# Clinical Outcomes of Knee Osteoarthritis Treated With an Autologous Protein Solution Injection

## A 1-Year Pilot Double-Blinded Randomized Controlled Trial

Elizaveta Kon,*† MD, Prof., Lars Engebretsen,‡ MD, Prof., Peter Verdonk,§ MD, Prof., Stefan Nehrer,‖ MD, Prof., and Giuseppe Filardo,¶# MD, PhD
*Investigation performed at the Rizzoli Orthopaedic Institute, Bologna, Italy; Department of Ortho Surgery, University of Oslo and OSTRC Oslo Sports Trauma Research Center, Norwegian School of Sports Sciences, Oslo, Norway; Monica Research Foundation, Antwerp Orthopaedic Center, Belgium; Center for Regenerative Medicine and Orthopedics, Danube University, Krems, Austria*

**Background:** Osteoarthritis (OA) is a debilitating disease resulting in substantial pain and functional limitations. A novel blood derivative has been developed to concentrate both growth factors and antagonists of inflammatory cytokines, with promising preliminary findings in terms of safety profile and clinical improvement.

**Purpose:** To investigate if one intra-articular injection of autologous protein solution (APS) can reduce pain and improve function in patients affected by knee OA in a multicenter, randomized, double-blind, saline-controlled study.

**Study Design:** Randomized controlled trial; Level of evidence, 2.

**Methods:** Forty-six patients with unilateral knee OA (Kellgren-Lawrence 2 or 3) were randomized into the APS group (n = 31), which received a single ultrasound-guided injection of APS, and the saline (control) group (n = 15), which received a single saline injection. Patient-reported outcomes and adverse events were collected at 2 weeks and at 1, 3, 6, and 12 months through visual analog scale (VAS), Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC), Knee injury and Osteoarthritis Outcome Score (KOOS), Short Form–36 (SF-36), Clinical Global Impression of Severity/Change (CGI-S/C), Patient Global Impression of Severity/Change (PGI-S/C), and Outcome Measures in Rheumatology–Osteoarthritis Research Society International (OMERACT-OARSI) responder rate. Imaging evaluation was also performed with radiograph and magnetic resonance imaging (MRI) before and after treatment (12 months and 3 and 12 months, respectively).

**Results:** The safety profile was positive, with no significant differences in frequency and severity of adverse events between groups. The improvement from baseline to 2 weeks and to 1, 3, and 6 months was similar between treatments. At 12 months, improvement in WOMAC pain score was 65% in the APS group and 41% in the saline group ($P = .02$). There were no significant differences in VAS pain improvement between groups. At 12 months, APS group showed improved SF-36 Bodily Pain subscale ($P = .0085$) and Role Emotional Health subscale ($P = .0410$), as well as CGI-C values ($P = .01$) compared with saline control. Significant differences between groups were detected in change from baseline to 12 months in bone marrow lesion size as assessed on MRI and osteophytes in the central zone of the lateral femoral condyle, both in favor of the APS group ($P = .041$ and $P = .032$, respectively). There were no significant differences between APS and control groups in other measured secondary endpoints.

**Conclusion:** This study provides evidence to support the safety and clinical improvement at 1-year follow-up of a single intra-articular injection of APS in patients affected by knee OA. Treatment with APS or a saline injection provided significant pain relief over the course of the study with differences becoming apparent at between 6 and 12 months after treatment.

**Trial Registration:** NCT02138890

**Keywords:** blood derivative; platelets; knee osteoarthritis; growth factors; cytokines; injection

The American Journal of Sports Medicine, Vol. XX, No. X
DOI: 10.1177/0363546517732734


A healthy joint requires not only a fine-tuned balance of molecular signals regulating homeostasis but also the ability to respond to damage, restoration, and remodeling. Biomechanical, metabolic, and biologic changes, as well as

trauma and isolated cartilage lesions, may lead to the loss of this homeostasis, resulting in degeneration of the articular surface and, ultimately, to osteoarthritis (OA).[1,17] With the population aging, the OA prevalence is increasing, along with its effect on society.[10] Thus, one of the goals of modern medicine is to extend the quality of life and years of activity of the population affected by cartilage lesions and OA.

Numerous approaches have been proposed as noninvasive treatments to avoid or delay the need for metal resurfacing, with variable success rates. But none has been clearly shown to modify the natural history of the disease and can be considered an ideal procedure for OA treatment.[4,13] Among the emerging treatment options, injective strategies based on the use of autologous blood derivatives have been the subject of vigorous scientific investigation and introduced into clinical use.[13,20,21]

In this landscape, autologous protein solution (APS), prepared using the nSTRIDE APS Kit (Zimmer Biomet), has been developed. This is a blood derivative that provides a milieu of bioactive factors (eg, EGF, IGF-1, PDGF-AB and -BB, VEGF, and TGF-β1) together with a high level of anti-inflammatory cytokines (eg, IL-1ra, sIL-1RII, sTNF-RI, and sTNF-RII) while ensuring low levels of proinflammatory molecules (eg, IL-1β and TNFα) due to the filtration of the platelet concentrate through polyacrylamide beads.[28] The APS Kit contains the APS Separator, which isolates platelets and white blood cells in a small volume of plasma, and the APS Concentrator, which concentrates the platelets, white blood cells, and plasma proteins further. It is this unique output of concentrated platelets, white blood cells, and plasma proteins prepared from patients' blood at the point of care that enables it to contain high concentrations of anti-inflammatory cytokines and anabolic growth factors, which differ from other biologic products. Platelet-rich plasma (PRP) mainly contains anabolic growth factors. Autologous conditioned serums contain lower concentrations of anti-inflammatory cytokines, require incubation, and usually use a series of injections. Finally, this procedure does not lead to stem cell concentration, thus differing from procedures aimed at taking advantage of concentrated or expanded stem cell properties, which, despite reported preclinical success, have not been proven to be an effective solution in treating OA.[9]

In vitro studies have shown that inhibition of the production of inflammatory cytokines and destructive proteases, together with the stimulation of cell proliferation in cartilage tissue likely due to the simultaneous delivery in APS of multiple anti-inflammatory and anabolic agents, is effective in preventing cartilage matrix degradation than are recombinant antagonists of inflammatory molecules.[24,28,34] A prospective randomized clinical trial in horses with naturally occurring OA demonstrated a decreased lameness at 14 days, with this benefit maintained up to 52 weeks.[5] Moreover, another study confirmed that high concentrations of anti-inflammatory molecules and growth factors can be obtained by all patients regardless of the degree of articular cartilage degeneration and age (with ages ranging from 22 to 85 years), thus prompting its use in OA patients.[27] Finally, a recent preliminary evaluation in a small cohort of patients showed the safety and confirmed the benefit of a single intra-articular injection in terms of pain improvement,[19] supporting the rationale to provide a proper concentration of white blood cells, platelets, and plasma to address OA and prompting further studies to validate these promising findings.

The hypothesis was that APS injection provides a superior outcome compared with saline. Thus, the aim of this study was to demonstrate if 1 APS injection can reduce pain and improve function in patients affected by knee OA in a prospective, randomized, double-blind, saline-controlled study.

## METHODS

### Patient Selection and Study Design

The present multicenter, double-blind, randomized, saline-controlled trial was approved by the hospital ethics committees, and each patient signed a written consent form (ClinicalTrials.gov identifier: NCT02138890). The study was conducted over a 2-year time span (2014-2016) in the inpatient departments or in sports medicine institutes and highly specialized referral centers for orthopaedics. Forty-six patients were included (CONSORT [Consolidated

#Address correspondence to Giuseppe Filardo, MD, PhD, NABI Laboratory, Rizzoli Orthopaedic Institute, Via Di Barbiano, 1/10–40136, Bologna, Italy (email: g.filardo@biomec.ior.it).

*Humanitas Clinical and Research Center, Milan, Italy.

†Humanitas University, Department of Biomedical Sciences, Milan, Italy.

‡Orthopedic Clinic, Oslo University Hospital and Oslo Sport Trauma Research Center, Oslo, Norway.

§Department of Orthopaedic Surgery, Monica Hospitals, Monica Research Foundation, Department of Orthopaedic Surgery, University Hospital, Antwerp, Belgium.

‖Dekan Fakultät Gesundheit und Medizin, Leiter Department für Gesundheitswissenschaften und Biomedizin, Leiter Zentrum für Regenerative Medizin und Orthopädie, Krems, Austria.

¶NanoBiotechnology Laboratory, Rizzoli Orthopaedic Institute, Bologna, Italy.

In the original online article, the Discussion section read ''Leukocyte-reduced PRP had a 43% WOMAC pain improvement at 52 weeks...'' This has been corrected to 57% online and in print.

One or more of the authors has declared the following potential conflict of interest or source of funding: Funding was received from Biomet Zimmer. E.K. is a paid presenter or speaker for Biomet, Fidia, and Finceramica and is a paid consultant and holds stock in Cartiheal. S.N. receives research support from Arthro Kinetics, Chroma Pharma, Biomet, and Braincon. L.E. receives research support from Arthrex, Biomet, and Smith & Nephew; is a paid consultant and receives IP royalties from Arthrex; and holds stock or stock options in iBalance. P.V. is a paid presenter for Active Implants, DePuy, and Smith & Nephew; is a paid consultant for Active Implants, DePuy, Smith & Nephew, and Conmed; and receives research support from Cartiheal. G.F. is a paid presenter for Cartiheal, Finceramica Faenza Spa, and GreenBone Ortho and receives financial or material support from IGEA Clinical Biophysics (Italy), BIOMET (US), Kensey Nash (US), Finceramica Faenza Spa (Italy), Fidia Farmaceutici Spa (Italy), Cartiheal (2009) Ltd (Israel), and EON Medica SRL (Italy).

Standards of Reporting Trials] flow diagram showing patients' inclusion and follow-up is reported in Figure 1) in the trial according to the following criteria: (1) male or female ≥40 years and ≤75 years, (2) willingness and ability to comply with study procedures and visit schedules and ability to follow oral and written instructions, (3) diagnosis of knee OA grade 2 or 3 according to the Kellgren-Lawrence grading scale based on a radiograph performed within 6 months before screening, (4) body mass index ≤ 40, (5) mean total score of the 5 pain subscale items together on the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) LK 3.1 questionnaire >1.75 and <4 at screening and at baseline; (6) failed at least 1 conservative OA therapy; (7) signed an independent ethics committee–approved informed consent form (exclusion criteria described in detail in Table 1). Patients could be screened into the study, but their WOMAC pain scores could be too low or too high on the day of treatment and become a screen failure. They are separate from the 24 excluded patients. The demographics and pretreatment score levels were similar between groups (detailed description in Table 2, where the data of the efficacy analysis population have been reported: 29 patients for APS group, 14 for saline group).

## Randomization and Blinded Treatment Procedure

Patients were randomized 2:1 in 2 treatment groups: the first one was treated by a single injection of APS, whereas the second group received a saline injection (0.9% sodium chloride solution). The 2:1 randomization was performed for ethical reasons, that is, to limit the number of patients receiving placebo and increase the number of patients receiving this promising therapy. A "blinding sleeve" was used to mask the syringe content to blind the participants. The randomization list was provided by an independent statistician, and sealed envelopes were kept in a dedicated office. One member of the physician team contacted this office just before the injection to learn the treatment allocation.

All patients had blood drawn, from which the APS was prepared for injection for patients randomized to the APS group. The nSTRIDE APS Kit with Anticoagulant Citrate Dextrose Solution–Formula A was used, a single-use device designed to concentrate growth factors and anti-inflammatory cytokines from whole blood in 2 steps: the first one with the nSTRIDE Cell Separator to separate the cellular components from plasma and red blood cells in whole blood, a suspension that was loaded in the second step in the nSTRIDE Concentrator, which uses filtration through polyacrylamide beads to concentrate the cytokines in the injectable output. The output of the APS Kit contains high concentrations of platelets, white blood cells, and plasma proteins. A detailed characterization of the output can be found in O'Shaughnessey et al.[27] After all joint fluid was aspirated, approximately 2.5 mL of APS or saline was injected into the joint via ultrasound guidance. The injector chose the position of the knee (eg, extended or bent) and the approach for the injection (eg, medial or lateral). At one study site (Rizzoli Orthopaedic Institute, Bologna, Italy), blood was drawn for 2 APS Kits from every patient (n = 17, 1 sample not recorded). The output of the first APS Kit was injected intra-articularly, and the output of the second APS Kit was stored in a –80°C freezer for further analysis. An APS sample was also prepared from patients enrolled in the saline control group and stored in a –80°C freezer for further analysis. After all patients were enrolled in the study, the concentrations of IL-1ra, sIL-1RII, IL-1β, sTNF-RII, and TNFα in APS from treatment and control participants were measured using ELISA kits (R&D Systems).

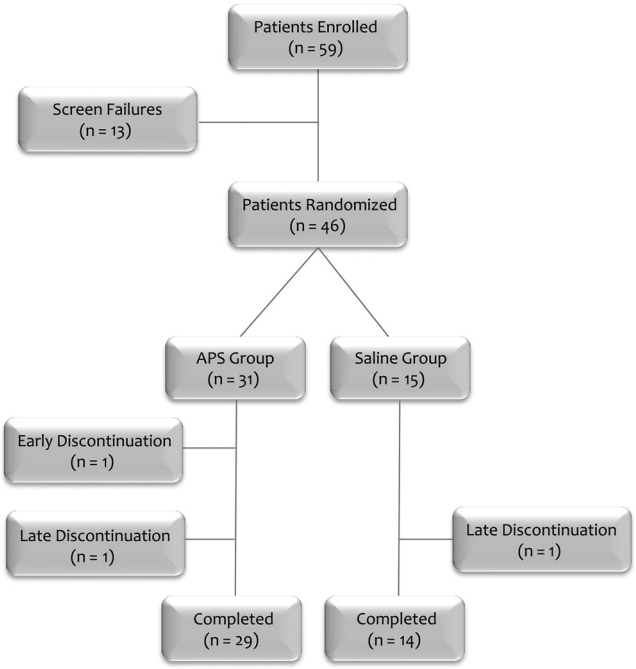



**Figure 1.** CONSORT flow diagram showing patients' inclusion and follow-up. Early discontinuation is indicative of lost to follow-up. APS, autologous protein solution; CONSORT, Consolidated Standards of Reporting Trials.

After the injection, patients were sent home with instructions to limit the use of the leg for at least 24 hours and to use cold therapy/ice on the affected area to relieve pain. After treatment, the use of nonsteroidal medication was forbidden, while allowed OA medication was standardized to be oral acetaminophen/paracetamol (maximum of 3 g per day) for all patients during the study. A gradual resumption of normal sport or recreational activities was allowed as tolerated.

## Clinical Assessment and Evaluation Items

The primary objective of this study was to evaluate the effect of a single dose of APS on pain in patients with knee OA. All patients were evaluated before the injection and then at 2 weeks and at 1, 3, 6, and 12 months after treatment by a physician not involved in and blind to the injective treatment to maintain the double-blind design of the study. Safety and tolerability were assessed for adverse events (AEs) and injection-site reactions, physical examinations, knee examinations, and vital signs were evaluated at baseline and postinjection up to 12 months. Clinical efficacy

TABLE 1
Exclusion Criteria

| No. | Exclusion Criteria |
|---|---|
| 1 | On day 1 (pre-injection), presence of active infection or abnormal effusion in the knee as noted by a physical examination |
| 2 | Presence of symptomatic osteoarthritis (OA) in the nonstudy knee |
| 3 | Diagnosis of rheumatoid arthritis (RA), Reiter's syndrome, psoriatic arthritis, ankylosing spondylitis, arthritis secondary to other inflammatory diseases or of metabolic origin |
| 4 | Diagnosis of isolated patellofemoral joint OA |
| 5 | Valgus/varus deformity judged by the investigator to be clinically significant |
| 6 | Disease of spine, hip, or other lower extremity joints of sufficient degree to affect assessment of the study knee |
| 7 | Untreated acute traumatic injury of the index knee |
| 8 | Presence of a symptomatic meniscal tear in the index knee |
| 9 | Limited daily activity for reasons other than OA |
| 10 | Presence of surgical hardware or other foreign body in the index knee |
| 11 | Arthroscopy (unless solely diagnostic in nature) or open surgery in knee within 6 months before screening |
| 12 | Intra-articular steroid injections in any joint within 3 months before screening |
| 13 | Intra-articular hyaluronic acid in any joint within 6 months before screening |
| 14 | Other intra-articular therapy in any joint within 6 months before screening |
| 15 | Taking systemic steroids within 2 weeks before screening |
| 16 | Planned/anticipated surgery of the knee during the study period |
| 17 | Any clinically significant results at screening (values or findings outside of normal ranges that are deemed clinically significant by the investigator) |
| 18 | Less than 5 years history free of malignancy other than nonmelanoma skin cancer |
| 19 | Any serious, nonmalignant, significant, acute, or chronic medical condition or active psychiatric illness that, in the investigator's opinion, could compromise patient safety, limit the patient's ability to complete the study, and/or compromise the objectives of the study |
| 20 | Skin breakdown at the knee where the injection is planned to take place |
| 21 | Pregnant or nursing mothers, or women likely to conceive a child and unwilling to use a reliable form of birth control for the duration of the study |
| 22 | Known recent history of drug or alcohol dependence |
| 23 | Use of any investigational drug or device within 30 days before screening, or 5 half-lives, whichever is longer |
| 24 | Use of any investigational biologic within 60 days before screening |

was assessed with the following evaluation tool outcomes: WOMAC LK 3.1 questionnaire and additional Knee injury and Osteoarthritis Outcome Score (KOOS) questions evaluated at baseline (pre-injection) and postinjection up to 12 months, plus additional assessments of pain with the visual analog scale (VAS) pain score, of health-related quality of life evaluated using the Short Form–36 (SF-36) survey and the clinician and patient global assessments of the severity of OA made at baseline (Clinical Global Impression of Severity [CGI-S], Patient Global Impression of Severity [PGI-S]), and assessment of change evaluated post-injection up to 12 months (Clinical Global Impression of Change [CGI-C], Patient Global Impression of Change [PGI-C]). The Outcome Measures in Rheumatology–Osteoarthritis Research Society International (OMERACT-OARSI) responder rate was calculated in each treatment group: The responders were defined as patients who achieved a high degree of improvement in either pain or function, or a moderate degree of improvement in 2 of the 3 response domains (pain, function, global assessment), according to the criteria defined by Pham et al[29] (Table 3).

Structural changes have been assessed by evaluating any changes on radiograph and magnetic resonance imaging (MRI) from baseline to 12 months and at 3 and 12 months after injection, respectively. In particular, all images were sent to an imaging core lab and were evaluated by 2 independent musculoskeletal radiologists blinded to each other's assessments. Disagreements between the primary reviewers were resolved by a third independent reviewer. The reviewers were blinded to the treatment group and did not have access to clinical outcomes data during the study. The change from screening to 3 and 12 months for each MRI Osteoarthritis Knee Score (MOAKS) parameter was evaluated to determine if there was a difference between treatment groups. In addition, joint space narrowing on radiographs was evaluated for differences between treatment groups.

## Efficacy Analysis Population

For analysis, the primary efficacy analysis population (intent-to-treat) group consisted of all participants for whom the treatment procedure was initiated and who had at least 1 baseline and post-baseline observation on the primary efficacy endpoint. Secondary efficacy analyses were performed on the per-protocol population, consisting of the intent-to-treat group modified to exclude subjects having major entry violations likely to affect outcome as determined by blind review (29 patients in APS group, 14 in saline group).

## Safety Analysis Population

For analysis, the safety analysis population included all participants who received an injection of either APS or saline. Analysis of safety endpoints included all data regardless of whether it was collected inside the protocol-defined window.

TABLE 2
Comparative Demographics and Baseline Scores of APS and Saline Groups[a]

| Characteristic | APS (n = 29) | Saline (n = 14) | *P* Value |
|---|---|---|---|
| Age, y (range) | 57 (41-68) | 54 (44-67) | NS |
| Sex | | | |
| Male | 18 | 9 | NS |
| Female | 13 | 6 | |
| Ethnicity | | | |
| Black | 1 | 0 | NS |
| White (non-Hispanic) | 30 | 15 | |
| Kellgren-Lawrence, % | | | |
| Grade 2 | 48 (n = 14) | 71 (n = 10) | NS |
| Grade 3 | 52 (n = 15) | 29 (n = 4) | |
| WOMAC | | | |
| Total | 51.2 | 54.9 | NS |
| Pain | 11.4 | 11.8 | NS |
| Stiffness | 4.8 | 5.0 | NS |
| ADL | 34.9 | 38.1 | NS |
| KOOS | | | |
| Pain | 39.9 | 37.9 | NS |
| Symptoms | 47.8 | 46.4 | NS |
| ADL | 48.6 | 44.0 | NS |
| Sports/Recreation | 23.1 | 14.3 | NS |
| Quality of Life | 26.5 | 22.3 | NS |
| VAS | 5.5 | 6.5 | NS |
| SF-36 | | | |
| Physical Functioning | 35.8 | 33.9 | NS |
| Role Physical | 38.5 | 37.8 | NS |
| Bodily Pain | 36.7 | 35.0 | NS |
| General Health | 49.6 | 49.8 | NS |
| Vitality | 51.7 | 50.6 | NS |
| Social Functioning | 43.7 | 44.5 | NS |
| Role Emotional | 44.0 | 39.5 | NS |
| Mental Health | 51.5 | 50.8 | NS |
| CGI-S, % | | | |
| Mild | 6.9 (2/29) | 0 (0/14) | NS |
| Moderate | 48.3 (14/29) | 42.9 (6/14) | |
| Marked | 37.9 (11/29) | 57.1 (8/14) | |
| Severe | 6.9 (2/29) | 0 (0/14) | |
| PGI-S, % | | | |
| Borderline | 3.4 (1/29) | 0 (0/14) | NS |
| Mild | 6.9 (2/29) | 0 (0/14) | |
| Moderate | 27.6 (8/29) | 21.4 (3/14) | |
| Marked | 41.4 (12/29) | 57.1 (8/14) | |
| Severe | 17.2 (5/29) | 21.4 (3/14) | |
| Extreme | 3.4 (1/29) | 0 (0/14) | |
| Rescue medication usage for osteoarthritis, % | 13.8 (4/29) | 35.7 (5/14) | NS |

[a]Patient-reported outcomes are from the efficacy analysis population. ADL, activity of daily living; APS, autologous protein solution; CGI-S, Clinical Global Impression of Severity; KOOS, Knee Injury and Osteoarthritis Outcome Score; NS, not statistically significant; PGI-S, Patient Global Impression of Severity; SF-36, Short Form–36; VAS, visual analog scale; WOMAC, Western Ontario and McMaster Universities Osteoarthritis Index.

### Statistical Analysis

The sample size was not statistically powered, as the trial was designed as a pilot study using an exploratory design to collect information about device efficacy, functionality, and safety, as well as the necessary data to power future studies.

For normally distributed data, *t* test or analysis of variance was used. Otherwise, the Wilcoxon test was used. Categorical variables were analyzed using the Fisher exact test (for 2 × 2 tables) and using the likelihood ratio chi-square test when more than 2 rows or columns were analyzed. For all tests, $P < .05$ was considered significant. All statistical analysis tests were performed using SAS version 9.2 for Windows.

TABLE 3
OMERACT-OARSI Responder Criteria[a]

| |
|---|
| Improvement in pain or function ≥50% |
| And absolute change of ≥20 on VAS pain score |
| Or improvement in at least 2 of the 3: improvement of pain, function, or patient's global assessment ≥20% |
| And absolute change of ≥10 on VAS |

[a]OMERACT-OARSI, Outcome Measures in Rheumatology–Osteoarthritis Research Society International; VAS, visual analog scale.

## RESULTS

Patient groups were homogeneous for demographics and baseline WOMAC LK 3.1, KOOS, VAS pain, SF-36 survey, and the global assessments of the severity of OA CGI-S and PGI-S (Table 2). In the APS group, 1 patient did not complete the 3-month follow-up visit and 1 patient did not complete the 12-month follow-up visit.

Cytokine analysis of the APS from patients at 1 site in the study contained high concentrations of anti-inflammatory cytokines and low concentrations of inflammatory cytokines (Table 4). In baseline blood, these concentrations have been reported to be IL-1ra: 7600 ± 2500 pg/mL; sIL-1RII: 9500 ± 2500 pg/mL; IL-1β: 3.3 ± 1.1 pg/mL; sTNF-RII: 1500 ± 490 pg/mL. The concentration of TNFα in whole blood, like APS, is below the range of the standard curve (15.6 pg/mL).[27] The APS Kit produced an output containing high concentrations of white blood cells (48.4 ± 16.7 k/μL).

No major complications related to the injections were observed during the treatment and follow-up period. No significant differences between treatment groups were observed in analyses of AE type (only knee pain and joint warmth were reported), severity, device relatedness, or procedure relatedness (Table 5). Three serious AEs were reported, of which 2 occurred in APS-treated patients (bladder cancer and kidney stone requiring urethral stent) and 1 in a saline-treated patient (meniscus tear in non-study knee). All 3 events were considered unrelated to the device or to the procedure. The 1 device-related AE was arthralgia, which the subject recovered from after treatment. There were 6 procedure-related AEs (all arthralgia). Procedure-related AEs included no device deficiencies, and no unexpected serious adverse device events were observed. The majority of patients did not use any acetaminophen/paracetamol either at baseline (APS,

TABLE 4
Concentrations (Mean ± SD) of Cytokines in Autologous Protein Solution Produced From Patients Enrolled at the Rizzoli Orthopaedic Institute (n = 16)

| Cytokines | Concentration, pg/mL |
|---|---|
| IL-1ra | 33,482 ± 16,013 |
| sIL-1RII | 27,874 ± 12,087 |
| IL-1β | 23.4 ± 28.6 |
| sTNF-RII | 6052 ± 1643 |
| TNFα | 0.6 ± 1.3 |

86.2%; saline, 64.3%) or at 12 months (APS, 96.6%; saline, 100%), with no significant difference between groups.

No significant difference in improvement in WOMAC pain scores in comparison to saline control was found at 2 weeks, 1 month, 3 months, and 6 months. However, at 12 months after the procedure, although both groups showed a significant improvement of WOMAC pain score over time (APS, $P < .0001$; saline, $P = .0012$), the APS group reported a mean 65% improvement, while the saline group had a mean 41% improvement over the same period, with a significant difference in percentage improvement between groups ($P = .02$) (Figure 2).

Also, no significant difference in improvement in VAS pain scores in comparison to saline control was found at 2 weeks, 1 month, 3 months, and 6 months. But after 12 months, VAS pain score had improved 49% in the APS group versus 13% in the saline group ($P = .06$) (Figure 3). The OMERACT-OARSI responder rate showed that the majority of participants (>50%) were considered responders at months 3, 6, and 12 in the APS group (12 months: APS, 65.5%; saline, 50.0%) (see Table 6 for details).

No difference between treatment groups was observed in the responder rate. However, it is important to note that the responder rate continued to increase over the 12-month time period in the APS group, while the maximum responder rate in the saline group was reached at month 6 (64.3%) and had declined by the 12-month time point. Longer term follow-up will be required to determine if this trend continues. Finally, at 12 months, in the SF-36 subscales Bodily Pain (APS, 47.0 ± 9.3; saline, 39.2 ± 7.0; $P = .0085$) and Role Emotional Health (APS, 49.6 ± 10.5; saline, 42.5 ± 9.8; $P = .0410$), a significant difference was observed between treatment groups, and the percentage of patients reported by the physicians (CGI-C) as being improved (minimally, much, or very much) was 79.3% and 50%, in the APS and saline groups, respectively. In this analysis, a significant difference between groups was observed ($P = .01$). There were not any significant differences in these measurements between groups at earlier time points. There were no significant differences in other outcome measures (SF-36, KOOS, PGI/CGI) at any time point.

The MRI analysis underlined significant differences between groups in change from baseline to 12 months in bone marrow lesion size and osteophytes. Osteophytes and bone marrow lesions in the central zone of the lateral femoral condyle got larger control participants, whereas they remained unchanged in APS-treated participants (Figure 4). In other compartments, no significant differences were found. At 3 and 12 months, MRI data did not show any difference between treatment groups in the change in any of the normalized quantitative measurements of T2 relaxation time, nor in any other measurements (see Section 2 of the Appendix, available in the online version of this article). No significant changes or differences between groups were detected in the radiograph data.

Finally, there was no significant correlation between the change in any of the semiquantitative assessments from MRI and the change in the WOMAC pain score, with the exception of the change in the size of cartilage loss in the central zone of the medial femoral condyle ($P = .025$). However, the proportion of subjects with changes in the qualitative assessments was small, so these $P$ values are only helpful in identifying possible trends in the data.

TABLE 5
AE Overview[a]

| | APS (n = 31) | Saline (n = 15) |
|---|---|---|
| Patients experiencing at least one AE | 14 (45.2%) | 6 (40.0%) |
| Total number of AEs | 48 | 17 |
| Screening | 6 | 0 |
| Week 2 | 13 | 5 |
| Month 1 | 4 | 2 |
| Month 3 | 10 | 5 |
| Month 6 | 8 | 2 |
| Month 12 | 7 | 3 |
| Device-related[b] AE (%) | 1 (3.2%) | 0 (0%) |
| Procedure-related[b] AE (%) | 4 (12.9%) | 2 (13.3%) |

[a]The safety analysis population has been considered. AE, adverse event; APS, autologous protein solution.
[b]"Related" refers to likely or definitely related to device or procedure.

## DISCUSSION

The main finding of the present study is that a single intra-articular APS injection is safe (similar to saline injection) and provides pain improvement at 1 year in patients affected by knee OA. Other measurements, including VAS, responder rate, KOOS, and SF-36, failed to show significant differences compared with a saline control injection. MRI analysis demonstrated few significant differences between treatment groups; however, there were significant differences between groups in change from baseline to 12 months in bone marrow lesion size and osteophytes in the central zone of the lateral femoral condyle, both in favor of the APS group as measured by MOAKS. No changes between groups were measured in cartilage; therefore, further studies are warranted to determine if changes in bone marrow lesion size and osteophytes could be attributed to APS or if these observations were caused by other factors.

This novel autologous therapy merges the advantages of different treatment strategies. The first attempt with

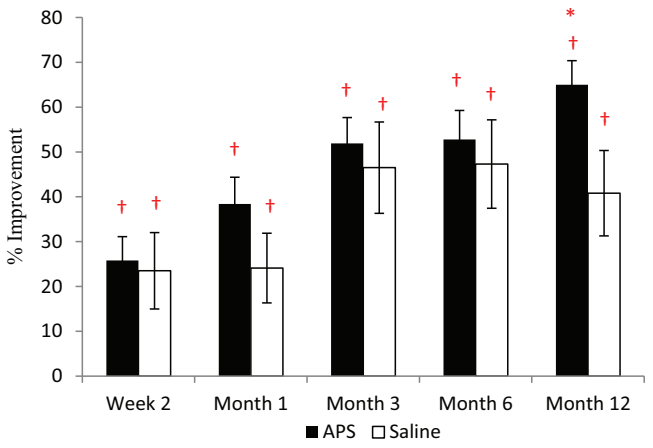



**Figure 2.** WOMAC percentage pain improvement from baseline. The figure shows the mean WOMAC percentage pain improvement from baseline (± standard error of the mean) at each follow-up visit until 12 months. *Indicates significant difference between treatment groups. †Indicates difference between follow-up time point and baseline. APS, autologous protein solution; WOMAC, Western Ontario and McMaster Universities Osteoarthritis Index.

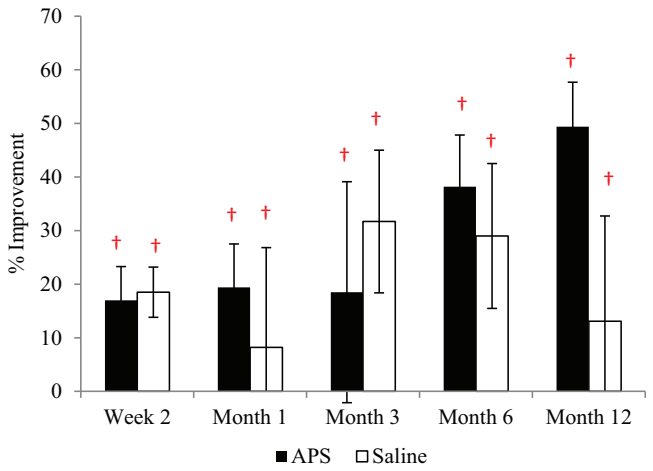



**Figure 3.** Visual analog scale percentage improvement (± standard error of the mean). †Indicates difference between follow-up time point and baseline. APS, autologous protein solution.

a blood-derived technology, targeted to the imbalance of pro- and anti-inflammatory cytokines driving cartilage degeneration, was performed in the mid-1990s. Based on the observations of Meijer et al,[25] who noted that exposure of blood to glass beads elicits a vigorous, rapid increase in the synthesis of several anti-inflammatory cytokines including IL-1Ra, autologous conditioned serum was developed in an attempt to generate an injectable product enriched in endogenous IL-1Ra as a novel therapeutic solution for OA. A randomized, double-blind, saline-controlled trial[3] showed a considerable improvement, with results even superior to those of hyaluronic acid. Nonetheless, these positive findings have not been successively confirmed,[35] and further analysis has shed doubts on the biological potential of this product, showing increased levels not only of anti-inflammatory but also pro-inflammatory cytokines, with a lack of net direct effect on cartilage metabolism.[31]

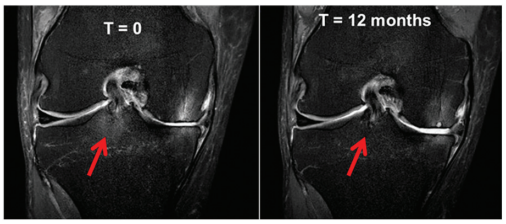
More recently, another blood-derived product, PRP, has gained increasing attention as a promising procedure, due to the pools of growth factors stored in platelet α-granules that have been found to take part in the regulation of articular cartilage.[14] Several in vitro studies and preclinical studies using different animal models provided the rationale for the clinical application of platelet concentrates, documenting positive effects and showing how intra-articular injections do not target only cartilage. PRP might also influence other tissues, such as menisci and synovia, and ultimately affect the entire joint environment, which may lead to the improvement reported in clinical practice.[14] An increasing number of studies have been published supporting the benefit of PRP for OA treatment. The results of this study compare favorably to PRP studies. For example, a recent randomized controlled study of a series of 3 leukocyte-reduced PRP injections failed to show statistical improvement in WOMAC pain compared with 3 injections of hyaluronic acid but did show improvements in International Knee Documentation Committee and VAS at 6 and 12 months. Leukocyte-reduced PRP had a 57% WOMAC pain improvement at 52 weeks in that study compared with a 65% WOMAC pain improvement measured after a single injection of APS. The time to onset of pain relief in this study was similar to that observed in PRP studies in which pain is statistically reduced within 2 weeks of injection.[7] However, the extent of the therapeutic benefit of PRP is still controversial,[11] with an increasing awareness of the need to optimize the biologic potential to clearly demonstrate the role of blood derivatives in OA treatment.

A strategy combining the concentration of both growth factors and anti-inflammatory molecules could offer a synergistic effect able to provide a significant therapeutic benefit. The uniqueness of APS is the content of concentrated platelets, white blood cells, and plasma proteins. PRP contains only concentrated platelets (and sometimes white blood cells), autologous conditioned serums contain plasma proteins with elevated cytokine levels, and other joint injections do not contain anabolic growth factors or cytokines. Research on blood derivatives still shows debate regarding whether or not white blood cells should be included in the autologous therapies. In particular, the presence of leukocytes is among the most discussed aspects concerning PRP formulations, as it has been suggested to be detrimental and impair the overall effects of PRP due to the release of metalloproteinases, other lytic enzymes, and reactive oxygen species that could stimulate an early inflammatory response within the joint environment.[14] However, other studies have shown more complex effects with less conclusive findings in terms of both molecule release and cellular influences on chondrocytes and synoviocytes,[2,6] and the only available analysis of synovial fluids collected in humans 7 days after leukocyte-rich PRP treatment suggested that the presence of leukocytes did

TABLE 6
OMERACT-OARSI Responder Analysis[a]

| | Yes | | No | | |
|---|---|---|---|---|---|
| Assessment | n | % | n | % | *P* Value |
| Week 2 | | | | | |
| APS | 5 | 17.2 | 24 | 82.8 | NS |
| Saline | 5 | 35.7 | 9 | 64.3 | |
| Month 1 | | | | | |
| APS | 9 | 31.0 | 20 | 69.0 | NS |
| Saline | 5 | 35.7 | 9 | 64.3 | |
| Month 3 | | | | | |
| APS | 14 | 50.0 | 14 | 50.0 | NS |
| Saline | 8 | 57.1 | 6 | 42.9 | |
| Month 6 | | | | | |
| APS | 17 | 58.6 | 12 | 41.4 | NS |
| Saline | 9 | 64.3 | 5 | 35.7 | |
| Month 12 | | | | | |
| APS | 19 | 65.5 | 10 | 34.5 | NS |
| Saline | 7 | 50.0 | 7 | 50.0 | |

[a]APS, autologous protein solution; NS, not statistically significant; OMERACT-OARSI, Outcome Measures in Rheumatology–Osteoarthritis Research Society International.




| Assessment | Visit | Saline | APS | *P* value |
|---|---|---|---|---|
| LFC - Bone Marrow Lesion Size (MOAKS) | 3 | 0\|0\|0\|14\|0\|1\|0 | 0\|0\|1\|29\|1\|0\|0 | NS |
| | 12 | 0\|0\|0\|10\|3\|1\|0 | 0\|0\|2\|26\|2\|0\|0 | .041 |
| LFC - Osteophytes (MOAKS) | 3 | 0\|0\|0\|15\|0\|0\|0 | 0\|0\|0\|31\|0\|0\|0 | NS |
| | 12 | 0\|0\|0\|10\|4\|0\|0 | 0\|0\|1\|28\|1\|0\|0 | .032 |

**Figure 4.** Imaging evaluation of patients in this trial. Top: reduction in bone marrow lesion size observed (indicated by arrows) at the 12-month time point in comparison with the screening time point. Bottom: The change relative to the screening visit was calculated for each patient at each time point. The format of the reported data is the number of patients with –3|–2|–1|zero|+1|+2|+3 changes. APS, autologous protein solution; LFC, lateral femoral condyle; MOAKS, MRI Osteoarthritis Knee Score; NS, not significant.

not induce a relevant in vivo up-regulation of pro-inflammatory mediators, in contrast with the evidence reported by in vitro studies.[23] The widely debated hypothesis that leukocytes in PRP might foster unwanted effects is sustained essentially in in vitro studies,[18,30] but this assertion remains a mere speculation since in vitro studies cannot completely mirror the complexity of the joint environment, where multiple different cellular populations are involved in the pathophysiology of articular compartment and their cross-talk with a particular network of soluble factors actively and collectively modulates the joint response.[15,22] In this landscape, the focus on the measurement of inflammatory cytokines in leukocyte-rich products provides an incomplete picture of the process that balances inflammation. This particularly applies to APS, where the cell solution produced in the first step is then processed in a concentration device containing polyacrylamide beads, which elicit the production of a highly anti-inflammatory solution.[27] Moreover, anabolic growth factors, including PDGF and IGF-1, are concentrated and act in concert to decrease IL-1β–induced NF-κB activation.[26] Many anabolic growth factors are secreted by platelets' alpha granules, and APS contains a statistically significant concentration increase of the following anabolic cytokines over baseline blood: EGF, IGF-1, PDGF-AB and -BB, VEGF, and TGF-β1, which play an important role in the cartilage repair pathways.[14,34] The pleiotropic effects of these bioactive molecules have been shown to lead to both chondroprotective effects, with the inhibition of matrix degradation, and even anabolic benefits on cartilage with an induced higher cellularity.[24] In addition, the synergistic interaction of the different cell components has been demonstrated by a clinical study of APS correlating the presence of white blood cells to a favorable cell release and, ultimately, positive clinical findings that last over time, extending the duration of the molecules' half-life itself.[19]

In this study, we confirmed the clinical improvement of OA patients 1 year after a single injection of APS. The study design included both randomization and double blinding, allowing us to account for the placebo effect ascribed to intra-articular injections and new fashionable procedures[12] and, therefore, to demonstrate the superiority of APS with respect to saline injections on some variables at 1 year.

The extended timeframe it took for significant differences to develop between treatment and control groups could be attributed to the long-term effects of APS. APS has been shown to potentially restore homeostasis in the joint by blocking matrix metalloprotease production,[34] production of inflammatory cytokines from macrophages,[28] and extracellular matrix degradation in inflammatory environments,[24] as well as by decreasing lameness in dogs and horses with OA.[5,33] Also, there are substantial placebo effects observed in almost every knee injection study, and it took some time for the potential changes in homeostasis in the joint induced by APS to overcome the waning placebo effect. It has been noted that the placebo effect is even greater in biologic trials where patients perceive they are getting a "regenerative medicine" therapy.[12] This effect was shown to affect scores differently: Control participants had a greater magnitude of improvement in VAS compared with WOMAC pain improvement scores, which could be attributed to previous observations that these scoring systems measure different components of the knee OA pain experience.[8]

This study has some limitations. The first is the lack of a power analysis. However, this study is in line with the recently published literature in this field with similar study designs,[32] and these data will be the reference point for properly powered studies in the future to confirm the clinical effects against other injective treatments for OA patients.

Moreover, the control group had fewer patients than did the treatment group: Based on the nature of the study (pilot, exploratory) and the nature of the investigated treatment (autologous therapy vs placebo), this 2:1 randomization was performed for ethical reasons (ie, to limit the number of patients receiving placebo). This asymmetric randomization procedure has been previously accepted in the literature, and according to US Food and Drug Administration guidelines (http://purl.access.gpo.gov/GPO/LPS117512, http://www.spirit-statement.org/trial-design/), increasing the number of patients receiving the injection is more likely to provide a clinical benefit according to the promising preliminary results.[19] Another limitation is the lack of analysis regarding possible malalignment, which could be a poor prognosticator for many nonoperative OA treatments, especially considering the MRI findings suggesting changes in a single compartment. However, this aspect was considered in light of the overall clinical evaluation, and it was deferred to the investigator's judgment.

Another interesting finding that deserves to be highlighted is that no differences could be detected between groups at 1, 3, and 6 months of follow-up. In fact, the lack of difference may be ascribed more to an unexpectedly high response to the saline injection than to a lack of improvement after APS injections. This strong saline effect may be related to a placebo effect, which may play an important role in injective treatments, as previously shown in similar studies on new regenerative injective treatments.[12,13] Whereas at this short follow-up few differences could be detected from the imaging evaluation at 1 year after evaluation, no signs of worsening could be detected at the last follow-up. Significant improvements were seen in some imaging parameters, and to the best of our knowledge, this is the first time such an observation has been observed in a human randomized controlled trial. In a case series of 15 patients with knee OA who received PRP injections, 12 of 15 patients did not get worse and 1 of 15 showed improvement in the medial compartment.[16] Although the half-life of proteins and cells is on the order of hours to days, the effect seen in pain relief and changes in osteophytes and bone marrow lesions developed over the course of months. These results suggest that APS may be restoring homeostasis to the joint. Further studies should focus on the evaluation of APS effects over time, to understand the duration of the clinical improvement and to determine if 1 injection may also provide a protective effect by improving the status of the chondral surface or delaying joint OA progression. Finally, further studies are warranted to determine the benefit of this new autologous treatment with respect to other available injective therapies and understand the role of APS for the treatment of patients affected by knee OA.

## CONCLUSION

This study supports the use and continued study of APS for knee OA. We demonstrated a similar safety profile to saline injection with a similar improvement in outcomes compared with baseline up to 6 months. At 12 months, we demonstrated a significant improvement in WOMAC pain score for APS compared with saline control.

## REFERENCES


1. Adams SB, Setton LA, Kensicki E, Bolognesi MP, Toth AP, Nettles DL. Global metabolic profiling of human osteoarthritic synovium. *Osteoarthritis Cartilage*. 2012;20(1):64-67.
2. Assirelli E, Filardo G, Mariani E, et al. Effect of two different preparations of platelet-rich plasma on synoviocytes. *Knee Surg Sports Traumatol Arthrosc*. 2014;23(9):2690-2703.
3. Baltzer AW, Moser C, Jansen SA, Krauspe R. Autologous conditioned serum (Orthokine) is an effective treatment for knee osteoarthritis. *Osteoarthritis Cartilage*. 2008;17(2):152-160.
4. Bennell KL, Buchbinder R, Hinman RS. Physical therapies in the management of osteoarthritis: current state of the evidence. *Curr Opin Rheumatol*. 2015;27(3):304-311.
5. Bertone AL, Ishihara A, Zekas LJ, et al. Evaluation of a single intra-articular injection of autologous protein solution for treatment of osteoarthritis in horses. *Am J Vet Res*. 2014;75(2):141-151.
6. Cavallo C, Filardo G, Mariani E, et al. Comparison of platelet-rich plasma formulations for cartilage healing: an in vitro study. *J Bone Joint Surg Am*. 2014;96(5):423-429.
7. Cole BJ, Karas V, Hussey K, Merkow DB, Pilz K, Fortier LA. Hyaluronic acid versus platelet-rich plasma: a prospective, double-blind randomized controlled trial comparing clinical outcomes and effects on intra-articular biology for the treatment of knee osteoarthritis. *Am J Sports Med*. 2017;45(2):339-346.
8. Creamer P, Lethbridge-Cejku M, Hochberg MC. Determinants of pain severity in knee osteoarthritis: effect of demographic and psychosocial variables using 3 pain measures. *Rheumatology*. 1999;26(8):1785-1792.
9. Cucchiarini M, deGirolamo L, Filardo G, et al. *Basic Science of Osteoarthritis*. Berlin: Springer; 2016.
10. Deshpande BR, Katz JN, Solomon DH, et al. Number of persons with symptomatic knee osteoarthritis in the US: impact of race and ethnicity, age, sex, and obesity. *Arthritis Care Res*. 2016;68(12):1743-1750.
11. Filardo G, DiMatteo B, DiMartino A, et al. Platelet-rich plasma intra-articular knee injections show no superiority versus viscosupplementation: a randomized controlled trial. *Am J Sports Med*. 2015;43(7):1575-1582.
12. Filardo G, Kon E. PRP: product rich in placebo? *Knee Surg Sports Traumatol Arthrosc*. 2016;24(12):3702-3703.
13. Filardo G, Kon E, Longo UG, et al. Non-surgical treatments for the management of early osteoarthritis. *Knee Surg Sports Traumatol Arthrosc*. 2016;24(6):1775-1785.
14. Filardo G, Kon E, Roffi A, Di Matteo B, Merli ML, Marcacci M. Platelet-rich plasma: why intra-articular? A systematic review of preclinical studies and clinical evidence on PRP for joint degeneration. *Knee Surg Sports Traumatol Arthrosc*. 2013;23(9):2459-2474.
15. Goldring MB, Otero M, Plumb DA, et al. Roles of inflammatory and anabolic cytokines in cartilage metabolism: signals and multiple effectors converge upon MMP-13 regulation in osteoarthritis. *Eur Cells Materials*. 2011;21:202.
16. Halpern B, Chaudhury S, Rodeo SA, et al. Clinical and MRI outcomes after platelet-rich plasma treatment for knee osteoarthritis. *Clin J Sport Med*. 2013;23(3):238-239.
17. Heijink A, Gomoll AH, Madry H, et al. Biomechanical considerations in the pathogenesis of osteoarthritis of the knee. *Knee Surg Sports Traumatol Arthrosc*. 2012;20(3):423-435.
18. Hraha TH, Doremus KM, McIlwraith CW, Frisbie DD. Autologous conditioned serum: the comparative cytokine profiles of two commercial methods (IRAP and IRAP II) using equine blood. *Equine Vet J*. 2011;43(5):516-521.
19. King WJ, vanDerWeegen W, vanDrumpt R, Soons H, Toler K, Woodell-May J. White blood cell concentration correlates with

increased concentrations of IL-1ra and changes in WOMAC pain scores in an open-label safety study of autologous protein solution. *J Exp Orthop*. 2016;3(1):9.
20. LaPrade CM, James EW, LaPrade RF, Engebretsen L. How should we evaluate outcomes for use of biologics in the knee? *J Knee Surg*. 2015;28(1):35-44.
21. LaPrade RF, Geeslin AG, Murray IR, et al. Biologic treatments for sports injuries, II: think tank—current concepts, future research, and barriers to advancement, part 1: biologics overview, ligament injury, tendinopathy. *Am J Sports Med*. 2016;44(12):3270-3283.
22. Liu-Bryan R. Synovium and the innate inflammatory network in osteoarthritis progression. *Curr Rheumatol Reports*. 2013;15(5):1-7.
23. Mariani E, Canella V, Cattini L, et al. Leukocyte-rich platelet-rich plasma injections do not up-modulate intra-articular pro-inflammatory cytokines in the osteoarthritic knee. *PLoS One*. 2016;11(6): e0156137.
24. Matuska A, O'Shaughnessey KM, King WJ, Woodell-May JE. Autologous solution protects bovine cartilage explants from IL-1a and TNFa induced cartilage degradation. *J Orthop Res*. 2013;31(12): 1929-1935.
25. Meijer H, Reinecke J, Becker C, Tholen G, Wehling P. The production of anti-inflammatory cytokines in whole blood by physico-chemical induction. *Inflamm Res*. 2003;52(10):404-407.
26. Montaseri A, Busch F, Mobasheri A, et al. IGF-1 and PDGF-bb suppress IL-1 beta-induced cartilage degradation through down-regulation of NF-kappaB signaling: involvement of Src/PI-3K/AKT pathway. *PLoS ONE*. 2011;6(12):e28663.
27. O'Shaughnessey K, Matuska A, Hoeppner J, et al. Autologous protein solution prepared from the blood of osteoarthritic patients contains an enhanced profile of anti-inflammatory cytokines and anabolic growth factors. *J Orthop Res*. 2014;32(10):1349-1355.
28. O'Shaughnessey K, Panitch A, Woodell-May JE. Blood-derived anti-inflammatory protein solution blocks the effect of IL-1b on human macrophages in vitro. *Inflamm Res*. 2011;60(10):929-936.
29. Pham T, van der HD, Altman RD, et al. OMERACT-OARSI initiative: Osteoarthritis Research Society International set of responder criteria for osteoarthritis clinical trials revisited. *Osteoarthritis Cartilage*. 2004;12(5):389-399.
30. Pifer MA, Maerz T, Baker KC, Anderson K. Matrix metalloproteinase content and activity in low-platelet, low-leukocyte and high-platelet, high-leukocyte platelet rich plasma (PRP) and the biologic response to PRP by human ligament fibroblasts. *Am J Sports Med*. 2014;42(5): 1211-1218.
31. Rutgers M, Saris DB, Dhert WJ, Creemers LB. Cytokine profile of autologous conditioned serum for treatment of osteoarthritis, in vitro effects on cartilage metabolism and intra-articular levels after injection. *Arthritis Res Ther*. 2010;12(3):R114.
32. Smith PA. Intra-articular autologous conditioned plasma injections provide safe and efficacious treatment for knee osteoarthritis: an FDA-sanctioned, randomized, double-blind, placebo-controlled clinical trial. *Am J Sports Med*. 2016;44(4):884-891.
33. Wanstrath AW, Hettlich BF, Su L, et al. Evaluation of a single intra-articular injection of autologous protein solution for treatment of osteoarthritis in a canine population. *Vet Surg*. 2016;45(6):10.
34. Woodell-May J, Matuska A, Oyster M, Welch Z, O'Shaughnessey K, Hoeppner J. Autologous protein solution inhibits MMP-13 production by IL-1beta and TNFalpha-stimulated human articular chondrocytes. *J Orthop Res*. 2011;29:1320-1326.
35. Yang KG, Raijmakers NJ, van Arkel ER, et al. Autologous interleukin-1 receptor antagonist improves function and symptoms in osteoarthritis when compared to placebo in a prospective randomized controlled trial. *Osteoarthritis Cartilage*. 2008;16(4):498-505.