CHAPTER 9

Knee Osteoarthritis: One versus Two Cycles of PRGF Infiltrations Treatment

AUTHORS

Vaquerizo V.[1], Ruiz de Castañeda MM.[1]

[1] Department of Orthopaedic Surgery, Príncipe de Asturias University Hospital, Alcalá de Henares, Spain

SUMMARY

Knee Osteoarthritis is a degenerative disease that produces an inflammatory response of the synovial membrane, cartilage and subchondral bone, and consequently produces pain and functional disability. Despite the enormous effort made to seek an early therapeutic intervention aimed at preventing progressive destruction of joint tissues or reversing the initial articular cartilage and bone damage, there is still a lack of disease-modifying osteoarthritis therapy. The application of the biological therapy PRGF has allowed significant improvement in the quality of life of patients, delaying implantation of prosthesis. According to the results shown in the various published studies, there are approximately 20% of patients do not respond to treatment with platelet rich plasma or the response is significantly lower than in other patients. There are several factors that determine this worse response such as the variance of level of degeneration, age, and the presence of alterations in terms of both platelet concentration and function. There is no straightforward agreement on the treatment to be followed. One of the options for improving the response could be to increase the number of infiltrations to get an effective response. Of late, there are no studies that determine the standard for the frequency and number of cycles that must be performed for the treatment of OA. Treatment with two cycles of PRGF show improved of quality of life in patients with knee OA, with greater reduction in pain and stiffness compared with one cycle, although these results are not statistically significant. In those patients with poor response to treatment an option is the application of intraosseous PRGF.

E. Anitua et al. (eds.), *Platelet Rich Plasma in Orthopaedics and Sports Medicine*,
https://doi.org/10.1007/978-3-319-63730-3_9

1. INTRODUCTION

Knee osteoarthritis (OA) is one of the most frequent causes of pain and loss of function in patients over 65 years, with an increasing prevalence given the increase in the older population itself. Osteoarthritis (OA) is a mechanically induced, cytokine and enzyme mediated clinicopathological syndrome, and characterized by the involvement of inflammatory events in early stages of the joint condition[1]. Inflammation affects joint tissues with neurovascular structures such as menisci, synovial membrane (SM), subchondral bone (SB), joint capsule, and ligaments[2-4] and where synovial fluid (FS) plays an important role in perpetuating a vicious cycle among knee joint`s tissues by maintaining a detrimental pro-inflammatory microenvironment for cells from SM and superficial articular cartilage to deep layers of articular cartilage, and to SB as well[2,5,6]. SM and SB are endowed with heat receptors, chemoreceptors, and mechanoreceptors from which the nociceptive stimulus may lead to peripheral pain. Indeed, this is the case in approximately 60% to 80% of patients with knee OA[7]. At the early and mild stages of OA, the pain is triggered by physical activity and relieved by rest[8]. Also included in the clinical assessment of OA patients is joint stiffness, and in conjunction with pain may well contribute to knee disability, ultimately resulting in a drastic reduction in patient quality of life.

Despite the enormous effort made to seek an early therapeutic intervention aimed at preventing progressive destruction of joint tissues or reversing the initial articular cartilage and bone damage, there is still a lack of disease-modifying osteoarthritis therapy, making joint replacement the only solution for these patients[9]. Based on the biological theory of OA, various therapies to modify the disease have been developed in recent years. Among the different biological therapies, the application of platelet-rich plasma has been an advance in the treatment, becoming a safe and effective autologous therapy[10-13]. With this biological approach, there is an increasing interest in autologous growth factor treatment such as the use of PRP. Platelets contain growth factors, cytokines, chemokines and lysosomal granules. The release of these may play a special role in cartilage repair including modulating inflammatory processes, cell proliferation, chemotaxis, migration, differentiation and syntheses of matrix. It has been shown that platelet rich plasma, PRGF, has a chondroprotective effect from both the hyaluronic acid secretion by synoviocytes[14] and the arresting of type II collagen, cleavage by the combination of TGFß and FGF. PRGF has been revealed as a mighty anti-inflammatory response that might be mediated on the basis of the high concentration of HGF present in PRP, besides being secreted by several cells, thereby inhibiting the intracellular signaling regulator of the inflammatory and stress-induced response pathway NF-kß. Some growth factors present in platelets such as PDGF and TGFß have been shown to promote the proliferation of osteoblasts[15,16]. PRP preparations facilitate bone repair by expressing the pro-osteogenic and angiogenic functions of endothelial cells, recruiting osteoblast precursors, and promoting expression of adhesion molecules (osteoprotegerin)[17].

The current molecular interventions mainly target the clinical hallmark of OA, namely, pain and subsequent loss of knee function. In fact, current studies on the application of platelet-rich plasma point to the efficacy of this treatment in the improvement of pain, stiffness and functional capacity compared to other conservative treatments[10-13,18-23]. There are several possible mechanisms that might likely link the pain reduction to PRP treatment. PRP and growth factors within it such as HGF, IGF-1, and PDGF suppress macrophage and fibroblast, and modifying the inflammatory sate of chondrocytes activation by inhibiting the NFκB pathway, thereby dampening the synovial and articular cartilage inflammatory response, and this could lead to decreased IL-β, TNF-α concentration and other proinflammatory cytokines in synovial fluid[24-26]. Another mechanism that has been reported is the significant amount of endogenous cannabinoids within PRP that might act as ligands for cannabinoid receptor 1 (CB1) and 2 (CB2) of chondrocytes, synovial cells and bone cells of OA patients, thereby support-

ing both a pain and inflammation reduction by targeting the endogenous cannabinoid systems[27].

In recent years there are many published studies have assessed the effectiveness of PRGF in patients with knee OA[21-23]. The results obtained are encouraging showing an improvement of quality of life in patients after long-term application compared with other conservative treatments. It is, however, complex to perform a meta-analysis due to the considerable heterogeneity of the PRP applied and the demographic differences of the patients. Variability in the OA degree including in the different studies, the significant difference of age groups, scales used, and PRP preparation themselves, conspire to make it difficult for us to reach a consensus on the standardization of treatment with PRP. However, it is clear that the studies performed by authors such as Sánchez et al show how the intra-articular application of PRGF improves patients' quality of life compared to accepted conservative treatments such as hyaluronic acid[10,12,13,18]. All papers show a significant reduction of pain and other symptoms such as stiffness, functional capacity, and mobility as measured according to the results of the WOMAC scales (Western Ontario McMaster Universities Osteoarthritis Index &), KOOS or Lequesne. This improvement was statistically significant. In addition, according to the OMERACT-OARSI clinical evaluation criteria, more than 80% of the patients presented a favorable response with reductions of over 50% in the scores[12,18]. As an autologous product, PRP is a safe treatment. In this regard, treatment with PRGF had no higher rate of adverse events than other treatments, all of which were related to injection technique. One of the problems of OA treatment is the limited effect over time, sometimes deriving adverse effects from chronic administration. In this sense the application of PRGF has not only shown effectiveness during the first 6 months, a beneficial effect has persisted for at least 12 months. The level of knee degeneration in all studies was one of the inclusion criteria. On the basis of this level, some studies reported more promising results with the use of PRP in knees with a lower level of joint degeneration. Although the response in patients over 65 years as well as in severe degrees of OA has been less studied, the work of Vaquerizo et al[12] shows that this improvement is lower in these patients although the patients were satisfied with the results obtained. According to the results reported in the various published studies, there are approximately 20% of patients do not respond to treatment with platelet rich plasma or the response is significantly lower than in other patients. There are several factors that determine this poor response such as the variable levels of degeneration, age, the presence of functional alterations, and in terms of preparations of platelet concentrations. In addition to this, there is no clear standard in the treatment to be followed. One of the options for improving the response could be to increase the number of infiltrations to get an adequate response. At the present, there are no studies, which determine the frequency and number of cycles that must be performed for the treatment of OA. In the light of this terra incognita, it was worth considering increasing the number of cycles to improve the symptoms of osteoarthritis such as pain, stiffness and functional capacity. We aimed to evaluate the effectiveness of two cycles of PRGF in the treatment of OA. The goal of this study was to assess, the clinical efficacy and safety of one cycle (OC) versus two cycles (TC) of intra-articular injections of PRGF on patients with knee OA. As in other studies we used WOMAC and Lequesne scores as outcomes measures. Our hypothesis was that treatment with 2 cycles of PRGF might add a greater clinical efficacy than a single cycle of PRGF in patients with OA, thus TC therapeutic dosage to improve associated symptoms of knee OA, pain stiffness and functional capacity. This study was performed during a second therapeutic open phase of the same randomized clinical trial[12], in the same centre in accordance with current law and regulatory rules, and the international guidelines for Good Clinical Practice (International Conference on Harmonization, June 1996), Declaration of Helsinki in its latest revised version (Fortaleza, 2013) was also followed. The study protocol was previously reviewed and approved by the institutional review board. All patients signed the informed consent prior to inclusion in the RCT. In the first blind phase of the RCT, an experimental group treated with PRGF (3

injections on a weekly basis) was compared with a control group receiving Viscous-supplementation with a follow-up of 48 weeks. In this posterior open phase of the RCT, after a washout period of 6 months at the end follow-up period, patients of the control group received treatment with 2 sequential cycles of PRGF (6 months separately), while patients in the control group previously had received a single cycle of three intra-articular infiltrations of PRGF (OC group).

The objective of this second phase was to compare the efficacy of these two different therapeutic regimens (one cycle of treatment vs. two cycles) of treatment with Endoret (PRGF) in OA. The study selection criteria were: over 50 years, osteoarthritis of the knee confirmed by radiographic (Kellgren-Lawrence classification grade II-IV), no severe mechanical deformity, no systemic autoimmune rheumatic disease or blood disorders, values of body mass index < 35, and no viscous-supplementation treatment in the past 6 months. Each patient also received a booklet that contained detailed instructions for the study and the Western Ontario and McMaster Universities Osteoarthritis Index WOMAC questionnaire.

PRGF was prepared following the technique described by Sanchez et al[18]. At each visit blood volume from each patient ranged from 36 to 72 mL, depending on the knees to be treated. Blood was collected in sterile conditions with a Sodium Citrate buffer. The blood was centrifuged for 8 minutes at 580g in a BTI System centrifuge. After centrifugation the BTI Plasma Transfer Device® was used to aspirate fractions of plasma enriched in platelets immediately above the buffy coat, taking care to avoid disturbing the buffy coat. Following activation of the PRGF with 50 microliters of Cl_2Ca 10% for every mL of plasma, the PRGF was infiltrated intraarticularly.

Clinical and demographic variables were analyzed (gender, age, body mass index (BMI), OA degree with Kellgren-Lawrence score, laterality and complications were collected at the beginning of the study.

The efficacy outcome measures were a reduction in the global score of the WOMAC Index (Western Ontario and McMaster University Osteoarthritis Index), as well as in the different sub-scales for pain, stiffness and physical function of this score, as well as the reduction, and in global LEQUESNE Index and its pain, MWD and ADL sub-scales, from baseline and at 6 and 12 months (48 weeks) of follow-up after treatment. At the end of the clinical trial and as a secondary objective, according to the guideline for Good Clinical Practice, all complications and/or adverse events were recorded at each patient visits. Severity grade, received treatment and evolution of all adverse events were assessed and documented. The use of rescue medication was also recorded daily in the patient's diaries.

In order to avoid bias in the analysis, an intention to treat (ITT) statistical analysis was performed for all variables, including all patients who received one or two cycles of intra-articular injections of Endoret (PRGF), and with at least one efficacy or safety assessment. Qualitative variables were expressed as absolute or relative frequencies and quantitative variables by either the mean and standard deviation or alternatively the median and its interquartile range in cases where normal distribution was not met. All comparisons between OC and TC groups were performed using the Student t-test or alternatively, with the Mann-Whitney U non-parametric statistical test for distributions other than normal. All statistical analysis was performed using the statistical program SPSS version 16.0.

90 patients were included in the study. Forty-eight patients had received one cycle of PRGF (OC group), while 42 patients received two cycles of PRG separated by 6 months (TC group) in this open phase. The mean age in the OC group was 63.55 years, and the mean body-mass index was 30.12, whereas in the two-cycle group (TC) mean age was 67.95 years and the mean body-mass index was 30.77. Comparing baseline values in both groups, no significant differences were observed in Kellgren grade or body mass index between groups, whereas a significant higher age was re-

corded in TC group. Moreover, a significantly higher Lequesne index and all WOMAC scores (global, pain, stiffness and function) were observed in patients of TC group (Table 1).

At 6 months of follow-up, all patients had a significant reduction in both scales (P <0.001). In both groups there was a clinical improvement of more than 30% in all subscales. Patients presented the best results at pain level (45%) and mobility (37%).

Results after both treatments showed a significant reduction at the end of follow-up (48 weeks) compared with the baseline values (p<0.001) for both treatment OC and TC groups. Patients in the OC group had a clinical worsening compared with the results obtained at 6 months in WOMAC and Lequesne score (Figure 1 and 2). This clinical worsening was not significant. On the other hand, patients in the TC group continued to improve at the end of follow-up, exceeding the results obtained by the OC group.

WOMAC AND LEQUESNE SCORE				
		OC	TC	P value
Patients		48	42	
Gender	Male	21	15	0.23
	Female	27	27	0.007*
Age (years)		63.6 ± 6.7	68 ±8.3	0.65
Laterality	Left	13	11	
	Right	22	17	
	Bilateral	13	14	
Kellgren-Lawrence		2.9 ± 0.7	2.9 ± 0.8	0.86
Body mass Index (kg/m^2)		30.1 ± 4	30.8 ±4.4	0.46
WOMAC scale	Pain score	9.7 ±2.5	11.01 ±3.4	0.024*
	Stiffness score	3.7 ±1.7	4.6 ±2	0.04*
	Function score	32.7 ±9.9	39.7 ± 12.2	0.005*
	Global	46 ± 12.7	55.4 ± 16.7	0.004*
Lequesne global Index		12.8 ± 3.8	15 ± 2.4	0.001*
*Statistical significance (P<0.05).				

TABLE 1. Demographic parameters and baseline values.

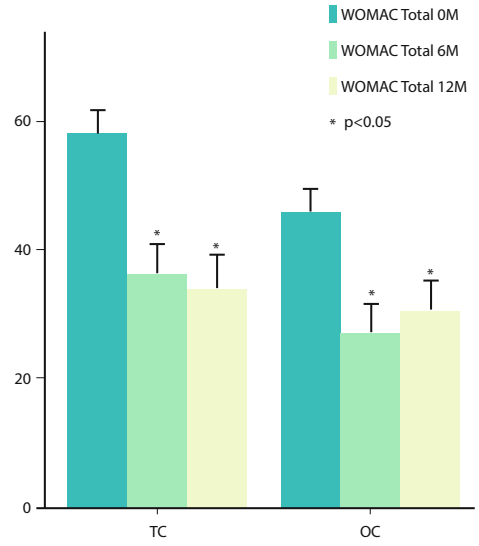

FIG. 1
Comparative Outcomes WOMAC score.

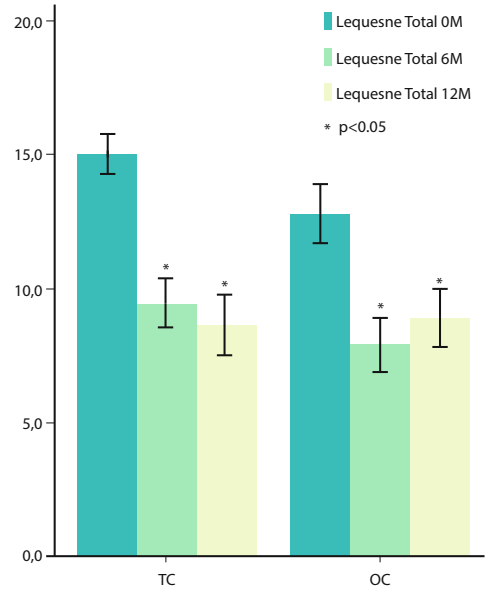

FIG. 2
Comparative Outcomes Lequesne score.

This reduction was observed for all WOMAC and Lequesne scales and sub-scales (Table 2). This substantial reduction from baseline was at least 24.6% (WOMAC stiffness score) for both groups and scores at the end of follow-up (48 weeks) as observed in Table 3.

Comparing the results in outcome measures in both OC/TCs treatment groups globally, the differences between groups were more relevant in the LEQUESNE score than in the WOMAC score as shown in Table 3. Regarding WOMAC score, patients of TC group showed a significantly higher reduction from baseline in WOMAC stiffness subscales compared with patients of OC group (P<0.05). In WOMAC global score and in pain and function sub-scales, despite a better response to treatment at the end of follow-up no significant differences between groups were detected. Regarding LEQUESNE index, a significantly higher

WOMAC AND LEQUESNE OUTCOMES					
		OC		TC	
		Mean ± SD	P value	Mean ± SD	P value
WOMAC Score	Pain score	9.7 ±2.5	<0.001	11.01 ± 3.4	<0.001
		6.3 ±3.3		6.7 ± 3.7	
	Stiffness score	3.7 ±1.7	<0.001	4.6 ±2	<0.001
		2.6 ± 1.4		2.7 ±1.6	
	Function score	32.7 ±9.9	<0.001	39.7 ± 12.2	<0.001
		21.9 ± 11.3		23.8 ± 12.8	
	Global	46 ± 12.7	<0.001	55.4 ± 16.7	<0.001
		30.8 ± 15.5		33.2 ± 17.5	
Lequesne Score	Pain score	5.6 ±1.4	<0.001	6.2 ± 1	<0.001
		4.1 ± 1.6		4.2 ± 1.9	
	MWD	2.8 ± 1.9	<0.001	3.3 ±1.6	<0.001
		1.5 ± 1.3		1.0 ± 0.9	
	ADL	4.5 ± 1.5	<0.001	5.6 ±0.9	<0.001
		3.3 ± 1.6		3.4 ± 1.7	
	Global	2.8 ± 3.8	<0.001	15 ± 2.4	<0.001
		8.9 ± 3.7		8.6 ± 3.7	

TABLE 2. WOMAC and Lequesne outcomes.

COMPARATIVE RESULTS				
		OC	TC	P value
WOMAC Score	Pain score	33.5 ± 30.9	33.5 ±30.9	0.96
	Stiffness score	24,7 ± 40.4	18.7 ±60.8	0.04*
	Function score	33.7 ± 28.7	37.1 ± 31.4	0.59
	Global	34 ± 27.6	36.5 ± 32.6	0.71
Lequesne Score	Pain score	25.3 ± 29	30.9 ±31.9	0.29
	MWD	29.7 ± 60.1	61.5 ± 53.6	0.006*
	ADL	25 ±30.7	39.7 ± 28.4	0.04*
	Global	30.2 ± 23	442 ± 24.7	0.02*

TABLE 3. Comparative results of outcome measures (%) between OC/TC groups. 48 weeks respect baseline.

reduction from baseline either in global score, in MWD sub-scale (maximum walking distance), in ADL (Activities of daily living) sub-scale, was observed. The improvement rate was 31.8 % higher for the TCs PRGF group compared with OC PRGF group (P<0.01) in MWD sub-scale. Specifically, in patients receiving two cycles of PRGF- (TC group), the improvement for the Lequesne global score was 11.84% higher than in the OC group (P<0.05), whereas in Lequesne MWD and ADL subscales the improvement compared to the OC group reached the 31.8% and 14.66% respectively (P<0.05). For pain assessment on the WOMAC scale, in Lequesne pain sub-scale patients in the TCs group had a greater pain reduction than patients in the OC group, although this difference was not significant.

Patients who have been treated with two cycles of PRGF (TC group) showed a higher pain reduction, compared with OC group, although this differ- ence was not significant. The reason might be related to the significant difference in baseline values observed for WOMAC and LEQUESNE pain score values between both groups (higher baseline values in TC group) among other reasons.

At the end of the study, there were no significant differences in rescue medication used in both groups. Only 7 patients in both groups presented pain during the first 24 hours. All of these events were related to post-injection pain. No new adverse events or complications in TC group were reported during this second therapeutic phase.

Biological therapies improve symptoms of OA, this is an obvious fact, but at present it is difficult to adopt a mechanism-based approach to pain management for several reasons, including the heterogeneity of the OA syndrome[28], the poor understanding of mechanisms underlying joint pain, the different tissue sources of pain, and the dual central and peripheral features of OA pain[2,28].

If we compare the results obtained in this clinical trial, assessed by both Lequesne and WOMAC scores in patients with severe knee OA treated with one or two cycles of intraarticular injections of PRGF, the significant improvement is consistent with the results reported previously by Sanchez et al[10,13,18] and Vaquerizo et al[12]. PRP has proven to significantly reduce pain and joint stiffness, and to improve physical function in patients with knee OA[21,23]. In the absence of pertinent studies, however, there is evidence that application of PRGF indirectly slows the progression of osteoarthritis, delaying surgery[11-13,18-23].

There are several potential mechanisms by which intraarticular injections of PRGF might reduce OA knee pain. Although pain is the clinical hallmark of OA, tissue inflammation and degeneration appear to underlie the molecular, cellular, and clinical phenomena characterizing the cluster of degenerative joint conditions known as OA[29]. In patients with severe knee OA, extracellular matrix fragments stemming from the degrading proteoglycans and cleaved collagen I may act as DAMPs (damage-associated molecular patterns)[30-32], and activate the intracellular signalling pathway known as nuclear factor kappa B (NFkB) on cells such as nociceptors, chondrocytes, synovial fibroblasts and macrophages[2,30-32]. This NFkB activation induces the gene expression of pro-inflammatory cytokines such as interleukin 1beta (IL-1B), interleukin 6 (IL-6), and tumor necrosis factor alpha (TNF-a). These are not only the major mediators of joint inflammation, they also contribute to generating and perpetuating inflammation-evoked pain by eliciting hyperalgesia and sensitizing joint tissue nociceptors for mechanical stimuli[24,25].

In vitro and in vivo studies have reported that PRP and growth factors within it such as HGF, IGF-1, and PDGF suppress macrophage, fibroblast, and chondrocyte activation by inhibiting the NFkB signaling pathway[15,16] and thereby breaking the catabolic loop, to dampen the synovial and articular cartilage inflammatory response when these cells are exposed to pro-inflammatory cytokines, abnormal mechanical stress and DAMPs, comprising the OA context[5]. In addition, the significant amount of endogenous cannabinoids within PRP might act as ligands for cannabinoid receptor 1 (CB1) and 2 (CB2) of chondrocyte and synovial cells of OA patients thereby supporting a pain reduction by targeting the endogenous cannabinoid system[5,7,9].

The greater degree of degeneration of structures may produce a lower initial response of patients to treatment. The fact that two cycles of PRGF treatment (TC group) did not add a significantly higher pain reduction in patients, compared with OC group treatment, might be related to the significant difference in baseline values observed for WOMAC and Lequesne pain score values between both groups (higher baseline values in TC group) among other reasons (Table 1).

However, patients treated with two cycles of Endoret (PRGF) underwent a significantly higher improvement in WOMAC stiffness, maximum walking distance, activities of daily living and both global sub-scales than patients receiving only one Endoret (PRGF) cycle (OC group) ($p<0.05$) at 6 months and at the end of follow-up. The sensation of knee stiffness is one of the six criteria evaluated in the WOMAC questionnaire, and although it is a symptom whose origin is complex, factors such as synovial fluid lubrication and composition, and periarticular muscle conditions play an important role in this symptom since these two joint elements are the most important shock absorbers at knee level. The anti-inflammatory effect of PRP on synovial membrane and articular cartilage of knee osteoarthritis patients may well reduce knee swelling which otherwise would trigger a spine reflex and inhibit the activation of periarticular muscle, thereby leading to muscle weakness and atrophy, and eventually contribute to knee stiffness. On the other hand, it has been shown by in vitro studies that PRP enhances the synthesis of hyaluronic acid by osteoarthritic synoviocytes even in the presence of IL-B 14. Moreover, another key component in knee lubrication and chondrocyte protection is lubricin, whose production by synovial cells and superficial zone chondrocytes decreased with age and after injury, and in knee OA is significantly enhanced by the application of PRP. Overall, the secretion of HA and lubricin together with a reduction in inflammatory synovial fluid, might well contribute to a reduction in knee stiffness.

In this study, patients undergoing two cycles of PRGF treatment showed a significantly higher improvement in efficacy outcomes such as maximum walking distance (MWD), activities of daily living (ADL) and Lequesne global sub-scale compared with patients of the OC group. This increase in tolerable physical load might entail a positive chondroprotective and anti-inflammatory effect since as several lines of evidence suggest, moderate mechanical loading of joints prevents cartilage degradation by suppressing the activation of NFkB 33. It is worth mentioning that the application of intraarticular infiltrations of PRGF does not entail any reduction in physical activity and patients resume their daily activities immediately after the procedure is performed. It can then be surmised that the increased physiological loading may well work in synergy with the anti-inflammatory effect of PRGF treatment, to reinforce as well as strengthen the periarticular muscles and contribute to a reduction in knee joint stiffness.

A limitation of this study includes the different WOMAC-Lequesne baseline values of both groups, which results are unfavorable for the TC group. Patients with worse degrees of osteoarthritis showed better results at the end of follow-up in TC group, although due to the initial differences these results were not significant. This fact was observed when comparing the results according to the patient age. However, in order to overcome this pitfall, the clinical improvement of WOMAC and Lequesne outcomes were shown in % relation from the baseline values for both treatment groups.

On the other hand, as with previous published studies, there are no biochemical data analyzing the synovial fluid composition. A more ideal study would entail a close examination of synovial fluid in terms of inflammatory mediators and lubricant components, as well as assessing peri-articular muscle to reveal the real impact on quality of life and improvement of knee stability.

This study indicates that although two cycles of PRGF treatment does not produce a measurable higher pain reduction compared with one cycle of PRGF treatment on patients, both modalities of treatment (OC and TCs groups) were safe and clinically effective, which significantly reduce all

assessed variables with WOMAC and LEQUESNE scores at the end of the follow-up period (48 weeks) compared with baseline values. In addition, patients treated with two cycles of PRGF showed a significant improvement in stiffness, maximum walking distance and activities of daily living, clearly indicating an improvement in life quality. Despite the best results obtained there are still other lines of research that may improve patient symptoms, and for this purpose the application of intraosseous PRGF can be a clear advance for the treatment of refractory cases. The application of intraosseous PRGF is explained below.

1. Little CB, Hunter DJ. Post-traumatic osteoarthritis: from mouse models to clinical trials. Nature reviews. Rheumatology. 2013;9:485-97.

2. Malfait AM, Schnitzer TJ. Towards a mechanism-based approach to pain management in osteoarthritis. Nature reviews. Rheumatology. 2013;9:654-64.

3. Felson DT. Clinical practice. Osteoarthritis of the knee. The New England journal of medicine. 2006;354:841-8.

4. Pelletier JP, Martel-Pelletier J, Abramson SB. Osteoarthritis, an inflammatory disease: potential implication for the selection of new therapeutic targets. Arthritis and rheumatism. 2001;44:1237-47.

5. Joos H, Wildner A, Hogrefe C, Reichel H, Brenner RE. Interleukin-1 beta and tumor necrosis factor alpha inhibit migration activity of chondrogenic progenitor cells from non-fibrillated osteoarthritic cartilage. Arthritis research & therapy. 2013;15:R119.

6. Scanzello CR, Goldring SR. The role of synovitis in osteoarthritis pathogenesis. Bone. 2012;51:249-57.

7. Dray A, Read SJ. Arthritis and pain. Future targets to control osteoarthritis pain. Arthritis research & therapy. 2007;9:212.

8. Felson DT. Developments in the clinical understanding of osteoarthritis. Arthritis research & therapy. 2009;11:203.

9. Martel-Pelletier J, Wildi LM, Pelletier JP. Future therapeutics for osteoarthritis. Bone. 2012;51:297-311.

10. Sanchez M, Anitua E, Azofra J, Aguirre JJ, Andia I. Intra-articular injection of an autologous preparation rich in growth factors for the treatment of knee OA: a retrospective cohort study. Clinical and experimental rheumatology. 2008;26:910-3.

11. Filardo G, Kon E, Pereira Ruiz MT, et al. Platelet-rich plasma intra-articular injections for cartilage degeneration and osteoarthritis: single- versus double-spinning approach. Knee surgery, sports traumatology, arthroscopy : official journal of the ESSKA. 2012;20:2082-91.

12. Vaquerizo V, Plasencia MA, Arribas I, et al. Comparison of intra-articular injections of plasma rich in growth factors (Endoret (PRGF)) versus Durolane hyaluronic acid in the treatment of patients with symptomatic osteoarthritis: a randomized controlled trial. Arthroscopy : the journal of arthroscopic & related surgery : official publication of the Arthroscopy Association of North America and the International Arthroscopy Association. 2013;29:1635-43.

13. Wang-Saegusa A, Cugat R, Ares O, Seijas R, Cusco X, Garcia-Balletbo M. Infiltration of plasma rich in growth factors for osteoarthritis of the knee short-term effects on function and quality of life. Archives of orthopaedic and trauma surgery. 2011;131:311-7.

14. Anitua E, Sanchez M, Nurden AT, et al. Platelet-released growth factors enhance the secretion of hyaluronic acid and induce hepatocyte growth factor production by synovial fibroblasts from arthritic patients. Rheumatology (Oxford). 2007;46:1769-72.

15. Bendinelli P, Matteucci E, Dogliotti G, et al. Molecular basis of anti-inflammatory action of platelet-rich plasma on human chondrocytes: mechanisms of NF-kappaB inhibition via HGF. Journal of cellular physiology. 2010;225:757-66.

16. van Buul GM, Koevoet WL, Kops N, et al. Platelet-rich plasma releasate inhibits inflammatory processes in osteoarthritic chondrocytes. The American journal of sports medicine. 2011;39:2362-70.

17. Cenni E, Ciapetti G, Granchi D, et al. Endothelial cells incubated with platelet-rich plasma express PDGF-B and ICAM-1 and induce bone marrow stromal cell migration. Journal of orthopaedic research : official publication of the Orthopaedic Research Society. 2009;27:1493-8.

18. Sanchez M, Fiz N, Azofra J, et al. A randomized clinical trial evaluating plasma rich in growth factors (Endoret (PRGF)) versus hyaluronic acid in the short-term treatment of symptomatic knee osteoarthritis. Arthroscopy : the journal of arthroscopic & related surgery : official publication of the Arthroscopy Association of North America and the International Arthroscopy Association. 2012;28:1070-8.

19. Gobbi A, Lad D, Karnatzikos G. The effects of repeated intra-articular PRP injections on clinical outcomes of early osteoarthritis of the knee. Knee surgery, sports traumatology, arthroscopy : official journal of the ESSKA. 2015;23:2170-7.

20. Gormeli G, Gormeli CA, Ataoglu B, Colak C, Aslanturk O, Ertem K. Multiple PRP injections are more effective than single injections and hyaluronic acid in knees with early osteoarthritis: a randomized, double-blind, placebo-controlled trial. Knee surgery, sports traumatology, arthroscopy : official journal of the ESSKA. 2017;25:958-65.

21. Meheux CJ, McCulloch PC, Lintner DM, Varner KE, Harris JD. Efficacy of Intra-articular Platelet-Rich Plasma Injections in Knee Osteoarthritis: A Systematic Review. Arthroscopy : the journal of arthroscopic & related surgery : official publication of the Arthroscopy Association of North America and the International Arthroscopy Association. 2016;32:495-505.

22. Laudy AB, Bakker EW, Rekers M, Moen MH. Efficacy of platelet-rich plasma injections in osteoarthritis of the knee: a systematic review and meta-analysis. British journal of sports medicine. 2015;49:657-72.

23. Dai Wi, Zhou AG, Zhang H, Zhang J. Efficacy of Platelet-Rich Plasma in the Treatment of Knee Osteoarthritis: A Meta-analysis of Randomized Controlled Trials. Arthroscopy: The Journal of Arthroscopic & Related Surgery. 2017;33:659-70.e1.

24. Schaible HG, von Banchet GS, Boettger MK, et al. The role of proinflammatory cytokines in the generation and maintenance of joint pain. Annals of the New York Academy of Sciences. 2010;1193:60-9.

25. Sellam J, Berenbaum F. The role of synovitis in pathophysiology and clinical symptoms of osteoarthritis. Nature reviews. Rheumatology. 2010;6:625-35.

26. Kapoor M, Martel-Pelletier J, Lajeunesse D, Pelletier JP, Fahmi H. Role of proinflammatory cytokines in the pathophysiology of osteoarthritis. Nature reviews. Rheumatology. 2011;7:33-42.

27. Descalzi F, Ulivi V, Cancedda R, et al. Platelet-rich plasma exerts antinociceptive activity by a peripheral endocannabinoid-related mechanism. Tissue engineering. Part A. 2013;19:2120-9.

28. Thakur M, Dickenson AH, Baron R. Osteoarthritis pain: nociceptive or neuropathic? Nature reviews. Rheumatology. 2014;10:374-80.

29. Fernandez-Tajes J, Soto-Hermida A, Vazquez-Mosquera ME, et al. Genome-wide DNA methylation analysis of articular chondrocytes reveals a cluster of osteoarthritic patients. Annals of the rheumatic diseases. 2014;73:668-77.

30. Sillat T, Barreto G, Clarijs P, et al. Toll-like receptors in human chondrocytes and osteoarthritic cartilage. Acta orthopaedica. 2013;84:585-92.

31. Gomez R, Villalvilla A, Largo R, Gualillo O, Herrero-Beaumont G. TLR4 signalling in osteoarthritis--finding targets for candidate DMOADs. Nature reviews. Rheumatology. 2015;11:159-70.

32. Houard X, Goldring MB, Berenbaum F. Homeostatic mechanisms in articular cartilage and role of inflammation in osteoarthritis. Current rheumatology reports. 2013;15:375.

33. Sanchez-Adams J, Leddy HA, McNulty AL, O'Conor CJ, Guilak F. The mechanobiology of articular cartilage: bearing the burden of osteoarthritis. Current rheumatology reports. 2014;16:451.