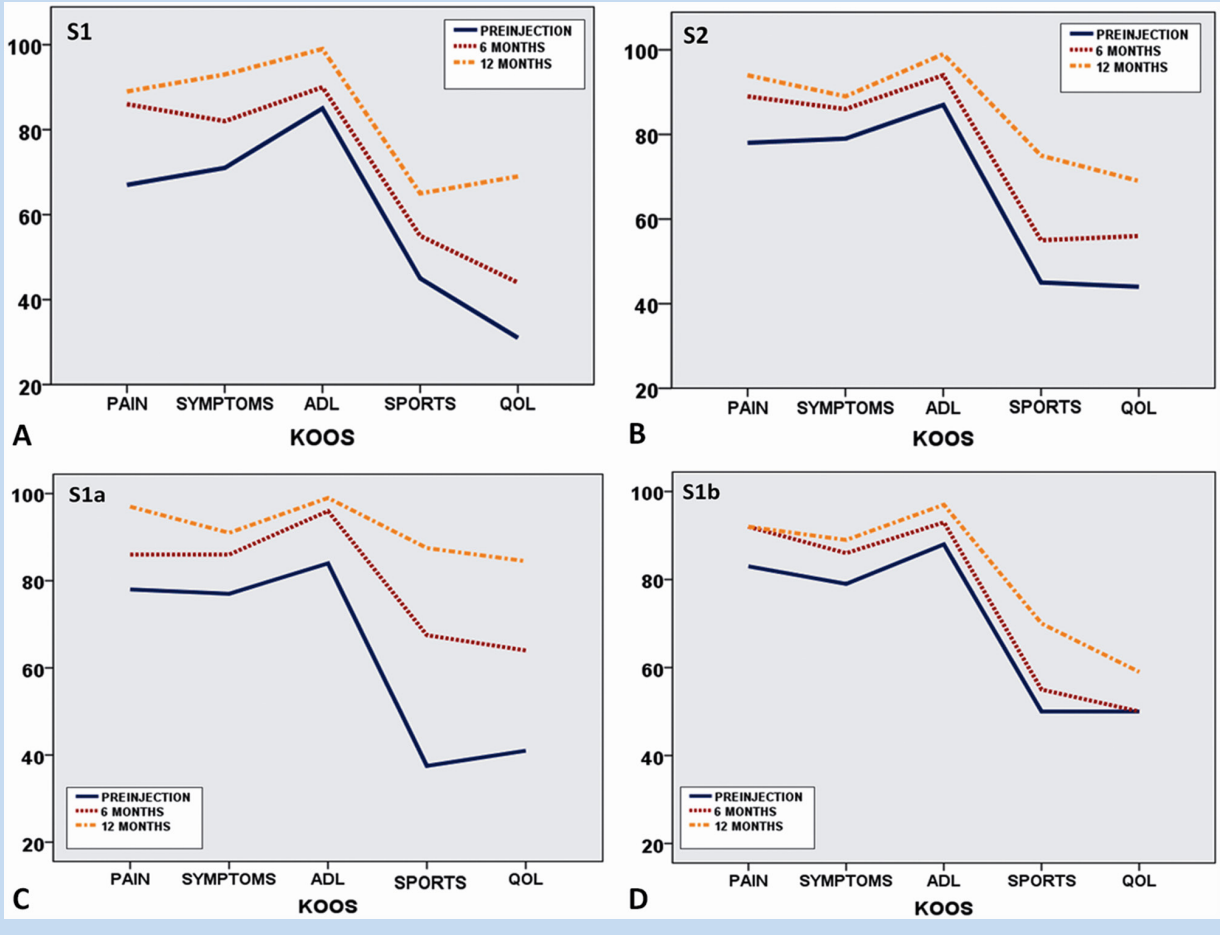

Figure 3. Diagrams showing Knee injury and Osteoarthritis Outcome Score improvement from preinjection evaluation to 6- and 12-month follow-up in patients ($P < 0.01$) (A) with previous operation, (B) without previous operation and in patients who had undergone (C) cartilage shaving and (D) microfracture.

Kon et al[30] reported interesting observations on PRP treatment in patients with chronic symptomatic degenerative condition of the knee. They demonstrated positive effects on function and symptoms, with an 85% improvement in scores for patients with a median age less than 60 years who were treated with 3 PRP intra-articular injections (one per week); in patients older than 60 years, the improvement was only 30%. Patients treated with PRP showed better results at 1-year follow-up than patients treated with hyaluronic acid; the results deteriorated over 12 to 24 months of follow-up.[16,31] Other authors[20,51,58] used intra-articular injections of PRP in knee OA patients and had good short-term results without provoking local or systemic adverse events. They demonstrated that PRP combined with proper nutrition (control of body mass index), exercise, and lifestyle can act as a preventive agent in chronic and degenerative musculoskeletal disease.[20] These results are in accordance with the preliminary results of the present study; all our patients showed significant improvement at 1-year follow-up. There was no deterioration of results at 1-year follow-up (Tables 4 and 5). In our study, patient ages ranged from 32 to 60 years, and patients with advanced OA were excluded. Patients did not have associated pathologies such as knee instability or tibiofemoral and patellofemoral malalignment, which can affect clinical outcomes and predispose to OA while increasing functional loads on the knee (Table 3).[10,22] Although worse results have been reported for female patients in other studies,[16] we found no significant difference in improvement between men and women (Mann-Whitney test). No adverse reactions (eg, acute pain and swelling) or major complications (eg, infection) were noted. This is in accordance with other study reports[30,51,58] and empowers the safety profile of autologous PRP intra-articular injections.

All our patients were active in sports, and they obtained more than 50% improvement in Tegner, Marx, and KOOS sports scores from pretreatment to final follow-up evaluation (Tables 4 and 5) and returned to their previous sporting activities. Patients were involved in sports in varied frequency;

Table 5. Clinical outcomes for patients with and without previous surgery.[a]

	S1: Patients With Previous Surgery						S2: Patients Without Previous Surgery						S1 vs S2,[b] *P*	
Variable	**PRE**	**6 mo**	**0-6 mo,[c] *P***	**12 mo**	**0-12 mo,[c] *P***	**6-12 mo,[c] *P***	**PRE**	**6 mo**	**0-6 mo,[c] *P***	**12 mo**	**0-12 mo,[c] *P***	**6-12 mo,[c] *P***	**0-6 mo**	**0-12 mo**
VAS	3.2 ± 1.4	1.9 ± 1.7	0.01	1.2 ± 1.1	< 0.01	0.01	4.4 ± 2.7	2.4 ± 1.9	< 0.01	1.3 ± 1.4	< 0.01	< 0.01	0.44	0.88
KOOS														
Pain	75.4 ± 9.9	84.5 ± 11.2	0.01	89.0 ± 9.7	< 0.01	0.02	70.0 ± 19.6	80.9 ± 17.4	0.01	86.1 ± 13.5	< 0.01	< 0.01	0.82	0.55
Symptoms	70.9 ± 13.9	80.0 ± 12.7	< 0.01	86.6 ± 10.6	< 0.01	< 0.01	70.2 ± 18.3	76.5 ± 18.3	0.42	83.1 ± 14.4	< 0.01	0.01	0.92	0.97
ADL	83.1 ± 9.4	91.3 ± 6.2	< 0.01	95.6 ± 2.1	< 0.01	0.01	77.8 ± 26.5	85.6 ± 20.2	< 0.01	92.8 ± 12.2	< 0.01	< 0.01	0.61	0.61
Sport	39.7 ± 22.8	58.2 ± 19.9	< 0.01	94.6 ± 9.0	< 0.01	0.02	46.5 ± 29.6	47.7 ± 32.9	0.83	61.7 ± 29.5	< 0.01	< 0.01	0.39	0.65
QOL	41.1 ± 15.3	56.7 ± 14.8	0.01	66.5 ± 24.5	< 0.01	0.02	41.0 ± 22.3	49.2 ± 20.9	0.03	73.1 ± 19.7	< 0.01	< 0.01	0.15	0.21
IKDC														
Subjective	48.6 ± 12.1	64.5 ± 10.6	< 0.01	64.0 ± 22.9	< 0.01	< 0.01	49.0 ± 14.9	64.9 ± 9.9	< 0.01	76.3 ± 16.8	< 0.01	< 0.01	0.97	0.16
Objective			< 0.01		< 0.01	0.02			< 0.01		< 0.01	< 0.01	0.71	0.23
A	0	9		14			0	7		15				
B	10	11		9			6	11		7				
C	10	5		2			13	5		3				
D	5	0		0			6	2		0				
Marx	3.2 ± 2.7	6.8 ± 2.9	< 0.01	8.8 ± 2.8	< 0.01	< 0.01	3.8 ± 0.36	7.1 ± 3.3	< 0.01	9.3 ± 3.4	< 0.01	< 0.01	< 0.83	0.71
Tegner	2.7 ± 1.7	3.8 ± 1.7	< 0.01	4.8 ± 2.3	< 0.01	0.02	3.0 ± 1.3	3.7 ± 1.5	0.01	4.9 ± 1.8	< 0.01	< 0.01	0.86	0.69

[a]Mean ± SEM. PRE, pretreatment; VAS, visual analog scale; KOOS, Knee injury and Osteoarthritis Outcome Score; ADL, activities of daily living; QOL, quality of life; IKDC, International Knee Documentation Committee.
[b]Intergroup comparisons between S1 and S2 groups (absolute values) were performed by Mann-Whitney test.
[c]Nonparametric Wilcoxon tests were performed to investigate the significance in improvement for each variable within time evaluation.

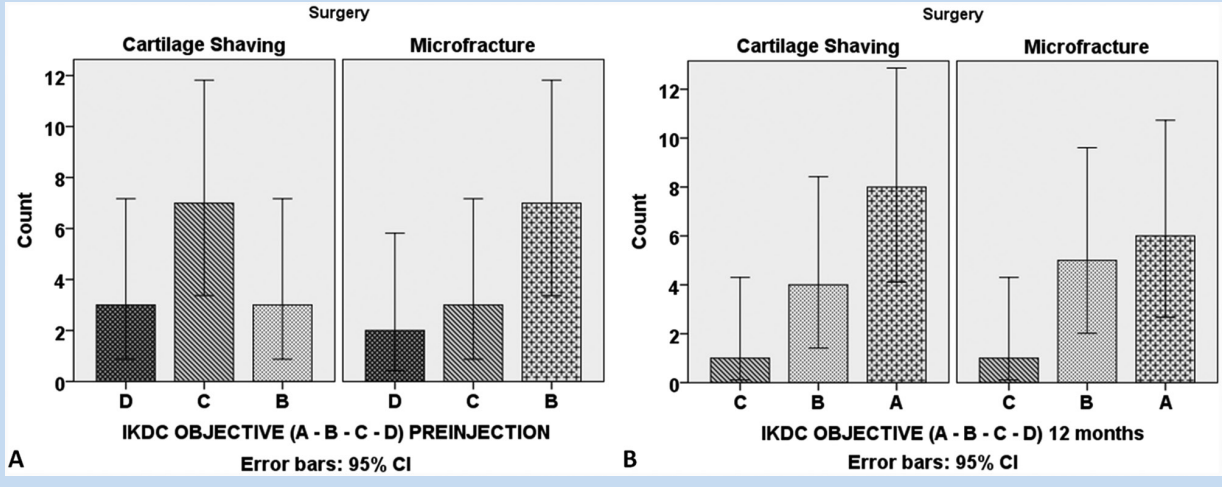

Figure 4. Graphs showing International Knee Documentation Committee objective score significant improvement ($P < .01$) from (A) preinjection to (B) 12-month follow-up for patients who had undergone either previous cartilage shaving (Sla) or microfracture (Slb).

therefore, we could not estimate any differences between our subgroups. Statistical analysis did not reveal any significant difference in improvement in Tegner, Marx, and KOOS sports scores between subgroups (Table 5). Our results are in accordance with other preliminary reports[12] and show that PRP injections can represent a valuable treatment in athletes as well.[33,53] Effective January 2011, the World Anti-Doping Agency and the US Anti-Doping Agency have removed PRP from their prohibited lists, following lack of current evidence concerning the use of these methods for performance enhancement beyond a potential therapeutic effect.[61]

Patients with previous cartilage shaving (S1a) and microfracture (S1b) showed significant improvement in all scores at 6 and 12 months ($P < 0.01$). Comparison of patients who underwent cartilage shaving and microfracture did not reveal any difference in improvement. Consequently, intra-articular PRP injections could improve postoperative clinical outcome in these patients. Cartilage shaving is known to provide symptomatic pain relief with no actual hyaline tissue formation.[39] However, this technique removes superficial cartilage layers, which include collagen fibers that are responsible for the tensile strength, thereby creating a less functional cartilage tissue.[39] Recent reports suggest that cartilage shaving is not effective in patients with severe cartilage lesions of 3 and 4 grade of International Cartilage Repair Society classification.[64] Microfracture may stimulate production of hyaline-like tissue with variable properties and durability by decreasing pain and disability. Recent studies demonstrate that these techniques produce fibrocartilaginous tissue, which degenerates with time.[21,42] Our patients, who had undergone microfracture at the time of PRP treatment, had OA of 2 and 3 grade of Kellgren-Lawrence classification (Table 2). We did not investigate the reason of microfracture failure, because the sample of the patients was not adequate for analysis. Regardless of the reason for previous surgery failure, all patients showed significant improvement at 6 and 12 months. Therefore, PRP injections could be considered as an adjuvant in postoperative treatment of these patients. Milano et al,[41] in an animal study, suggested that PRP showed a positive effect on cartilage repair and restoration after microfracture, although none of their experimental treatments produced hyaline cartilage. In our patients, we did not investigate the improvement of cartilage lesions utilizing magnetic resonance imaging and/or biopsy at final follow-up.

Platelet concentration varies widely in end-product PRP prepared by the different commercially available systems,[37] and the impact on the efficacy of the PRP product is not known. The differences in PRP products (centrifugation, platelets concentration, and presence of leukocytes and erythrocytes) could be a reason for the different results in various clinical applications.[26] In our study, we used a commercially available system (Regen ACR-C), which is a leukocyte-rich and PRP according to the Dohan Ehrenfest et al classification.[14] The pretreatment blood analysis of our patients showed an average platelet count of 261 000 platelets/μL (ranging from 164 000 to 305 000 platelets/μL). After centrifugation of 8 mL of peripheral blood, we had a platelet recovery of > 95% and a leukocyte recovery of 58% (mononuclear cell recovery, 93%) in 4 mL of PRP; therefore, we obtained approximately a 2-fold increase of platelets.[36] The system we used did not include a second centrifugation step to further concentrate platelets by removing poor platelets plasma. The advantage was that we avoided manipulation-induced platelet stress by second centrifugation and did not remove GFs contained by poor platelets plasma.[37] Therefore, we obtained a PRP preparation with a high platelet recovery and a good GF content from a small volume of blood.[36,37] Additionally, the close circuit system we utilized contributes to the safety of the procedure. We did

not activate PRP prior to injection with induce rapid fibrin clot formation, because activation could actually decrease their availability, compared with collagen activation of platelets.[5,26] Platelet concentration in our PRP solution is similar to the PRP concentration obtained by the Anitua[5] technique and that utilized by other researchers (approximately 2.5-fold increase).[58] This level of platelet count may provide optimal benefit. Studies have shown that too high a concentration of platelets may have paradoxical inhibitory effects.[23,59,63] The dose-response relationship between GF concentration and the biological processes that GFs stimulate is not linear. Once cell surface receptors for a specific GF are occupied, additional concentrations of GFs provide no additional effect.[2] GFs can exert an inhibitory effect once a high-enough concentration is reached.[2,47] Clinical efficacy of PRP preparations is expected to show, at minimum, a 2- to 6-fold increase of platelet count from baseline value.[15,16,30,37,58,59]

The main limitation of our study was that we did not include a control group. A second limitation was that we followed our patients for a minimum of only 12 months; long-term follow-up should also be carried out.

CONCLUSIONS

A number of viable biological approaches have been made available to prevent progression to OA. PRP represents a user-friendly therapeutic application that is well tolerated and shows encouraging preliminary clinical results in active patients with knee OA. Patients who underwent previous cartilage shaving and/or microfractures also showed favorable results, indicating that PRP could be an additional therapy for these patients.

Standardization of PRP protocols, long-term follow-up, and prospective blinded randomized studies should clarify questions regarding PRP effectiveness and durability of clinical improvement.

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