The PRP Question

Platelet-rich plasma (PRP) has been a focus of researchers and clinicians for quite some time with mixed results reported. From stunning laboratory animal success to disappointing clinical studies, a clear picture has not emerged.² Yet, the enthusiasm for cell-based therapies is understandable. The potential to unlock a magic array of growth factors from readily available cell sources generates excitement for those treating injuries and musculoskeletal degeneration or performing surgical procedures. Added pressure to use these preparations comes from the public that is well aware of PRP’s potential because of reports like that on the front page of a New York newspaper detailing the miraculous recovery of a Pittsburgh Steeler before the 2009 Super Bowl.³ Unfortunately, moving the positive developments from laboratory animals and Petri dishes to the patient is a daunting, complex transformation.

Consequently, the need for well-designed PRP translational research has never been more apparent. When moving from the easily controlled environs of the laboratory, the magnitude of the challenge expands. Patients come in all shapes, sizes, and ages. Their medical complexities highlight the challenges of streamlining an environment for a clinical trial. Controlling the multitude of human factors and variables appears almost impossible at times. It takes a monumental effort, usually by an experienced skilled research team, to design a trial that can often only answer one clinical question: Can the therapy improve a patient’s status when confounding variables are controlled?

My hat is off to those who accept the challenge, design a trial, and begin the task of securing funding. The review process for clinical research studies at the federal level is daunting. The time, effort, and commitment are staggering—much more than what most outside of research realize. So, it is no surprise that modern medicine is not as evidence based as most leaders in medical care would like to see. The paucity of properly designed randomized clinical trials is certainly understandable given the resources and effort needed to bring them to fruition.

So, in today’s world, short of randomized clinical trial-generated Level I evidence, practitioners often rely on the best evidence available. This is where a good clinician is invaluable: one who is able to balance the needs of patients with the risks and potential of new therapies. Too often this step is undervalued. This is where knowledge, practical experience, and ethics should merge for patient care. Unfortunately, this is also where the dark side of medicine can appear. Subjecting patients to unproven therapies unnecessarily or failing to balance the risk:benefit ratio because of financial incentives should not happen, but does. Clinicians must always remember their pledge to “do no harm.” When clinicians lose sight of what’s best for the patient, the health care delivery system is harmed, and these events encourage government agency intrusion into the practice of medicine to protect the public.

While the regulatory role of the Food and Drug Administration has a place in our medical delivery system, it must not distort the patient-physician relationship that is the basis for modern medicine.

Two initial studies set the stage for the frenzy that now surrounds PRP in sports medicine. The 2006 study by Mishra and Palvelko⁴ with resistant elbow tendinosis and the Achilles surgical repair report by Sanchez et al⁵ highlighted the potential of these preparations. Consequently, PRP is being added to the treatment protocol for many surgical and nonsurgical problems that involve muscle, tendon, ligament, and bone.

One of the most recent targets of PRP treatment has been the ubiquitous problem of early osteoarthritis (OA) after joint injury, especially in athletic joint injury. The study by Gobbi et al⁶ is an excellent example of the clinical evaluation process in working order: a credible research team with an exciting intervention tackling a daunting, expanding clinical problem—osteoarthritis. As the authors clearly point out, it is not a perfect study because of its limitations: mainly, no control group. The lack of a control group makes it difficult to put this study into perspective. Without a control group of OA patients in the same environment, it is hard to know how much better this PRP treatment was than the natural history of OA. Even so, it’s difficult to dismiss the 6- and 12-month improvement seen in the Knee injury and Osteoarthritis Outcome Score, International Knee Documentation Committee, and Marx scores for males and females, with or without surgery. Similar results were reported very recently in a Level II evidence study by Filardo et al⁷ of 144 symptomatic patients treated with 3 PRP injections. Prospective follow-up at 2, 6, and 12 months showed significant clinical improvements.

Are these results too good to be true? I hope not, but we can’t simply rely on this level of proof to justify large-scale inclusion of PRP into OA treatment protocols. These authors did a reasonable job of “translating” the process of clinical research further down the road. We clearly need larger, better-designed studies with adequate controls to fully answer the question of where this sits in the treatment of OA. Level I and II studies can
supply better clinical information to help decide if and when PRP has a role in the treatment of OA. Scientific methods of investigation should always be the cornerstone of our research efforts. Balance of the risk:benefit ratios for those in dire straits is frequently difficult because of the “slippery slope” on which many lie. Throughout this process, we must be willing to divulge any potential conflicts to our research endeavors. We are human and capable of bias and poor judgment. If, however, we are transparent in our efforts, others will be capable of fairly judging our work. As physicians, if we don’t follow these principles in deciding treatment protocols, we can expect the decision making to be taken out of our hands. That would be a sad day for modern medicine!

—Edward M. Wojtys, MD

Editor-in-Chief

REFERENCES


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. Once cartilage is injured, it gradually degenerates, leading to osteoarthritis (OA).

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...
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.7,24,25,27,65 The high cost of bone and cartilage pathologies has influenced the trend toward preventive interventions and therapeutic options that regenerate tissue homeostasis3,4 and retard progression to OA. Platelet-rich plasma (PRP) is one therapeutic application with promising preliminary clinical results.30,31,38

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.26,38 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, batroxobin36), 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 proportionally 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,57 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.5,36 GFs mediate the biological processes necessary for repair of soft tissues,9,10 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.32,35 In vitro studies in animal and human chondrocytes4,6 have demonstrated that PRP-secreted GFs stimulate proliferation and collagen synthesis. Animal studies have demonstrated clear benefits in terms of accelerating healing8,9 and anti-inflammatory action. More interesting, their positive effect in OA-affected animal joints, by stimulating cartilage matrix metabolism, has been reported.17,40 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.50,60,62,66,67 Recent studies 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,
coagulation profile, and test for transmissible 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 Marx35 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 step36,37 (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 analysis was used to perform the comparisons between the 2 groups and the subgroups (S1, S1a, S1b, S2). The ANOVA model included the following factors: (1) the treatment group (S1, S1a, S1b, S2) and (2) the follow-up period (pretreatment, 6-month, 12-month). Within each subgroup, the scores were compared by using the Wilcoxon rank sum test. The level of significance was set at P < 0.05.

---

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

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

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

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

*At the time of surgery.
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 χ² 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 α = 0.05 and power = 0.80, considering a standard deviation of 20.54 Therefore, we included 50 patients in our study.

RESULTS

The χ² 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

<table>
<thead>
<tr>
<th>Inclusion</th>
<th>Exclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age between 30 and 60 years, body mass index &lt; 30, normal results for complete blood count and coagulation control, minimum follow-up of 1 year</td>
<td>Patients with blood diseases, systemic metabolic, immunodeficiency, hepatitis B or C, HIV-positive status, infection and sepsis, local infection</td>
</tr>
<tr>
<td>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)</td>
<td>Patients with advanced and tricompartmental osteoarthritis, rheumatoid or polyarticular arthritis, symptomatic hip osteoarthritis, or symptomatic contralateral knee osteoarthritis</td>
</tr>
<tr>
<td>Patients with severe pain and under anti-inflammatory treatment without improvement &gt; 3 months</td>
<td>Significant joint swelling or clinical signs of acute inflammation (possible inflammation or infection)</td>
</tr>
<tr>
<td>Patients with stable knees, normal tibiofemoral alignment, or patellofemoral tracking</td>
<td>Varus-valgus malalignment above 5°, patellofemoral maltracking or untreated instability, and total or subtotal meniscectomy (&gt; 2/3 excised)</td>
</tr>
<tr>
<td>Patients with or without previous cartilage shaving and microfracture (other interventions were excluded)</td>
<td>Pretreatment blood platelets value 25% below the reference value or alcoholism, smoking, drugs</td>
</tr>
<tr>
<td>Patients who gave consent for treatment with platelet-rich plasma per our protocol</td>
<td>Treatment with corticosteroids &lt; 3 months or medication &lt; 7 days that could interfere with platelet aggregation</td>
</tr>
</tbody>
</table>
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.
Recent studies have documented the effectiveness of GFs in chondrogenesis and prevention of joint degeneration 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. 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 successfully cultured porcine chondrocytes with PRP, showing higher cell proliferation and proteoglycans and collagen synthesis. Moreover, Wu et al 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, clinical and histologic improvement has been reported in OA-affected joints after treatment with platelet rich plasma. Frisbie et al reported clinical and histologic improvement in OA-affected joints of horses after treatment with PRP. Saito et al 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.

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

\(^a\) 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.
Kon et al. 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. Other authors 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. 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). Although worse results have been reported for female patients in other studies, we found no significant difference in improvement between men and women (Mann-Whitney test). No adverse reactions (e.g., acute pain and swelling) or major complications (e.g., infection) were noted. This is in accordance with other study reports 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. *

<table>
<thead>
<tr>
<th>Variable</th>
<th>S1: Patients With Previous Surgery</th>
<th>S2: Patients Without Previous Surgery</th>
<th>S1 vs S2, (^*) P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PRE</td>
<td>6 mo</td>
<td>0-6 mo, (^*) P</td>
</tr>
<tr>
<td>VAS</td>
<td>3.2 ± 1.4</td>
<td>1.9 ± 1.7</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>KOOS Pain</td>
<td>75.4 ± 9.9</td>
<td>84.5 ± 11.2</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Symptoms</td>
<td>70.9 ± 13.9</td>
<td>80.0 ± 12.7</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>ADL</td>
<td>83.1 ± 9.4</td>
<td>91.3 ± 6.2</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Sport</td>
<td>39.7 ± 22.8</td>
<td>58.2 ± 19.9</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>QOL</td>
<td>41.1 ± 15.3</td>
<td>56.7 ± 14.8</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>IKDC Subjective</td>
<td>48.6 ± 12.1</td>
<td>64.5 ± 10.6</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Objective</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>0</td>
<td>9</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>B</td>
<td>10</td>
<td>11</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>C</td>
<td>10</td>
<td>5</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>D</td>
<td>5</td>
<td>0</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Marx</td>
<td>3.2 ± 2.7</td>
<td>6.8 ± 2.9</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Tegner</td>
<td>2.7 ± 1.7</td>
<td>3.8 ± 1.7</td>
<td>&lt; 0.01</td>
</tr>
</tbody>
</table>

*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.

*Intergroup comparisons between S1 and S2 groups (absolute values) were performed by Mann-Whitney test.

*Nonparametric Wilcoxon tests were performed to investigate the significance in improvement for each variable within time evaluation.
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 and show that PRP injections can represent a valuable treatment in athletes as well. 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.

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. 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. 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. 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. 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., 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, 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. 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. 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. 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. Therefore, we obtained a PRP preparation with a high platelet recovery and a good GF content from a small volume of blood. Additionally, the close circuit system we utilized contributes to the safety of the procedure. 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., 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, 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. 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. 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. 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. Therefore, we obtained a PRP preparation with a high platelet recovery and a good GF content from a small volume of blood. Additionally, the close circuit system we utilized contributes to the safety of the procedure. 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., 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.
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.5,10 Platelet concentration in our PRP solution is similar to the PRP concentration obtained by the Anitua technique and that utilized by other researchers (approximately 2.5-fold increase).58 This level 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.

ACKNOWLEDGMENTS
We thank Andrea Primo, professional statistician, for statistical analysis of our study and Dr Anup Kumar, fellow at OASI Bioresearch Foundation, for his help.

REFERENCES
58. Tiwari A, Rooyackers OA, Stasko AL, Burns AL, Seker R. Platelet-derived growth factors (PDGF), basic fibroblast growth factor (bFGF) and insulin-like growth factor-I (IGF-I) stimulate the proliferation of intervertebral disc cells in vitro via the activation of the ERK and Akt signaling pathways. *Eur Spine J*. 2007;16(11):1858-1866.
64. Yamaguchi R, Tsushima H, Yoneyama S, Tadano S, Ohkohchi N. Effects of platelet-rich plasma on intestinal anastomotic healing in rats. PRP concentration is a key factor [published online ahead of print November 2, 2010]. *J Surg Res*.