The Effects of Platelet-Rich Plasma (PRP) in the Management of Knee Osteoarthritis
A Review of Current Literature with Long Term Follow-Up

A Capstone Project submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements for the degree of Master of Physician Assistant Studies

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Abstract

Background: Symptomatic osteoarthritis (OA) of the knee is a common and debilitating degenerative condition causing significant pain and functional limitations. Previous conservative modalities have been unable to provide longer-term symptomatic relief. Autologous blood products, such as platelet-rich plasma (PRP), may provide extended benefits to patients with knee OA due to the proposed disease-modifying and anti-inflammatory effects. This literature review aims to investigate and consolidate current literature on the potential long-term therapeutic effects of PRP (greater than twelve months) in osteoarthritis of the knee. A secondary goal is to identify which demographic of patients sustain longer-lasting results and thus may optimally benefit from conservative management with intra-articular PRP.

Methods: A review of the literature was undertaken utilizing the PubMed database to identify articles examining the duration of beneficial effects following intra-articular PRP injection on knee osteoarthritis. Primary studies were reviewed within the past ten years (2011-2021) with follow-up periods greater than twelve months. A total of five articles were included in the final literature review.

Results: There was significant variability in the overall study design of the five articles of interest included in this review. All studies utilized clinical scoring systems, which demonstrated an improvement in scores post intra-articular PRP injection. However, a decline in scores was observed at follow-up periods beyond twelve months. The longest duration of action was found in male patients with less advanced degrees of osteoarthritis.

Conclusion: PRP is a promising and safe option for the management of knee osteoarthritis. However, the long-term therapeutic effects were observed to be ill-sustained beyond twelve months post-injection in the studies of interest. Younger male patients with mild grades of

radiographic osteoarthritis demonstrated superior outcomes post intra-articular PRP injection. Repeated cyclical injections, intra-articular/intra-osseous injection protocols, and combined PRP and hyaluronic acid regimens may provide further symptomatic improvement. Due to the high degree of variability between PRP products and injection regimens utilized, a universally accepted classification system must be adopted. Additionally, high-quality studies are necessary to further delineate the proposed biological interactions of PRP in knee osteoarthritis and define future treatment protocols.

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Introduction

Osteoarthritis of the Knee: Epidemiology, Prevalence and Pathophysiology

Osteoarthritis (OA) is a complex and progressively degenerative condition causing significant pain and functional limitations in adult populations worldwide (1)(2). It is estimated that 1 in 10 Canadians suffer from osteoarthritis, with the prevalence expected to double by the year 2031 due to the advancing age of the population and the rising incidence of obesity (3). The knee joint, the largest synovial joint in the human body, is the most commonly affected, constituting 29% of Canadian cases of symptomatic osteoarthritis (2)(3). The pathophysiology of osteoarthritis is complex and poorly understood, involving an interplay of genetic, mechanical, and cellular factors, ultimately resulting in the failure of chondrocytes to maintain the intra-articular balance between cartilage matrix synthesis and degradation (2)(4). Subsequently, subchondral bone remodelling occurs with the development of vascular channels containing sensory nerve terminals and osteoblasts (2). Vascular channels facilitate communication between cartilage and bone via biochemical mediators, which initiate a cycle of cartilage destruction, triggering an inflammatory response within the synovium, resulting in further joint damage and the progression of osteoarthritis over time (2). Osteoarthritis may be defined and diagnosed clinically, radiographically, or pathologically; however, integration of these factors is optimal as not all patients presenting with radiographic findings of OA may experience symptomatic osteoarthritis (2). Despite this, plain radiography remains the mainstay of diagnosis (2)(5). The Kellgren-Lawrence (KL) radiographic grading system is commonly used in both clinical and research settings to stratify the progression of the disease by degrees of increasing severity based on progressive joint space narrowing, development of osteophytes, sclerosis and bone contour deformity as outlined in Figure 1 below (2)(4-6).

Figure 1: Kellgren-Lawrence Radiographic Grading System of Osteoarthritis (5)(6).

Grade	Radiologic Findings
0	No radiological findings of osteoarthritis
I	Doubtful narrowing of joint space and possible osteophytic lipping
II	Definite osteophytes and possible narrowing of joint space
III	Moderate multiple osteophytes, definite narrowing of joint space, small pseudocystic areas with sclerotic walls and possible deformity of bone contour
IV	Large osteophytes, marked narrowing of joint space, severe sclerosis and definite deformity of bone contour

Current Treatment Options for Knee Osteoarthritis

As osteoarthritis of the knee is a complex and progressive disorder involving an interplay of modifiable and non-modifiable factors, there are no existing curative therapies for the disease (1). Current management strategies for symptomatic knee osteoarthritis are divided into conservative and surgical interventions, with total knee arthroplasty (TKA) being the end-stage solution for severe symptomatic osteoarthritis (1)(7). While total knee arthroplasty is effective in treating severe knee osteoarthritis in older patients who are optimal surgical candidates, concerns regarding implant longevity in younger age groups have been raised (1). Approximately 20% of patients suffering from osteoarthritis fall into the treatment gap, defined as the time between the exhaustion of conservative modalities and eligibility for surgical intervention (3). These patients are often active individuals under 60 years of age with mild to moderate symptomatic osteoarthritis (KL stage 1-3) (3). Additionally, it has been suggested that patients less than 70 years of age who undergo surgical intervention are more likely to be dissatisfied with their results due to ongoing pain and inability to return to pre-treatment levels of activity (3). This has opened the door for conservative modalities to provide symptomatic relief, delay the time to

surgical intervention and optimize the benefits of total knee arthroplasty by reducing the need for a second revision surgery (1).

Conservative modalities composing the current standard of care for patients suffering from symptomatic knee OA include pharmacological strategies such as oral or topical anti-inflammatories, physical therapy, activity modification, weight loss, bracing and intra-articular injections (3)(8). Corticosteroid intra-articular injections are traditionally widely used in treating symptomatic knee OA; however, hyaluronic acid viscosupplementation and platelet-rich plasma (PRP) have been gaining increasing popularity over the last decade (1). The use of intra-articular corticosteroids strictly offers short-term symptomatic improvement in pain (less than six months), and it has been suggested that frequent use is correlated with a loss of intra-articular cartilage volume (3)(10). Hyaluronic acid (HA), a naturally-occurring constituent of synovial fluid that acts as an intra-articular shock absorber, has been shown to be deficient in knees affected by osteoarthritis (3). Thus, intra-articular viscosupplementation with HA aims to provide symptomatic relief by increasing joint lubrication, restoring viscoelastic properties and decreasing inflammation within the joint space (3)(1). Multiple formulations of HA exist with variations in molecular weight, cost and injection timing resulting in heterogeneity amongst existing randomized controlled trials studying the effectiveness of viscosupplementation in the treatment of knee osteoarthritis (3).

Platelet Rich Plasma: The Theory Behind PRP and Implications in the Treatment of Knee Osteoarthritis

Previous intra-articular options have been unable to adequately target the inflammatory cascade while promoting cartilage and bone matrix bio-synthesis and thus do not provide longer-

term symptomatic relief. This has opened the door for research initiatives investigating autologous blood products such as PRP for their potential to improve outcomes in patients with knee OA (8). PRP is an autologous blood product harvested from a venous sample of whole blood which undergoes centrifugation to achieve a platelet concentration of at least 1000 x 10^3 platelets/μL, or a three to five-fold increase compared to peripheral blood (1)(11). The therapeutic effects of PRP have been suspected to be largely due to the promotion of growth factors released from the alpha granules of platelets and the inhibition of pro-inflammatory factors, which in turn play a role in reducing inflammation while enhancing cartilage and bone matrix synthesis (11)(8). Growth factors which have been identified include platelet-derived growth factor (PDGF), transforming growth factor β-1 (TGFβ-1), fibroblast growth factor (FGF), hepatocyte growth factor (HGF) and vascular endothelial growth factor (VEGF) (11)(12). These act synergistically to shift the intra-articular balance favouring increased anabolic activity leading to chondral remodelling, synthesis of type II collagen, and stimulation of HA production while consequently decreasing potent inflammatory mediators such as Interleukin-1 (IL-1) and metalloproteinases, namely MMP-13 (11).

There has been significant prior research investigating the effectiveness of intra-articular PRP in osteoarthritis of the knee, and comparisons between PRP and existing intra-articular injective options, such as hyaluronic acid and corticosteroids, with promising results. However, as there are various formulations of PRP due to differences in preparation processes, variability in injection timing, and delivery methods, a standardized therapeutic protocol for its use does not exist at present (11). Additionally, current research initiatives have investigated the effectiveness of intra-articular PRP in treating knee osteoarthritis with a focus predominantly on the short-term therapeutic evaluation (less than twelve months follow-up). The purpose of this literature review

is to investigate and consolidate current research on the potential long-term therapeutic effects of PRP (greater than twelve months) in patients with knee osteoarthritis to establish whether the biological approach may yield superiority over existing intra-articular modalities. A secondary goal of this review is to identify which demographic of patients sustain superior long-term results and may optimally benefit from conservative management with intra-articular PRP.

Methods

A review of the literature was initiated in December 2021 by broadly searching the PubMed database to identify articles examining the duration of effectiveness of intra-articular PRP injection on knee osteoarthritis. Search terms used included "PRP" OR "platelet rich plasma" AND "knee osteoarthritis" within the past ten years (2011-2021). Primary studies of any design were examined with no language limits applied, yielding 78 unique articles. Titles and abstracts were screened, and 20 full-text articles were individually assessed for eligibility to determine their relevance to the topic and identify papers with longer-term follow-up periods (greater than twelve months). Reference lists of eligible full-text articles were reviewed to identify additional relevant studies to which one article was added. Subsequently, 5 articles were included in the final literature review. The comprehensive study selection process is outlined in Figure 1 (appendix).

Results

Study Characteristics and Population Demographics Included and Excluded

All the relevant studies selected for inclusion in this review had follow-up periods greater than twelve months. One study evaluated patients at a final assessment period of 18 months, and the remaining four extended the final assessment to at least 24 months. Research performed by

Di Martino et al. (9) had the longest follow-up time, assessing patients until a final evaluation mean of 64.3months (5.3 years) post PRP injection.

Individual population demographics in the relevant studies had slight variances in inclusion criteria such as age, BMI, severity of osteoarthritis (as defined by the Kellgren-Lawrence radiographic system) and gender. The study by Di Martino et al. (9) encompassed the broadest age range of patients, including patients aged 18-80 years. All studies of interest had a similar mean patient age of approximately 55.5 years. The Body Mass Index (BMI) of patients also varied between studies from 18-32. Additionally, all studies utilized the Kellgren-Lawrence (KL) radiographic grading system to classify osteoarthritis severity; however, the grade of osteoarthritis varied between individual studies. Filardo et al. (13) included all grades of osteoarthritis from KL-0 (degenerative chondropathy without definitive radiological findings of osteoarthritis) to IV (severe osteoarthritis). Additionally, as the most severe radiographical grades of osteoarthritis were included in this study, the authors did not exclude patients who underwent previous knee surgery. Di Martino et al. (9) opted to omit patients KL grade IV, Gobbi et al. (14) included KL grades I-II. At the same time, Xu et al. (15) and Su et al. (16) restricted their study to mild or moderate osteoarthritis (KL grades II-III). It should be noted that radiographical evidence of osteoarthritis as defined by the KL system is deemed definitively present at grade II (6). Lastly, the predominant gender was largely heterogeneous in the respective studies. Three of five articles showed a male predominance, with only studies performed by Xu et al. (15) and Su et al. (16) having a largely female participant population.

Exclusion criteria common to the selected studies were systemic diseases such as immunodeficiency, hematological or cardiovascular disease, local or systemic infection and use of non-steroidal anti-inflammatories prior to injection. All studies with the exception of Xu et al.

(15) explicitly omitted patients on active anticoagulation or antiplatelet therapy. Di Martino et al. (9) and Filardo et al. (13) additionally excluded patients with hemoglobin values less than 11g/dL and platelet counts less than 150,000/m^3, while the remaining three studies did not comment on initial blood counts of patients.

Study Design, Sample Size, PRP Composition and Comparative Interventions

Of the five articles of interest, there was significant variability in the overall study design. The study performed by Di Martino et al. (9) was an extended follow up of a previously conducted randomized controlled trial where patients who showed no significant difference between intra-articular injection of PRP and HA in the initial twelve month period were followed to a mean of 64.3 months (5.3 years). Leukocyte-rich PRP (LR-PRP) was obtained using a double centrifugation protocol from a 150mL venous blood sample. The comparison group consisted of high molecular weight hyaluronic acid. A total of 192 patients were randomized, with 96 patients allocated into PRP treatment groups and HA control groups respectively, each receiving a total of three weekly intra-articular injections.

The study by Xu et al. (15) was a double-blind prospective cohort study that compared three groups receiving PRP, HA or PRP and HA for 24 months via subjective scoring systems. Additionally, synovial fluid composition (of Interleukin-1β (IL-1β), tumour necrosis factor-α (TNF-α), matrix metalloproteinase-3 (MMP-3) and tissue inhibitor of metalloproteinase-1 (TIMP-1)) as well as ultrasound evaluation were performed but limited by a shorter follow up period of twelve months. Leukocyte-poor PRP (LP-PRP) was obtained by a double spin approach from a 36mL sample of venous blood (72mL if bilateral injections were required). Each group received three intra-articular injections spaced two weeks apart with either 4mL of PRP, 2mL of HA or 4mL of PRP plus 2mL of HA in the combination group. 150 knees

underwent initial randomization with 50 knees in each treatment group. 78 patients (44 patients receiving bilateral injections) were assessed at the final 24 month follow.

Research performed by Filardo et al. (13) consisted of an extended 24-month follow-up of a previous pilot study to determine if the beneficial effects observed with intra-articular PRP injections were persistent over time. No comparative agent was used, and 90 patients (24 with bilateral knee osteoarthritis) were treated with three intra-articular PRP injections spaced 21 days apart. Due to the lack of a placebo or comparison agent in this pilot study, blinding of patients or researchers was not possible. No comment as to the leukocyte constituent of the final PRP product was made by the authors.

The study by Gobbi et al. (14) was a prospective randomized open-label trial which aimed to determine whether cyclical intra-articular PRP injections repeated at one year would produce superior results when followed until 24 months. 93 patients (119 knees) were enrolled in the initial study and received a minimum of one cycle of intra-articular PRP injection consisting of three injections spaced one month apart. 38 patients (50 knees) were randomly selected prior to the first injection to receive a second treatment cycle at twelve months. Leukocyte-poor PRP (LP-PRP) was obtained from an 8mL venous blood sample via a single centrifugation protocol. After twelve months, 10 patients (17 knees) in group 2 who were initially randomized to receive the second cycle of treatment crossed over to group one (single injection cycle) as they felt a second cycle of treatment was not warranted due to symptomatic improvement. The final analysis at 24 months involved 51 patients (69 knees) receiving a single PRP cycle and 28 patients (33 knees) receiving two cycles of PRP.

Research performed by Su et al. (16) was a prospective randomized open-label trial with a follow-up period of 18 months comparing three groups receiving either intra-articular injection

of PRP, HA or a combination approach of intra-articular and intra-osseous PRP injection. 86 patients were randomized into the three treatment groups. Leukocyte-rich PRP (LR-PRP) was obtained from a 45mL venous blood sample with a double centrifugation protocol. The PRP group received 6mL of intra-articular PRP repeated at two weeks, while the HA group received five 2mL high molecular weight hyaluronic acid injections spaced one week apart. The combined intra-articular/intra-osseous approach consisted of 2mL intra-articular PRP in addition to 2mL intra-osseous PRP injected into the medial tibial plateau and medial femoral condyle and repeated at a two-week interval.

Individual study primary outcomes

All studies examined utilized subjective clinical scoring systems to investigate the duration of beneficial effect of PRP over time; however, due to heterogeneity of study design, primary outcomes varied between studies. All studies reported an improvement in subjective scores post-injection; however, the effects of PRP at long-term follow-up were ill-sustained.

In the study performed by Di Martino et al. (9), statistically significant improvement in the PRP group was demonstrated in all subjective clinical scores (International Knee Documentation Committee (IKDC), EuroQol Visual Analog Scale (EQ-VAS) and Tegner) up to 24 months followed by a gradual return to baseline, with the exception of the IKDC score which was found to remain significantly higher than baseline scores at the 2-year mark (60.5 +/- 19.0 at 24 months vs 53.3 +/- 14.3 at baseline $P < .001$). However, no significant intergroup differences between clinical scores at any time frame in the two study groups were identified, with a median duration of beneficial effect of twelve months in the PRP group and nine months in the HA group. The only significant difference between PRP and HA treatment arms was found in the re-intervention rate, defined as the percentage of patients who sought a new injective or surgical

treatment at the 24-month follow-up. Patients receiving HA injections were found to have a significantly higher re-intervention rate at 37.1%, with 5.6% of patients seeking surgical management with prosthesis. In comparison, 22.6% of patients treated with PRP underwent a new surgical treatment, with 3.2% undergoing intervention with prosthesis. Unfortunately, no correlation between patient demographic factors or the degree of osteoarthritis was commented on within this study.

The 24-month follow up by Xu et al. (15) reported a worsening of the subjective pain score (Visual Analog Scale (VAS)) in the PRP group (from 4.33 +/- 0.66 to 4.85 +/- 0.62) after one-month post-injection followed by a significant decrease in pain at the six and twelve month follow-ups. No significant difference was reported at the final 24-month follow-up in the PRP group. The HA group had significant improvement in VAS pain score at one month only (4.23 +/- 0.70 to 2.82 +/-0.83), with no lasting effects at 6, 12 or 24 months. The combined PRP and HA group had improved outcomes, with significant improvement reported at each follow-up interval (1, 6, 12 and 24 months). Similar trends were demonstrated in the analysis of the subjective functional scores (Lysholm, WOMAC and Lequesne), with significant improvement noted in the PRP group at six and twelve months, while lasting effects at 24 months were demonstrated only in the combined PRP and HA group. Complications were highest within the PRP group, with five patients reporting increased pain after injection, corresponding to the initial worsening of VAS scores. Two patients in the combined PRP and HA group and no patients in the HA group experienced local complications. Lastly, synovial fluid composition and ultrasound evaluation were performed, although limited by a shorter follow-up period of twelve months. Ultrasound evaluation of cartilage and synovial thickness and blood flow demonstrated that PRP combined with HA more effectively inhibited synovial inflammation than PRP alone

but did not produce significant changes in the cartilage thickness. The synovial fluid analysis demonstrated decreases in the inflammatory markers (IL-1β, TNF-α, MMP-3 and TIMP-1) in both PRP and the combined PRP and HA groups at six months, with mild lasting inhibition at twelve months in the combination group only. No changes were demonstrated in the synovial fluid analysis at any time frame in the HA group.

Research performed by Filardo et al. (13) focused specifically on identifying which patient characteristics such as age, sex, BMI and degree of joint degeneration influenced the results using objective and subjective IKDC scores and EQ-VAS. The mean duration of action of PRP was reported to be 11 +/- 8 months with a median of nine months. However, when stratified by patient characteristics, the longest duration of action was found to be a mean of 13.7 months in patients with less advanced OA (KL-0) and male patients (mean of 12.6 months). All scores were significantly lower at the 24-month follow-up period; however, they remained improved from pre-treatment values. Only one patient experienced post-injection pain and swelling after PRP injection. 80% of patients reported being satisfied with their PRP treatment results at twelve and 24 months, and 46% of satisfied patients requested a second cycle of injections after twelve months. However, it should be noted that due to the unblinded nature of this study, it is difficult to interpret whether this data is unduly influenced by placebo or the true effects of PRP.

As previously mentioned, the study performed by Gobbi et al. (14) was additionally unblinded, aiming to identify if a second injection cycle performed at twelve months would yield a significant improvement in scores at the 24-month follow-up. The scoring measurements used for evaluation included the Knee Injury and OA Outcome Score (KOOS), VAS, Tegner, and Marx scoring systems. At twelve months, significant improvement was demonstrated in all scores compared to pre-treatment values. The second injection cycle group showed significant

improvement in all scores except for Tegner and the KOOS symptoms subscale at 18 months, with subsequent worsening of all scores at the 24-month follow-up in both single and double injection cycle groups. Similar to the Filardo et al. (13) study, results must be interpreted cautiously as the effect of placebo cannot be negated due to the unblinded nature of this study. However, in contrast, a large group of patients did not wish to receive a second cycle of injections and consequently crossed over to the single injection cycle group. Patient satisfaction or post-injection complications were not explicitly commented on.

Lastly, the study performed by Su et al. (16) demonstrated that a combined approach utilizing intraosseous (IO) and intra-articular (IA) PRP injection was more effective than intra-articular PRP or HA alone using subjective VAS and WOMAC scoring systems. However, the effects of the novel IO and IA PRP injection protocol proved to be ill-sustained at the 18-month follow-up period, and the most beneficial improvements in pain and function were demonstrated within three to twelve months. Additionally, the intra-articular PRP group demonstrated significant improvement in pain, stiffness and function after 3 months compared to HA. However, similarly to the above studies, the effects decreased by twelve months, although final evaluation values at 18 months remained improved from pre-treatment values. In terms of adverse effects, eight patients in the PRP intra-articular group experienced adverse effects, including post-injection pain and swelling. Five patients in each of the combined IA and IO PRP and HA groups experienced local complications. However, it should be noted that the only patient who withdrew from the study due to pain and swelling was in the combined IA and IO treatment group.

A summary of the pertinent study information and primary outcomes may be found in Table 1 (appendix).

Discussion

Importance of Findings

The primary goal of this paper was to examine the literature on the effects of PRP in the management of knee osteoarthritis to determine whether the proposed biological effects of this conservative treatment yielded longer-lasting results than existing intra-articular modalities. As previously mentioned, delaying the time until exhaustion of conservative modalities prior to surgical intervention is of importance due to the limitations of TKA in younger patients with less severe symptomatic osteoarthritis (1). Theoretically, due to the proposed biological effects, PRP should have a longer duration of action when compared to existing injective treatments (9). These studies demonstrate that intra-articular PRP injection is a viable, safe, and effective option for the treatment of knee OA; however, the therapeutic effects are ill sustained beyond twelve months following the initial injection cycle. This raises the question as to the extent of the proposed disease modifying effects of PRP. The biological effects of PRP in knee osteoarthritis are supported by basic in-vitro studies, which have shown that growth factors such as PDGF and TGF-β found in PRP are capable of increasing chondrocyte proliferation (17), and stimulating HA production, while decreasing cartilage catabolism within the joint space (1)(18). Only one of the five studies included in this review conducted by Xu et al. sought to evaluate the cellular mechanism of action of PRP, demonstrating a reduction in synovial fluid inflammatory markers after PRP injection, with the greatest inhibition in the combined PRP and HA group (15). However, no significant changes in cartilage thickness were observed via ultrasound evaluation in the six and twelve month follow-up intervals, and the cellular effects on growth factor concentrations were not examined (15). The majority of studies included in this review demonstrated a decrease in subjective scores at long-term follow-up, suggesting that the

therapeutic effects of PRP are temporary and likely due to anti-inflammatory properties rather than regenerative capabilities (15)(11). As the pathophysiology of osteoarthritis is increasingly complex, further detailed studies are required to delineate interactions at the cellular level in osteoarthritis of the knee.

Interestingly, although the effects of PRP as extrapolated through clinical scores declined after twelve months, scores remained improved from pre-treatment baselines. Additionally, the significantly lower re-intervention rate following PRP injection in the study by Di Martino et al. is clinically relevant, demonstrating lower rates of surgical management in comparison to HA injection (9). Although intra-articular injective treatments are common and considered minimally invasive, they carry a small but relevant risk of infection (9). Thus modalities which offer longer-term symptom management should be prioritized regardless of the reversative effects on the degree of joint damage (9). A recent retrospective study and survival analysis conducted by Sanchez et al. examining the effects of PRP on delaying definitive surgical management via total knee arthroplasty (TKA) found that 74.1% of patients treated with intra-articular PRP delayed TKA by more than 1.5 years with a median delay of 5.3 years (7). However, it should be noted that many considerations such as PRP composition, number of injection cycles, administration route and patient-specific factors, including age, gender and degree of osteoarthritis, have a significant impact on outcomes (7). Repeated cyclical injections at twelve months, intra-articular/intra-osseous injection protocols, as well as combined PRP and HA regimens, have all demonstrated further positive effects on symptomatic improvement (7)(14-16). Repeated cyclical PRP injections have been proposed to maintain the biological balance within the joint space, while the addition of intra-osseous administration allows penetration of PRP into subchondral bone, further enhancing the effects of PRP (16)(14). The benefit of combined PRP and HA

regimens is suspected to be partly due to the synergistic release of growth factors, with the addition of hyaluronic acid serving as a scaffolding material allowing for increased PRP adherence and thus extended anti-inflammatory effects (15).

A secondary goal of this review was to identify which demographic of patients would optimally benefit from conservative management with PRP. Filardo et al. demonstrated that younger male patients with milder degrees of cartilage degeneration had improved outcomes at longer-term follow-up (13). The mean duration of therapeutic benefit in males was found to be 12.6 months which was extended to 13.7 months in patients with KL grade 0 (degenerative chondropathy) in contrast to increasingly advanced grades of osteoarthritis (13). This finding coincides with the literature, including a double-blind RCT conducted by Cole et al., where patients with early evidence of osteoarthritis were shown to have superior responses post PRP injection compared to patients with KL grades 2-3 (8)(19). With respect to patient age, it has been suggested that not only do younger patients typically present with less severe degrees of osteoarthritis, they also likely produce PRP with lower pro-inflammatory constituents, as inflammatory markers in plasma have been demonstrated to increase with age (20)(7).

Lastly, with respect to the composition of PRP used, the studies included in this review used a combination of both leukocyte-poor PRP (LP-PRP) and leukocyte-rich PRP (LR-PRP). The variable composition of PRP and the role of leukocytes in impairing anti-inflammatory effects is a highly debated aspect in the literature (9). It has been suggested that LP-PRP yields improved outcomes compared to LR-PRP (21), and in-vitro studies have indicated a correlation between the inclusion of leukocytes and the release of pro-inflammatory mediators; however, further in-vivo studies are required to delineate this relationship (22-23)(9). At long-term follow-up, no dramatic differences between LP-PRP and LR-PRP were observed in the studies of

interest included in this review (9)(13-16). Due to the high degree of variability in both PRP preparation and injection protocols, these results are difficult to interpret, and further high-quality research is required to define this relationship (7)(9).

Limitations and Future Directions

The large degree of heterogeneity demonstrated within the studies of interest in this review primarily reflects the existing literature surrounding PRP. This variability has been a significant barrier to the integration of PRP into clinical practice. Many studies utilizing highly variable PRP formulations, injection protocols, control groups and outcomes have been conducted, making the interpretation of results and development of evidence-based treatment regimens challenging (1). Multiple classification systems have been attempted to allow for concise characterization of PRP, such as PAW (Platelet, Activation and WBC), Dohan Ehrenfest, DEPA (dose of injected platelets, efficiency of production, purity of PRP and activation), Sports Medicine Platelet Rich Plasma Classification System as well as many others including a recent coding system proposed by Kon et al. (24-28). A systematic review performed by Chahla et al. reported that only 10% of studies provided satisfactory reporting of the PRP preparation protocol, and a mere 16% provided quantitative values of the final PRP product, such as leukocyte and platelet counts (29). Within the studies of interest included in this review, only the study performed by Gobbi et al. specifically utilized a classification system (PAW and Dohan Ehrenfest) to characterize the PRP product used (14). Unfortunately, no single classification system has been universally adopted to date, emphasizing the need for future developments to standardize PRP utilization (1).

Secondly, this review was limited by the sparse number of studies investigating the effects of PRP at longer-term follow-up. Consequently, there was significant variability within

the study design, with three of the studies conducted as open-label trials with no blinding. Additionally, the large trial by Di Martino et al. (9) unfortunately unblinded patients after twelve months of treatment, limiting the quality of results available for long-term analysis. The inherent effect of placebo, which is of particular relevance with respect to both intra-articular injections and knee osteoarthritis research, cannot be ignored (30). Additionally, sample sizes were relatively small with low statistical power, and no comparison to intra-articular corticosteroid injection was made. The largest study included in this review conducted by Di Martino et al. (9) included 192 patients with 167 patients available at long-term follow-up. At present, 2019 OARSI (Osteoarthritis Research Society International) guidelines do not recommend intra-articular PRP injection for the management of knee osteoarthritis due largely to the lack of high-quality double-blind randomized controlled trials (31)(1). Future directions in PRP research, in addition to the need for high-quality trials, involve adopting a universally accepted PRP classification system and considering longer-term follow-up of patients who are likely to be optimal candidates for biological intra-articular therapy.

Conclusion

Based on the available research on the long-term effects of PRP examined in this review, PRP may be a promising and safe option for the management of knee osteoarthritis. However, there is limited evidence on the therapeutic effects beyond twelve months post-injection. Many factors such as PRP-composition, injection protocol, administration route, and patient-specific factors significantly impact outcomes. Annually repeated cyclical injections, intra-articular/intra-osseous injection protocols, as well as combined PRP and HA regimens have all demonstrated further positive effects on extending symptomatic improvement. In terms of patient demographics, younger male patients with mild grades of radiographic osteoarthritis may have superior

outcomes post intra-articular PRP injection. It has been proposed that LP-PRP has improved anti-inflammatory properties; however, no definitive trends were uncovered at long-term follow-up in the studies included in this review, and the inclusion of leukocytes remains a highly debated aspect in the literature. Due to the high degree of variability between PRP products and injection regimens utilized, a universally accepted classification system must be integrated into future research surrounding PRP to develop a common language between clinicians and researchers. Additionally, further high-quality trials are necessary to further delineate the proposed biological interactions of PRP in knee osteoarthritis and define future treatment protocols.

Appendix

Figure 2: Comprehensive Study Selection Process

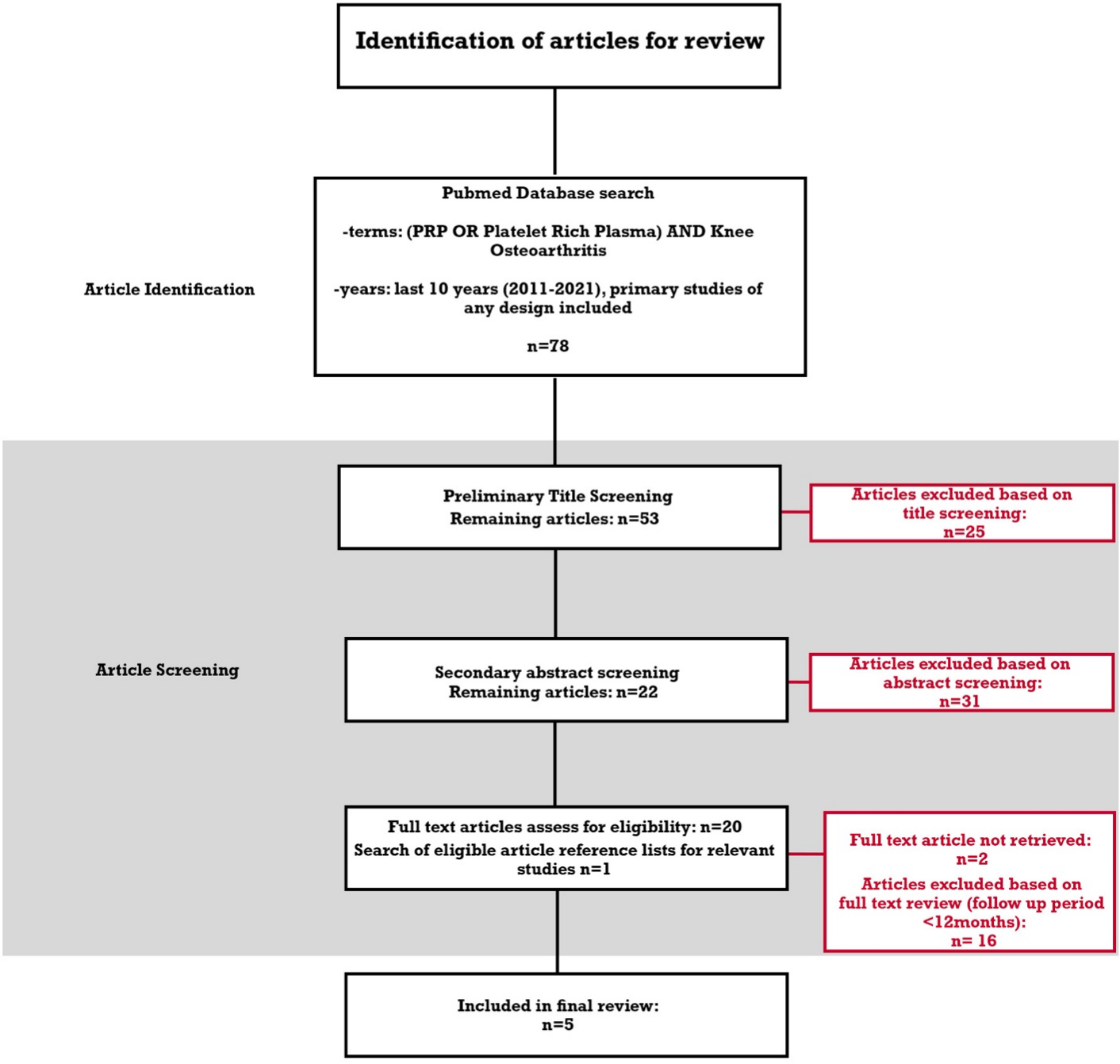

Table 1: Summary of Pertinent Study Information and Primary Outcomes

	Di Martino et al. (2019)	**Xu et al. (2021)**	**Filardo et al. (2011)**	**Gobbi et al. (2015)**	**Su et al. (2018)**
Study Design	Follow-up of a double blind RCT	Prospective double blind cohort study	Follow -up of original pilot study	Prospective randomized open label trial	Prospective randomized open label trial
Sample Size	192 patients **Final follow up** • PRP Group: 85 patients • HA Group: 82 patients	150 knees **Final follow up:** 78 patients (44 bilateral OA) • PRP Group: 30 patients (40knees) • HA Group: 20 patients (34 knees) • PRP+ HA Group: 28 patients (48 knees)	90 patients (24 patients with bilateral OA, 114 knees)	93 (119 knees) **Final follow up:** • Single injection cycle: 51 patients (69 knees) • Double injection cycle 28 patients (33 knees) Note: 10 patients (17 knees) crossed over from group 2 to the single injection cycle group.	86 patients **Final follow up** • PRP IA + IO Group: 27 patients • PRP Group: 25 patients • PRP + HA Group: 30 patients
Study Duration	Mean 64.3 (+/- 7.8) months Patients evaluated at 2, 6, 12, 24 and a mean of 64.3 months post injection	24 months Patients evaluated at 1, 6, 12 and 24 months (pain & functional scores) • Synovial fluid analysis & US evaluation limited to 6 & 12 months only	24 months Patients evaluated at 2, 6, 12 and 24 months	24 months Patients evaluated at 12, 18 and 24 months	18 months Patients evaluated at 1, 3, 6, 12 and 18 months
Severity of OA (KL grade)	KL 0-III (mean II)	KL II-III	KL 0-IV	KL I-II	KL II-III
PRP Preparation + Volume Injected	• LR-PRP, double spin protocol from a150mL sample of venous blood. • Yielded 4x 5mL samples. 3 frozen at -30°C, 1 sent for quality testing • Thawed and activated with 10% calcium chloride prior to injection • PRP platelet count: 4.6 +/- 1.4 x baseline value • leukocyte count: 1.1 +/- 0.5 x normal blood values • **volume of PRP injected:** 5mL	• LP-PRP, double spin protocol from a 36mL (or 72mL if bilateral injections) sample of venous blood. 4mL of citrate dextrose added (8mL if bilateral injections). • PRP platelet count: 5.13x baseline value • **volume of PRP injected** - PRP group: 4mL - PRP + HA group: 4mL PRP + 2mL HA	• No comment as to LP/LR-PRP, double spin protocol from a 150mL sample of venous blood, sodium citrate added. • Yielded 4x 5mL samples. The first sent for quality testing, the second was activated with 10% calcium chloride prior to injection. • The remaining 2 samples were frozen at -30degC for future injections • PRP platelet count: 600% compared to whole blood (per mL), 6.8	• LP-PRP, single spin protocol from an 8 mL sample of venous blood. • Yielded 1x 4mL sample. • PRP platelet count:>80% platelet recovery (2-fold increase) • leukocyte count: "below the normal specific granulocyte depletion >95% (mostly mononuclear cells recovered). PAW Classification: P2Bß • **volume of PRP injected**: 4mL	• LR-PRP double spin protocol from a 45mL sample of venous blood with sodium citrate added. • activated with calcium chloride prior to injection. • PRP platelet count: 789.68 +/- 17.80 x 10^9/L (5.61-fold greater than whole blood). • Leukocyte count: 29.92 +/- 1.54 x 10^9/L • **volume of PRP injected:** - PRP IA + IO group: 2mL IA + 2mL IO - PRP group: 6mL IA

			billion platelets administered per injection • **volume of PRP injected**: 5mL		
Comparison agent/Control Group	Hyaluronic Acid: (Hyalubrix, 30mg/2mL, MW: >1500kDa) -volume of HA injected 2mL	Hyaluronic Acid (SOFAST 20mg/2mL, MW: 2500kDa) -volume of HA injected: 2mL -	No comparison agent used	No comparison agent used	Hyaluronic Acid (Freda Shandong China, 20mg/2mL, MW 0.6-1.5 million Daltons) -volume of HA injected: 2mL
Injection protocol	3 intra-articular injections administered 1 week apart (HA + PRP groups)	3 intra-articular injections administered 2 weeks apart (HA, PRP + HA/PRP groups)	3 intra-articular injections administered 3 weeks apart	3 intra-articular injections administered 1 month apart	2 intra-articular/intra-osseous PRP injections administered 2 weeks apart -HA group: 5 injections spaced 2 weeks apart
Primary Outcomes/ Summary of Findings	• PRP median effect duration 12 months • HA median effect duration 9 months • reintervention rate statistically significant, HA: 37.1%, PRP 22.6%	• PRP + HA combination improved pain & function scores with lasting effects at 24 months. • HA group only demonstrated short term benefits (1month) • PRP group: significantly higher scores at 6 + 12 months, which worsened at 24 months. • Local complications (pain after injection + swelling) highest in PRP group (5 patients) • Greatest reduction in synovial fluid inflammatory markers demonstrated in the PRP group and PRP+HA group at 6 months. • PRP+ HA more effectively inhibited synovial inflammation. No change in cartilage thickness.	• Median beneficial effect duration for PRP: 9 months (mean: 11 +/- 8 months) • all evaluated parameters were lower at the final 24 month follow up however remained above baseline pre-treatment levels. • younger male patients with lower degrees of cartilage degeneration presented improved results at 24 month follow up. • 1 patient reported local complications (pain/swelling post injection)	• Plateau in results after 1 year for patients receiving a single cycle of treatment. • deterioration after 18 months for second cycle of PRP repeated at 12months. • effects at 24 month follow up ill sustained however remained higher than baseline pre-treatment values in both groups.	• IO + IA PRP injection was more effective than IA injection of PRP or HA alone. • Worsening effects at 12 months follow up but substantially higher than PRP or HA alone. • PRP IA group: highest number of reported local complications (post injection knee pain/swelling)

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