Knee Surg Sports Traumatol Arthrosc (2018) 26:2615–2621
https://doi.org/10.1007/s00167-017-4565-z

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# Two cycles of plasma rich in growth factors (PRGF-Endoret) intra-articular injections improve stiffness and activities of daily living but not pain compared to one cycle on patients with symptomatic knee osteoarthritis

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Received: 30 August 2016 / Accepted: 3 May 2017 / Published online: 19 May 2017


**Abstract**
*Purpose* To assess the clinical efficacy and safety of a treatment based on one cycle versus two cycles of intra-articular injections of plasma rich in growth factors (PRGF-Endoret) on patients with knee osteoarthritis (OA).
*Methods* Ninety patients with knee OA were included and evaluated. A total of 48 patients received one cycle (OC group) (3 injections on a weekly basis), while 42 patients received two cycles of PRGF-Endoret (TC group) spaced 6 months between them. Patients were evaluated with LEQUESNE and WOMAC scores before treatment and after 48 weeks. Safety assessment was also performed.
*Results* A significant reduction of all assessed outcome measures was shown for both groups at 48 weeks compared with baseline values ($P < 0.001$). Patients of TCs group showed a significantly higher reduction ($P < 0.05$) in WOMAC stiffness subscales. Regarding LEQUESNE INDEX, a significantly higher reduction was observed in the TC group in all subscales except in pain score. In the maximum walking distance subscale (MCD), the improvement rate was 31.8% higher for the TCs group compared with the OC group ($P < 0.01$). In addition, the TC group showed a significant improvement in LEQUESNE activities of daily living (ADV) and global subscales of 14.7 and 11.8% ($P < 0.05$) higher, respectively, than the OC group.
*Conclusions* Treatment with two cycles of PRGF did not show a significantly higher pain reduction compared with one cycle treatment. However, two cycles of PRGF showed a significant improvement in WOMAC stiffness, LEQUESNE MCD, LEQUESNE ADV and LEQUESNE global subscales. Therefore, patients treated with two cycles present an improvement in quality of life.
*Level of evidence* II.

**Keywords** PRGF · Osteoarthritis · Regeneration · Growth factors · Pain

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## Introduction

Osteoarthritis (OA) is a cytokine- and enzyme-mediated clinicopathological disease, in which different initial pathological phenotypes are grouped under the umbrella term OA [25], and characterized by the involvement of inflammatory events in early stages of the joint condition [13]. Inflammation affects joint tissues with neurovascular structures such as menisci, synovial membrane, subchondral bone, joint capsule and ligaments [13, 27, 32] and where synovial fluid 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 [20, 27, 38]. Synovial membrane and subchondral bone 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 60–80% of osteoarthritis patients [10]. At the early and mild stages of OA, the pain is triggered by physical activity and relieved by rest [14]. Also included in the clinical assessment of OA patients is joint stiffness, and in conjunction with pain may well contribute to knee disability [31], 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 [28]. The current molecular interventions mainly target the clinical hallmark of OA, namely, pain and subsequent loss of knee function [7].

Among the several biologic agents in the symptom-modifying OA treatment, intra-articular injection of plasma rich in growth factors (PRGF) has emerged as a safe and efficacious autologous therapy [16, 36, 41, 43]. This platelet concentrate within a plasma suspension forms an in situ-generated fibrin matrix and acts as a delivery system of growth factors, cytokines, and morphogens [3] (IGF-1, TGFB1, HGF, PDGF, VEGF, NGF, BDNF, CTGF, BMPs, Vitronectin, Fibronectin, SDF-1, PF4 among others), which have been shown to exert a chondroprotective, anabolic, anti-inflammatory and immunomodulatory effect [1, 5, 8, 30, 34, 44].

The assessment of the clinical efficacy and safety of one cycle (OC) versus two cycles (TC) of intra-articular injections of PRGF on patients with knee OA using WOMAC and LEQUESNE scores as outcomes measures was the main aim of this study. Treatment with two cycles of PRGF could add a greater clinical efficacy than a one cycle of PRGF in patients with OA was the main hypothesis, thus improving patients' life quality.

## Materials and methods

This study was performed during a second therapeutic open phase of the same randomized clinical trial [41], in the same centre in accordance with current law regulatory rules, and the international guidelines for Good Clinical Practice. The study protocol was previously reviewed and approved by the institutional review board (EC11-026).

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 visco-supplementation. In this posterior open phase of the RCT, and once the first 12-month follow-up period finished, patients of the control group received treatment with two sequential cycles of PRGF (6 months separately). The objective of this second phase was to compare the efficacy of these two different therapeutic regimens (one cycle vs two cycles) of treatment with PRGF-Endoret in OA.

### Patient selection

All patients signed the informed consent prior to inclusion in the RCT. Study selection criteria were: over 50 years, all patients reported knee pain within the last 6 months, lasting at least 1 month, requiring pain medication, osteoarthritis of the knee confirmed by radiographic (Kellgren-Lawrence classification grade 2–4), and no visco-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 OA Index WOMAC questionnaire.

### Interventions

In this open phase, patients in the experimental group received two cycles of three intra-articular injections of PRGF-Endoret® spaced 6 months apart (TC group), while patients in the control group had previously received only one cycle of three intra-articular injections of PRGF-Endoret® (OC group).

PRGF-Endoret® was prepared following the technique described by Anitua et al. [37]. At each visit, blood volume from each patient ranged from 36 to 54 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 min at 580 g 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 µL of $Cl_2Ca$ 10% for every ml of plasma, the PRGF was infiltrated intra-articularly.

## Outcome measures

Clinical and demographic variables (sex, age, body mass index (BMI), osteoarthritis degree with Kellgren-Lawrence score, laterality and complications were collected at the beginning of the study. All measures were performed at baseline and at 6 and 12 months of follow-up.

### *Efficacy assessments*

The efficacy outcome measures were the reduction in the global score of the WOMAC Index (Western Ontario and McMaster University Osteoarthritis Index) [4] and also in the different subscales for pain, stiffness and physical function of this score, as well as the reduction in global LEQUESNE Index [24] and its pain, maximum walking distance (MCD) and Activities of daily living (ADV) subscales, from baseline and 12 months (48 weeks) of follow-up after treatment.

### *Safety assessments*

Severity grade, received treatment and evolution of all adverse events were assessed and documented at each visit. The use of rescue medication was also recorded daily in the patient's diaries.

## Statistical analysis

In order to calculate the number of patients, the parameters obtained in the Wang-Saegusa study [43] were taken as reference. A sample size of 48 subjects per group was estimated to provide at least 80% power, at a 5% level of significance, taking into consideration 10% possible losses. 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 PRGF-Endoret®, 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's *t* test or alternatively, with the Mann–Whitney *U* nonparametric statistical test for distributions other than normal. The level of statistical significance was set at $P = 0.05$. All statistical analysis was performed using the statistical program SPSS version 16.0.

**Table 1** Patients demographic parameters and baseline values of LEQUESNE and WOMAC scores

| | One cycle | Two cycles | *P* value |
|---|---|---|---|
| Number of patients (*n*) | 48 | 42 | |
| Gender (M/F) | 21/27 | 15/27 | n.s. |
| Age (years) | 63.6 ± 6.7 | 68.0 ± 8.3 | 0.007* |
| Laterality (L/R/bilateral) | 13/22/13 | 11/17/14 | n.s. |
| Kellgren-Lawrance | 2.9 ± 0.7 | 2.9 ± 0.8 | n.s. |
| Body mass index (Kg/m$^2$) | 30.1 ± 4.0 | 30.8 ± 4.4 | 0.462* |
| WOMAC score | | | |
| Pain score | 9.7 ± 2.5 | 11.0 ± 3.4 | 0.024* |
| Stiffness score | 3.7 ± 1.7 | 4.6 ± 2.0 | 0.041* |
| Function score | 32.7 ± 9.9 | 39.7 ± 12.2 | 0.005* |
| Global | 46.0 ± 12.7 | 55.4 ± 16.7 | 0.004* |
| Lequesne index | 12.8 ± 12.8 | 15.0 ± 2.4 | 0.001* |

* denotes *P* value is significant

### *Results*

Forty-eight patients had received one cycle of PRGF-Endoret (3 injections on a weekly basis) (OC group), while 42 patients received two cycles of PRGF-Endoret separated by 6 months (TC group) in this open phase (Table 1).

Results after both treatments showed a significant reduction at the end of the follow-up period (48 weeks) compared with the baseline values ($P < 0.001$) for both OC and TC groups. This reduction was observed for all WOMAC and LEQUESNE scales and subscales (Table 2). This substantial reduction from baseline was at least 24.6% (in 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 TCs 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 subscales, no significant differences between groups were detected. Regarding LEQUESNE index, a significant higher reduction was observed from baseline either in global score, in MCD subscale (maximum walking distance), and in ADV (Activities of daily living) subscale ($P < 0.05$). The improvement rate was 31.8% higher for the TCs PRGF-Endoret group compared with OC PRGF-Endoret group ($P < 0.01$) in MCD subscale. Specifically, in patients receiving two cycles of PRGF-Endoret (TCs group), the improvement for the LEQUESNE global score was 11.8% higher than in the OC group ($P < 0.05$), whereas in LEQUESNE

**Table 2** WOMAC and LEQUESNE outcomes in OC and TCs groups

| | One cycle | | Two cycles | |
|---|---|---|---|---|
| | Mean ± SD | *P* value | Mean ± SD | *P* value |
| WOMAC score* | | | | |
| Pain score | | | | |
| Basal | 9.7 ± 2.5 | <0.0001 | 11.1 ± 3.4 | <0.0001 |
| 48 weeks | 6.3 ± 3.3 | | 6.7 ± 3.7 | |
| Stiffness score | | | | |
| Basal | 37 ± 1.7 | <0.0001 | 4.6 ± 2.0 | <0.0001 |
| 48 weeks | 2.6 ± 1.4 | | 2.7 ± 1.6 | |
| Function score | | | | |
| Basal | 32.7 ± 9.9 | <0.0001 | 39.7 ± 12.2 | <0.0001 |
| 48 weeks | 21.9 ± 113 | | 23.8 ± 12.8 | |
| Global | | | | |
| Basal | 46.0 ± 127 | <0.0001 | 55.4 ± 16.7 | <0.0001 |
| 48 weeks | 30.8 ± 15.5 | | 33.2 ± 17.3 | |
| LEQUESNE score† | | | | |
| Pain score | | | | |
| Basal | 5.6 ± 1.4 | <0.0001 | 6.2 ± 1.0 | <0.0001 |
| 48 weeks | 4.1 ± 1.6 | | 42 ± 1.9 | |
| MCD | | | | |
| Basal | 2.8 ± 1.9 | <0.0001 | 33 ± 1.6 | <0.0001 |
| 48 weeks | 1.5 ± 13 | | 1.0 ± 0.9 | |
| ADL | | | | |
| Basal | 43 ± 13 | <0.0001 | 5.6 ± 0.9 | <0.0001 |
| 48 weeks | 3.3 ± 1.6 | | 3.4 ± 17 | |
| Global | | | | |
| Basal | 12.8 ± 3.8 | <0.0001 | 15.0 ± 2.4 | <0.0001 |
| 48 weeks | 8.9 ± 37 | | 8.6 ± 37 | |

*, † denotes *P* value is significant

**Table 3** Comparative results of outcome measures (%) between OC/TCs groups

| | One cycle | Two cycles | *P* value |
|---|---|---|---|
| Number of patients | 48 | 42 | |
| WOMAC score | | | |
| Pain score | 34.0 ± 31.1 | 31.9 ± 41.7 | n.s. |
| Stiffness score | 24.6 ± 40.4 | 30.0 ± 60.8 | 0.040* |
| Function score | 33.7 ± 28.8 | 37.1 ± 31.4 | n.s. |
| Global | 34.1 ± 27.6 | 36.5 ± 32.6 | n.s. |
| Lequesne index | | | |
| Pain score | 25.3 ± 29.0 | 30.9 ± 31.9 | n.s. |
| MCD | 29.7 ± 60.1 | 61.5 ± 53.6 | 0.006* |
| ADV | 25.0 ± 30.7 | 39.7 ± 28.4 | 0.042* |
| Global | 30.2 ± 23.0 | 42.0 ± 24.7 | 0.021* |

Variation between baseline values and 48 weeks

* Statistical significance ($P < 0.05$)

MCD and ADV subscales, the improvement compared to the OC group reached 31.8 and 14.6%, respectively ($P < 0.05$). In LEQUESNE pain subscale reduction, no differences were detected between both treatment groups.

Seven adverse events were reported in the OC group. All of them were related with post-injection pain. No new adverse events or complications in the TC group were reported during this second therapeutic phase.

## Discussion

The most important finding of the present study was that two cycles of PRGF improve quality of live in patients with Knee OA. Pain reduction improved in patients treated with two cycles of PRGF compared with the patients treated with one cycle and other symptoms as stiffness. At present, it is difficult to adopt a mechanism-based approach to pain management for several reasons, including the heterogeneity of the OA [15], the poor understanding of mechanisms underlying joint pain, the different tissue sources of pain, and the dual central and peripheral features of OA pain [27, 39].

The significant clinical improvement assessed by both LEQUESNE and WOMAC scores in patients with severe knee OA treated with one or two cycles intra-articular injections of PRGF-Endoret observed in this study is consistent with the results reported previously by Sanchez et al. [36, 41, 43]. PRP has proven to significantly reduce pain [16, 36, 37, 41, 43] and joint stiffness, and to also improve physical function [17–19, 22, 29, 41, 43] in patients with knee OA. There are several potential mechanisms by which intra-articular injections of plasma rich in growth factors (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 [21].

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 signalling pathway [2, 5, 8, 12, 30, 40] 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 [38]. In addition, the significant amount of endogenous cannabinoids within PRP [9] might act as ligands for cannabinoid receptor 1 (CB1) and 2 (CB2) of chondrocyte and synovium cells of OA patients [10, 33] thereby supporting a pain reduction by targeting the endogenous cannabinoid system [9, 10, 28].

As it can be observed, patients who have been treated with two cycles of PRGF (TC group) showed a higher pain reduction, compared with OC group, although this difference 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 (Table 1).

However, patients treated with two cycles of PRGF-Endoret underwent a significant higher improvement in WOMAC stiffness, LEQUESNE maximum walking distance, LEQUESNE activities of daily living and both global subscales than patients receiving only one PRGF-Endoret cycle (OC group) ($P < 0.05$). The sensation of knee stiffness is one of the six criteria evaluated in the WOMAC questionnaire [31], 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 [6] 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 [6, 14], 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 [1]. Moreover, another key player in knee lubrication and chondrocyte protection [42] is lubricin, whose production by synovial cells and superficial zone chondrocytes decreased after injury, and in an osteoarthritic knee [11, 26] is significantly enhanced by the application of PRP [34]. 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 PRP showed a significantly higher improvement in efficacy outcomes such as maximum walking distance (MCD), activities of daily living (ADV) and LEQUESNE global subscale compared with patients in the OC treatment 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 [23, 35]. A limitation of this study includes the different WOMAC-LEQUESNE baseline values of both groups, which results unfavourable for the TC group. 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. A more ideal study would entail a close examination of synovial fluid composition in terms of inflammatory mediators and lubricant components, as well as assessing peri-articular muscle to reveal the real impact of maximum walking distance (MCD), and activities of daily living (ADV) improvement on knee stability.

## Conclusions

This study concludes 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.

**Acknowledgements** We would like to thank Verónica Moreno and Esther Montes for their contribution to the paper. Authors wish to thank BTI Biotechnology Institute for their active contribution.

**Compliance with ethical standards**

**Conflict of interest** Vaquerizo V. and Aguirre JJ. declares that have no conflict of interest. Anitua E. is the Scientific Director and GO, SP and LB are scientists at BTI Biotechnology Institute, a dental implant company that investigates in the fields of oral implantology and PRGF-Endoret technology.

**Funding** This work was supported by the Biomedical Research Foundation of Principe de Asturias University Hospital.

**Ethical approval** This study was performed in accordance with current law regulatory rules, and the international guidelines for Good Clinical Practice. The study protocol was previously reviewed and approved by the institutional review board (EC11-026).

**Informed consent** Informed consent was obtained from all individual participants included in the study.

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