Abstract

The purpose of our study was to determine the efficacy of Platelet-Rich Plasma (PRP) treatment in symptomatic patients with early knee osteoarthritis.

Material and Methods: We prospectively followed up 80 patients with knee OA (Kellgren-Lawrence classification grade 1-3) for a minimum follow up of 24 months. Mean age of patients was 47.7 years. All patients were treated with 2 intra-articular injections at monthly interval with autologous PRP. Twenty-two of the patients repeated the 2 infiltrations at 6 and 12 months and 25 of the patients repeated the 2 infiltrations at 12 and 18 months, while 33 patients had only the initial 2 infiltrations. Half of the patients included in this study had undergone a previous operative intervention for cartilage lesions; previous cartilage interventions included cartilage shaving and microfracture. KOOS, VAS, Tegner, IKDC and MARX scores were collected at pre-treatment evaluation and at 6, 12, 18 and 24-month follow-up.

Results: All patients showed significant improvement in all the scores at final follow up (p < 0.005) and returned to previous activities. There was no significant difference in improvement between operated and non-operated patients as well as between male and female patients. Patients who repeated the treatment protocol showed significant better clinical outcome at final follow-up.

Conclusion: This study shows that PRP intraarticular knee injections is effective both in operated and non-operated arthritic knees and can act as a preventive agent of OA, by diminishing pain and improving symptoms and quality of life. Patients who repeated PRP treatment preserved a better clinical outcome, indicating that PRP injections can provide a better outcome when repeated.

Key Words: Knee, Cartilage, Chondral Lesion, PRP
INTRODUCTION

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 a limited intrinsic healing potential due to the fact that it is avascular and the presence of 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 over 40 years of age) and often causes persistent pain. OA incidence increases steadily with age, affecting 12.1% of the population from 25 to 74 years of age, and is the leading cause of physical disability in people over the age of 65 years. Community-based studies have shown that 10% of the population over the age of 55 has troublesome knee pain, and among these 25% is severely disabled.

Many conservative treatment options, such as oral and topical non-steroidal anti-inflammatory drugs (NSAIDs), 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. The high cost of bone and cartilage pathologies has influenced the trend towards preventive interventions and therapeutic options that regenerate tissue homeostasis and retard progression to OA. Platelet-rich plasma (PRP) is one therapeutic application with promising preliminary clinical results. Platelet-rich plasma can be defined as the volume of the plasma fraction from autologous blood with platelet concentration above baseline count (200,000 platelets/µl). Platelets contain many important bioactive proteins and growth factors (GF). These factors regulate key processes in tissue repair, including cell proliferation, chemotaxis, migration, cellular differentiation and extracellular matrix synthesis. The rationale for the use of PRP is to stimulate the natural healing cascade and tissue regeneration by a “supra-physiological” release of platelet-derived factors directly at the site of treatment. Autologous PRP can be obtained from simple blood extraction using 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 animals derived), calcium chloride or pro-coagulant enzyme, i.e. Batroxobin, 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 intraarticular collagen. GF have a half-life from minutes to hours. Previous thrombin activation
could actually decrease their availability when compared to collagen activation of platelets. \(^5,^{26}\) In general, the amount of GF delivered is not necessarily proportional to the platelet count, because of their high variability in platelets among individuals. \(^11,^{61}\) The concentration of platelets and platelets-derived GF is different in commercially available medical devices to prepare PRP according to manufacturer \(^39\) 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 two- to six-fold of platelets count from baseline value. \(^15, 38, 39, 60\)

Platelets α-granules contain a variety of GF including transforming growth factors (TGF-β1), platelet-derived growth factors (PDGF-BB), hepatocyte growth factor (HGF), basic fibroblast growth factors (b-FGF). Epidermal growth factor (EGF), vascular endothelial growth factors (VEGF) and insulin-like growth factor 1 (IGF-I). \(^39\) GF 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 growth-factor-receptor that, 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 GF stimulate proliferation and collagen synthesis. Animal studies have demonstrated clear benefits in terms of accelerating healing \(^6,^{40}\) and anti-inflammatory \(^8\) action. More interestingly, 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 \(^30, 51,^{59}\) 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 have undergone a previous operative intervention for cartilage lesions (cartilage shaving and/or microfracture), and patients who did not undergo any previous operative intervention for the knee.

**MATERIALS AND METHODS**

We prospectively followed 80 patients with symptomatic knee OA of grade 1-3 of Kellgren-Lawrence classification (Table 1). All patients (49 males and 31 females) were treated with two intra-
articular injections (once monthly) with autologous PRP (Regen® ACR-C, Regen Lab, Switzerland) and followed up for a minimum period of 24 months. Based on senior author’s previous observations and experience, our 2 PRP-injections protocol was repeated in patients who had persistent symptoms. Although there was no significant deterioration of the clinical outcome 20 of the patients repeated the 2 infiltrations at 6 and 12 months, 25 of the patients repeated the 2 infiltrations at 12 and 18 months and 35 patients had only the initial 2 infiltrations. The mean age of patients was 47.7 years, ranging from 30 to 60 years, and BMI was 25.5 (SD 2.7). All patients were involved in various sports activities, but not at a professional level. Inclusion-exclusion criteria are reported in Table 2. Forty patients (50%) had undergone a previous operative intervention for cartilage lesions of grade 3 and 4 of ICRS classification (Table 1) repair on ipsilateral knee at least one year before PRP treatment, while 40 patients did not undergo any previous operative intervention for the knee. Average time from previous surgery to treatment was 20.2 months (SD 15.1), ranging from one to three years. Previous operative interventions for cartilage included cartilage shaving and microfracture for grade 3 and 4 cartilage lesions (ICRS classification).

Table 1: Kellgren-Lawrence and ICRS Classification

<table>
<thead>
<tr>
<th>Grade</th>
<th>Kellgren-Lawrence</th>
<th>ICRS</th>
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<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>
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<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>
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The standard radiographic evaluation included a standing AP long-leg radiograph, including both hips and ankles, standing AP/lateral views of knees, skyline patellofemoral and standing 45 degrees flexion knee views and magnetic resonance imaging (MRI). Standard blood investigations including complete blood count (CBC), coagulation profile, and test for transmittable diseases were done before treatment. Visual analogue scale (VAS) for pain (0=no pain at all to 10=worst pain), International Knee Documentation Committee subjective and objective score (IKDC), Knee Injury and Osteoarthritis Outcome Score
(KOOS), 48 Tegner, 57 and Marx 34 scores were collected at pre-treatment evaluation and at 6, 12 and 24-month follow-up.

Table 2: Inclusion-Exclusion Criteria

<table>
<thead>
<tr>
<th>Inclusion Criteria</th>
<th>Exclusion Criteria</th>
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<tr>
<td>Age between 30 and 60 years, Body Mass Index (BMI) &lt; 30, normal Complete Blood Count (CBC) and Coagulation control, minimum follow up of one year</td>
<td>Patients with: blood diseases, systemic metabolic, immunodeficiency, Hepatitis B or C, HIV positive, infection and septicemia, local infection</td>
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<td>Patients with symptomatic osteoarthritic knees (Kellgren-Lawrence grade 1-3 based on x-rays findings) and partial- or full-thickness cartilage lesions (ICRS grade 3-4 based on MRI findings)</td>
<td>Patients with: advanced and tricompartmental OA, rheumatoid or polyarticular arthritis, symptomatic hip OA or symptomatic contralateral knee OA</td>
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<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>
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<tr>
<td>Patients with stable knee, normal tibio-femoral 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>Pre-treatment blood platelets value 25% below the reference value / alcoholism, smoking, drugs</td>
</tr>
<tr>
<td>Patients who gave consent for treatment with PRP as per our protocol</td>
<td>Treatment with: corticosteroids &lt; 3 months / medication That could interfere with platelet aggregation &lt; 7 days</td>
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Technique

All patients were treated with two intra-articular injections of autologous PRP (one month interval between injections). After extraction of 8 ml of peripheral blood, the sample was centrifuged for nine minutes at 3,500 rpm according to recommendations of the manufacturer. The system that we used did not include a second centrifugation step 38, 39 (Figures 1A-B). Subsequently, we obtained 4 ml of PRP and we proceeded to the intrarticular infiltration by a supra-patellar approach under sterile aseptic conditions (Figures 1C-D). A topical anesthetic skin refrigerant was applied locally prior to the injections. We did not activate PRP prior to injection to induce rapid fibrin clot formation. After treatment, patients were allowed
weight-bearing and local ice application was recommended 20 minutes every 2-3 hours for 24 hours. We recommended restriction of vigorous activities of the knee for at least 48 hours.

**Statistical Analysis**

Statistical analysis was performed by an independent statistician (AP), who was blinded to the sample and subgroups, using SPSS software (SPSS 17.0, SPSS, Chicago, Illinois, USA). General Linear Model-Repeated Measure test was performed to investigate time improvement in KOOS, VAS, Tegner, IKDC, and MARX scores from pre-treatment to 6 and 12 and 24 months follow-up. A post-hoc test with Bonferroni adjustment for multiple comparisons was performed to investigate the improvement for each variable for the total sample. A Chi-Square test was performed in order to investigate whether S1 and S2 subgroups were homogenous regarding Kellgren-Lawrence grade of OA. Post-hoc tests were performed with the Wilcoxon rank test to evaluate improvement from pre-treatment to 6, 12 and 24-month follow-up for each subgroup. The nonparametric Mann-Whitney test was performed to analyze the difference in improvement between operated and non-operated patients and between male and female patients. Mann-Whitney test was used to detect any difference in improvement between patients who underwent cartilage shaving and microfracture. Reported p-values are two-tailed with an alpha level of 0.05 indicating significance. A power analysis determined the number of required patients. International Knee Documentation Committee (IKDC) subjective score was defined as the primary parameter. An improvement of 10 points was considered clinically important. A sample size of 43 patients was required for alpha=0.05 and power = 0.80 considering a standard deviation of 20.

**Figure 1:** PRP preparation: A) blood aspiration B) centrifugation of the blood sample for 9 min C) fraction of PRP after centrifugation (yellow upper part in tube) and D) knee intrarticular infiltration.
RESULTS

All patients showed significant improvement in all scores at 6, 12 and 24-month follow-up (p < 0.05) and returned to previous activities including recreational sports. No adverse reactions (like swelling or acute pain) or any major complication (like infection) were noted. Each subgroup showed significant improvement from pre-treatment to 6, 12 and 24-month follow-up as well (p < 0.05). Patients who repeated PRP treatment preserved a better clinical outcome at 2 year follow-up. Patients who did not have previous surgery did not show improvement in KOOS-symptoms and KOOS-sports (from pre-treatment to six months). Mann-Whitney test showed significant difference in improvement between operated and non-operated patients only in KOOS (Sport) and KOOS (QOLS) scores; S1 group patients showed higher reduction in KOOS (Sport) and S2 group patients showed higher improvement in KOOS (QOL) score, at 24-month follow-up. Patients treated with cartilage shaving did not show a significant difference in improvement from patients who were treated with microfracture. There was no significant difference in improvement between male and female patients. All patients returned to their previous level of sporting activity, which varied between patients. Statistical analysis did not reveal any significant difference in Tegner, Marx, and KOOS scores between S1 and S2 subgroups at 6, 12 and 24-month follow-up.

DISCUSSION

The purpose of this study was to investigate the effectiveness of intrarticular PRP injections in active patients with symptomatic knee OA in terms of diminishing pain, improving quality of life and return to previous activities. All patients showed significant improvement in all scores at 6, 12 and 24 months follow-up (p < 0.05) displaying that PRP injections could 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 GF in chondrogenesis, by controlling the synthesis and degradation of extracellular matrix proteins. Their mode of action is to bind to the extracellular domain of a target growth-factor-receptor that, in turn, activates the intracellular signal-transduction pathways. The elucidation of some of the functions of GF 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\(^6\) in an experimental animal study, showed the effectiveness of intrarticular 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 histological 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 platelet rich plasma. Shaito et al\(^{9}\) reported significantly suppressed progression of OA morphologically and histologically in a rabbit model after administration of intrarticular injections of PRP in gelatin hydrogel microspheres. These preventive effects were attributed to stimulation of cartilage matrix metabolism caused by the GF contained in PRP. Anitua et al\(^3\) 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\(^{45}\) has 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.

Kon et al\(^{30}\) have reported interesting observations on PRP treatment in patients with chronic symptomatic degenerative condition of the knee. They demonstrated positive effects on the function and symptoms with an 85% improvement in scores for patients treated with three PRP intra-articular injections weekly with median age less than 60 years. 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 (HA); the results deteriorated over 12-24 months of follow-up.\(^{16,31}\) Other authors\(^{20,51,59}\) also 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 BMI), exercise and lifestyle, can act as a preventive agent in chronic and degenerative musculoskeletal disease.\(^{20}\) These results are in accordance with the results of the present study; all our patients showed significant improvement at 1 and 2-year follow-up; however, although there was no significant deterioration of the results at 6 and 12-month follow up 20 patients repeated our protocol at 6 and 12 months, while 25 of the remaining patients repeated the infiltrations at 12 and 18 months to preserve the good clinical outcome. In our study, patient ages ranged from 30 to 60 years, and patients with advanced osteoarthritis were excluded. Patients did not have associated pathologies such as knee instability or tibiofemoral and patellofemoral
malalignment that can affect the clinical outcome and predisposes to OA while increasing functional loads on the knee.\textsuperscript{10, 22} Although worse results have been reported for female patients in other studies,\textsuperscript{16} we found no significant difference in improvement between male and female patients (Mann-Whitney test). No adverse reactions like acute pain and swelling or major complications like infection were noted. This is in accordance with other study reports,\textsuperscript{30, 51, 59} and empowers the safety profile of autologous PRP intrarticular injections.

All our patients were active in sports and they obtained more than 50% improvement in Tegner, Marx, and KOOS-sports scores from pre-treatment to final follow-up evaluation and returned to their previous sporting activities. Patients were involved in sports in varied frequency; therefore, we could not estimate any differences between the subgroups we studied. Statistical analysis did not reveal any significant difference in improvement in Tegner, Marx, and KOOS-sports scores between subgroups. Our results are in accordance with other preliminary reports\textsuperscript{12} and show that PRP injections could represent a valuable treatment in athletes as well.\textsuperscript{33, 52} Effective January, 2011, WADA and USADA (International and United States anti-doping agencies) 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\textsuperscript{58}.

Patients with previous cartilage shaving and microfracture showed significant improvement in all scores at 6, 12 and 24-month follow-up (p<0.05). Comparison of patients who underwent cartilage shaving and microfracture did not reveal any difference in improvement. Consequently, intrarticular PRP injections could improve post-operative 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 creating a less functional cartilage tissue.\textsuperscript{36} Recent reports suggest that cartilage shaving is not effective in patients with severe cartilage lesions of 3 and 4 grade of ICRS classification.\textsuperscript{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.\textsuperscript{21, 42} Our patients, who had previously undergone microfracture at the time of PRP treatment, had OA of 2 and 3 grade of Kellgren-Lawrence classification. 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, 12 and 24-month follow-up. 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 MRI 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 leucocytes and erythrocytes) could be a reason for the different results in various clinical applications. In our study, we used a commercially available system, which is a leukocyte-PRP according to the Dohan Ehrenfest, et al classification. The pretreatment blood analysis of our patients showed an average platelet count of 220,000 platelets/µl (ranging from 170,000 to 352,000 platelets/µl). After centrifugation of 8 ml of peripheral blood, we had a platelet recovery of >95% and leukocytes recovery of 58% (mononuclear cells recovery 93%) in 4 ml of PRP, therefore we obtained approximately a two-fold increase of platelets. The system we used did not include a second centrifugation step to further concentrate platelets by removing poor platelets plasma (PPP). The advantage was that we avoided manipulation-induced platelet stress by second centrifugation and we did not remove GF contained by PPP. Therefore we obtained a PRP preparation with a high platelet recovery and a good growth factor content from a small volume of blood. Additionally the close circuit system we utilized contributes to the safety of the procedure. We did not activate PRP prior to injection to induce rapid fibrin clot formation because activation could actually decrease their availability compared to collagen activation of platelets. Platelet concentration in our PRP-solution is similar with PRP concentration obtained by the Anitua technique and utilized by other researchers (approximately 2.5-fold increase). This level of platelet count may provide optimal benefit. Studies have shown that too high a concentration of platelets may have paradoxical inhibitory effects. The dose-response relationship between GF concentration and biological processes they stimulate is not linear. Once cell surface receptors for a specific GF are occupied, additional concentrations of GF provide no additional effect. GF can exert an inhibitory effect once a high enough concentration is reached. Clinical efficacy of PRP preparations is expected to show, at minimum, a two- to six-fold increase of platelets count from baseline value.
A number of viable biological approaches have been made available to prevent progression to OA. PRP represents a user friendly therapeutic application, which is well-tolerated and shows encouraging preliminary clinical results in active patients with knee OA. Patients who repeated PRP treatment preserved a better clinical outcome, indicating that PRP injections can provide a better outcome when repeated. Standardization of PRP protocols, long-term follow-up and prospective, blinded, randomized studies should clarify some of the questions regarding PRP effectiveness, and durability of clinical improvement.

REFERENCES


