Efficacy and Tolerability of Progen, a Nutritional Supplement Based on Innovative Plasma Proteins, in ACL Reconstruction

A Multicenter Randomized Controlled Trial

Emilio López-Vidriero,* MD, PhD, Ramón Olivé-Vilas,† MD, PhD, David López-Capapé,‡ MD, PhD, Luisa Varela-Sende,§‖ PharmD, MSc, Rosa López-Vidriero,* MD, and Luís Til-Pérez,† MD

Investigation performed at CST Consorci Sanitari de Terrassa, Barcelona, Spain; ISMEC, Sevilla, Spain; and Clinica CEMTRO, Madrid, Spain

Background: New biologic strategies are arising to enhance healing and improve the clinical outcome of anterior cruciate ligament (ACL) reconstruction.

Purpose: To evaluate the efficacy of a new oral nutritional supplement (Progen) that contains hydrolyzed collagen peptides and plasma proteins, a hyaluronic acid–chondroitin sulfate complex, and vitamin C.

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

Methods: The study included patients who underwent ACL reconstruction with hamstring autografts using the same fixation method. All patients received the same analgesia and physical therapy (PT) protocol and were randomized to receive either the nutritional supplement (supplemented group) or no additional therapy (control group). Patients were followed up at days 7, 30, 60, and 90. Pain was assessed by use of a visual analog scale (VAS) and by analgesic consumption. Clinical outcome was assessed via International Knee Documentation Committee (IKDC) score and the number of PT sessions. Perceived efficacy and tolerability were rated on a 5-point Likert scale. Graft maturation was assessed by a blinded musculoskeletal radiologist using magnetic resonance imaging. The number of adverse events (AEs) was recorded.

Results: The intention-to-treat analysis included 72 patients, 36 allocated to the supplemented group and 36 to the control group, with no significant differences regarding demographic and preoperative characteristics. Both groups showed significant improvement in pain and function (measured by VAS and IKDC scores) during the 90-day follow-up period ($P < .001$ for both), without significant differences between groups. The supplemented group had fewer patients that needed analgesics (8.5% vs 50.0%; $P < .05$) and attended fewer PT sessions (38.0 vs 48.4 sessions; $P < .001$) at 90 days and had a higher IKDC score at 60 days (62.5 vs 55.5; $P = .029$) compared with the control group. Patient- and physician-perceived efficacy was considered significantly higher in the supplemented group at 60 and 90 days ($P < .05$). Perceived tolerability of the overall intervention was better in the supplemented group at 30, 60, and 90 days ($P < .05$). Graft maturation showed more advanced degrees (grades 3 and 4) in the supplemented group at 90 days (61.8% vs 38.2%; $P < .01$). No intolerance or AEs associated with the nutritional supplement treatment were reported.

Conclusion: The combination of the nutritional supplement and PT after ACL reconstruction improved pain, clinical outcome, and graft maturation. Nutritional supplementation showed higher efficacy during the second month of recovery, without causing AEs.

Registration: NCT03355651 (ClinicalTrials.gov identifier).

Keywords: anterior cruciate ligament tears; dietary supplementations; platelet-rich plasma; collagen; chondroitin sulfate; hyaluronic acid; vitamin C

Anterior cruciate ligament (ACL) tears are common injuries in sports medicine.[46] They are disabling, and the injury may end an athlete's career. Owing to the relevant impact on the patient's life, there is an increasing trend toward surgical treatment options,[46] with overall

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satisfactory outcomes. However, results may differ depending on the technique, biomechanics of the construct, rehabilitation program, concomitant injuries, and biological features of the healing process.[28]

In recent years, various strategies have been proposed to improve surgical outcomes and achieve an earlier return to play and a decrease in failure rates and secondary surgeries. These strategies include the revision of the tunnel placement[29] and the implementation of accelerated rehabilitation programs.[27] Furthermore, the field of sports medicine—and particularly ACL surgery—has raised interest in the use of platelet-rich plasma (PRP), including growth factors and other cytokines to enhance healing.[28,41]

The use of PRP to enhance the healing process of ACL reconstruction and accelerate the patient's return to play in safe conditions is an emerging research topic.[41] Recently, a systematic review showed promising results in terms of intra-articular maturation of grafts, although results regarding graft integration in the tunnels are controversial.[15] The divergence in the reported results might be due to lack of consensus regarding PRP protocols, which vary in terms of preparation, application, timing, and dose.[28]

Various nutritional supplements, including hyaluronic acid (HA),[22] glucosamine sulfate (GS),[43] chondroitin sulfate (CS),[33,43] and collagen,[10,45] have shown efficacy and safety in managing hip and knee osteoarthritis. However, results in this regard are heterogeneous, and studies investigating the use of nutritional supplements as adjuvants to improve ACL postsurgical results are scarce. To date, scientific evidence has not shown any benefits of oral supplementation with chondroprotective substances such as GS[14] or with substances aimed at enhancing muscle strength and performance during recovery (eg, creatinine,[51] leucine,[24] antioxidants such as vitamins C and E,[6] or even high doses of carbohydrates and proteins[21]).

The use of injected intra-articular PRP and viscosupplementation with HA is well accepted in the orthopaedic and rheumatologic literature, and good results have been reported in randomized studies and systematic reviews in terms of pain in knee and hip osteoarthritis.[9,26] However, injections are invasive procedures with notable drawbacks, such as risk of infection, the need for a sterile environment, pain and other side effects (eg, edema and swelling), and health care costs.

To overcome the aforementioned limitations, new strategies have been developed to deliver these substances orally and avoid the drawbacks of injection. Progen, the nutritional supplement evaluated in the current study, is a combination of hydrolyzed collagen (CH), porcine plasma proteins, a complex of HA and CS (HC-15), and vitamin C.[1] The rationale behind this combination is that collagen peptides and plasma proteins enhance the healing cascade of the ACL graft, mainly ligamentization.[17,40] HA and CS have been assessed as chondroprotective agents and have shown promising results regarding pain reduction.[22,35,49,50] Finally, vitamin C is an antioxidant that may play a role in decreasing proinflammatory cytokines by increasing interleukin 1.[7]

To our knowledge, this is the first study evaluating the effect of this oral supplement in the clinical and biological environment of ACL postsurgical reconstruction.

Our hypothesis was that the use of this oral nutritional supplement would improve postoperative symptoms and clinical outcomes, enhance ligamentization, and provide safe treatment with minimal side effects.

METHODS

Study Design

This was a prospective, multicenter, randomized, open-label, unblinded postauthorization clinical study to assess the efficacy of supplementation with plasma proteins, CH, a complex of HA-CS (HC-15), and vitamin C in patients who underwent arthroscopically assisted functional ACL reconstruction with hamstrings using the same fixation method, analgesic therapy, and a physical therapy (PT) protocol. No placebo was used for comparison.

Patients were recruited between March 2015 and January 2017 from 3 Spanish centers, including 2 general hospitals and 1 sports medicine center. The study protocol was approved by the ethics committee of Catalan Sports Council, and all patients gave their written informed consent before entering the study. All data were managed in agreement with local personal data protection law (LOPD 15/1999).

ACL reconstruction was performed with the same procedure in the 3 centers by a single expert surgeon in each center (E.L.-V., R.O.-V., D.L.-C.) as follows: Both hamstring tendons were harvested, doubled, and prepared. A knee arthroscopy was performed to fix any intra-articular disorder. After arthroscopy, femoral and tibial tunnels were drilled according to the graft diameters, and the double hamstring graft was passed through and secured with a suspension device on the femur and an interference screw in the tibia. Patients did not use any orthoses and were allowed partial weightbearing as tolerated.

‖Address correspondence to Luisa Varela-Sende, MSc, Plaça d'Europa 13-15, 08908 L'Hospitalet de Llobregat, Spain (email: lvarela@opko.com).
*ISMEC, Sevilla, Spain.
†CST Consorci Sanitari de Terrassa, Barcelona, Spain.
‡Clinica CEMTRO, Madrid, Spain.
§OPKO Health, Barcelona, Spain.

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Ethical approval for this study was obtained from the Catalan Sports Council, Barcelona, Spain.

Upon entering the study, patients were consecutively assigned a randomization number from a centralized randomization list (Random Allocation Software 1.0). Based on the randomization number, patients were allocated into one of the following 2 groups: the control group was treated with the rehabilitation protocol and analgesics only, and the supplemented group was treated with rehabilitation, analgesics, and the nutritional supplement (1 sachet daily). Each sachet contained 2500 mg of CH, 300 mg of porcine PRP, 50 mg of HC-15, and 40 mg of vitamin C (70% of total protein content and 328 kcal/100 g of energy value). Treatment for analgesia consisted of paracetamol 1 g (up to 3 doses per day) at the patient's discretion. Treatment was administered for 90 days. The patients, therapists, and treating physicians were not blinded as to randomization group. Only the radiologists were blinded throughout the entire study.

The rehabilitation program started on the seventh day after surgery and was the same for both groups. It consisted of 2 phases lasting 6 weeks each: The first 6-week phase aimed at controlling pain, decreasing swelling, and restoring range of motion, and the second 6-week phase included specific exercises focused on restoring strength and proprioception. Concomitant treatment with analgesics was permitted. The surgery was performed at day 0 (baseline). Assuming a regular rehabilitation time for PT programs of 8 to 12 weeks,[27] patients were followed up with visits at days 7, 30, 60, and 90 after starting treatment. Compliance with supplemented treatment was assessed by the number of nonused sachets returned at each follow-up visit and at the end of the study.

Study Population

The study population included patients aged between 18 and 55 years with partial or complete ACL rupture, diagnosed clinically and by magnetic resonance imaging (MRI), requiring reconstructive surgery. Recruitment was limited to patients with symptoms of acute or subacute ACL rupture (such as inflammation of the knee and pain with leg movement) or chronic ACL tear, including instability of the knee. Other inclusion criteria were ACL ruptures without osteochondral lesions requiring additional surgery and patients who, according to their medical records, were responders to analgesic agents.

Patients with concomitant osteochondral abnormality were excluded from the study. Other exclusion criteria were treatment with intra-articular injections of corticosteroids and/or PRP or with oral glucosamine, CS, HA, or CH in the 2 months prior to surgery. Patients with systemic diseases, those treated with antibiotics or other drugs that might alter the healing process, and those who had undergone arthroscopic lavage in the 90 days prior to surgery were also excluded.

Study Endpoints and Variables

The primary endpoint was the change in pain throughout the follow-up period (ie, from baseline to 30, 60, and 90 days), assessed by use of a 100-mm visual analog scale (VAS) and by means of analgesic consumption. Knee function was assessed by use of the International Knee Documentation Committee (IKDC) score. The final score was interpreted as a measure of function, with higher scores representing higher levels of function.[2,11] The VAS and IKDC forms are both validated tools for the assessment of patients with ACL injury.

Secondary endpoints included indirect measures of functional improvement, such as the number of required rehabilitation sessions, which was determined at the physician's discretion based on achievement of the rehabilitation goals: full range of joint motion, reduction of muscular atrophy, decrease of joint effusion, and full weightbearing gait without pain. The maturation and ligamentization of the graft were assessed by MRI on the days the treatment started and ended by a blinded musculoskeletal radiologist using a standard procedure based on ACL oblique parasagittal T1-weighted sequences.[38] The signal intensity of the graft was compared with the posterior cruciate ligament (typical hypointensity) and graded. Grafts were classified according to the hypointensity percentage, associated with maturation, into 4 grades: grade 1 was considered below 25% of hypointensity, grade 2 between 25% and 50%, grade 3 between 50% and 75%, and grade 4 between 75% and 100%. Grafts of grades 3 and 4 were deemed to be in advanced degrees of maturation or ligamentization.[12,48]

Perceived efficacy and tolerability of the whole treatment (ie, PT with or without oral supplementation) were assessed by patients and physicians using an ad hoc single-item questionnaire and rated on a 5-point Likert scale, where higher scores indicated better efficacy and tolerability. Last, treatment safety was assessed by means of all adverse events (AEs) reported throughout follow-up.

Statistical Analysis

The sample size was estimated considering that 20 mm on the 100-mm VAS was the minimum clinically relevant difference to detect a relevant change in pain scores in patients with knee diseases, with a standard deviation (SD) of 23.38 (data reported in previous studies).[8] Under these assumptions, a study sample of 72 patients (36 in each group) would provide a statistical power of 90% with a significance α level of .05.

The analysis was performed as an intention-to-treat (ITT) analysis; hence, patients lost to follow-up after the first visit were included in the analysis. Clinical and demographic characteristics of study patients were described by use of means and standard deviations as quantitative variables. The mean values of the baseline characteristics in each group were compared by use of an unpaired t test, whereas the percentages were compared via Fisher exact test. Differences between 2 given visits were analyzed by use of a paired-samples t test, whereas differences between the study groups throughout the study were compared by analysis of variance of repeated measures. In contrast, the physicians' and patients' perceptions of tolerability and efficacy in each study group were compared via the nonparametric Mann-Whitney U test. The analysis was performed on available data only, and thus no imputations were made for missing values, which were eliminated

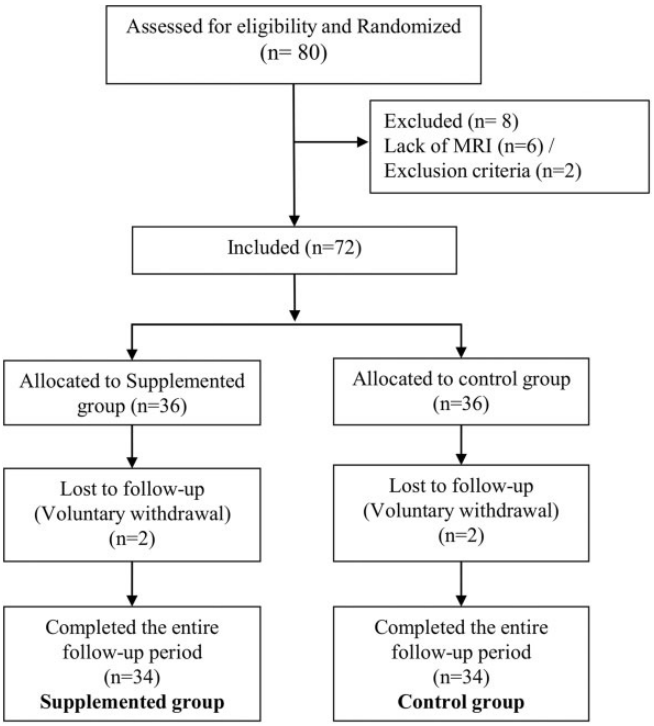

Figure 1. Flow diagram of the patients included in the study. MRI, magnetic resonance imaging.

pairwise. The significance threshold was set at an α value of .05, and all analyses were computed by an external statistical team (Onmedic) using the SAS System statistics software (version 9.4).

RESULTS

Patient Characteristics

Between March 2015 and January 2017, a total of 80 patients were considered for randomization. Of these, 6 patients were excluded because they lacked a previous MRI and 2 patients because they did not fulfill the inclusion criteria after the surgery; thus 72 patients (83% males) with a mean ± SD age of 33.7 ± 9.9 years were included in the study (Figure 1). Four patients were lost to follow-up (voluntary withdrawal), 2 in each group of patients. Their available data were included in the analysis to comply with the ITT strategy. Ultimately, 68 patients completed the entire follow-up (34 in each group). All patients assigned to the supplemented group completed treatment with the nutritional supplement according to the established schedule.

At baseline, patients allocated to the supplemented group and the control group showed no significant differences in their demographic characteristics (Table 1). Likewise, the scores of all scales used for the assessment of knee pain and function (VAS and IKDC score) were equal between the groups. Most patients were taking analgesics at the beginning of the study (75% of them occasionally), with no significant differences between groups.

Main Efficacy Scales

Patients in both groups experienced a significant improvement in VAS and IKDC scores during the follow-up period ($P < .001$, paired t test at the end of the study). By the 90-day follow-up, the VAS score had improved in both groups, decreasing –3.32 ± 2.79 cm in the control group and –3.91 ± 2.72 cm in the supplemented group, with no significant difference between groups (Figure 2). The corresponding improvement in the IKDC score was 20.32 ± 15.61 in the control group and 26.21 ± 19.44 in the supplemented group, respectively. The IKDC score at the 60-day follow-up was significantly higher in the supplemented group (62.5 ± 11.7 vs 55.5 ± 11.1; $P = .029$); however, no significant differences were found at other the visits (Figure 2).

Patients in both groups reported a similar consumption of analgesic drugs during the first 30 days of treatment. After that time, analgesic consumption, irrespective of the frequency, was significantly greater in the control group; analgesics were taken by 50% to 56% of patients in the control group versus 9% to 11% in the supplemented group. At the final follow-up, 91.4% of patients in the supplemented group did not require any analgesics, whereas the corresponding proportion in the control group was 50.0% (Figure 3).

Indirect Measures of Efficacy

The cumulative number of rehabilitation sessions required for recovery, used as an indirect measure of functional improvement, was significantly greater in the control group (Figure 4). By the 90-day follow-up, patients in the control group had attended greater number of rehabilitation sessions than those in the supplemented group, with a mean of 48.4 ± 11.3 versus 38.0 ± 9.3 sessions, respectively ($P < .001$).

Regarding MRI examination, at the final follow-up, 61.7% of patients in the supplemented group showed advanced degrees (grades 3 and 4) of graft maturation; the corresponding percentage in the control group was 38.4% ($P = .05$) (Table 2).

Perception of Efficacy and Tolerability

Based on the physician's perception, combined treatment with PT and the nutritional supplement was significantly more effective than that based on rehabilitation only, at day 30 of the follow-up period (Figure 5A). Patients rated the efficacy of combined treatment significantly greater at days 60 and 90 (Figure 5B).

Both physicians and patients rated the tolerability of treatment with PT and the nutritional supplement higher than that based on PT only, with significant differences at days 30 and 60 according to the physicians' assessments (Figure 5C) and at days 30, 60, and 90 according to the patients' assessments (Figure 5D).

Safety

During the follow-up period, no AEs associated with the nutritional supplement were reported. Overall, 4 patients (5.6%)

TABLE 1
Baseline Characteristics of Study Patients[a]

	Overall (N = 68)	Supplemented Group (n = 34)	Control Group (n = 34)	*P* Value[b]
Demographic characteristics				
Age, y, mean ± SD	33.71 ± 9.88	32.89 ± 9.23	34.53 ± 10.56	.485
BMI, kg/m^2, mean ± SD	24.59 ± 3.11	24.64 ± 3.70	24.54 ± 2.43	.897
Sex, n (%)				
Males	60 (83.33)	31 (86.11)	29 (80.56)	.753
Females	12 (16.67)	5 (13.89)	7 (19.44)	
Clinical characteristics				
Affected knee, n (%)				
Right	39 (54.2)	20 (55.56)	19 (52.78)	≤.999
Left	33 (45.8)	16 (44.44)	17 (47.22)	
VAS score, mean ± SD	5.00 ± 2.50	5.04 ± 2.44	4.96 ± 2.60	.904
IKDC score, mean ± SD	45.35 ± 13.33	44.83 ± 15.09	45.88 ± 11.50	.740
Basal analgesic consumption, n (%)				
Any consumption	41 (56.9)	21 (58.3)	20 (55.6)	≤.999
Frequency				
Often	10 (25.0)	4 (19.1)	6 (31.6)	.484
Occasionally	31 (75.6)	17 (81.0)	14 (70.0)	

[a]Percentages of patients in categorical variables were calculated for each group. BMI, body mass index; IKDC, International Knee Documentation Committee; VAS, visual analog scale.
[b]*P* values correspond to the comparison between the supplemented group and the control group (*t* test for quantitative variables and Fisher exact test for categorical variables).

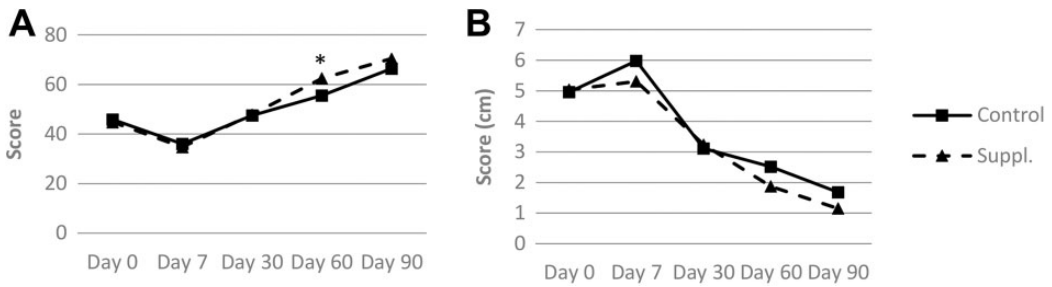

Figure 2. Efficacy assessments during the follow-up in the control and supplemented groups. (A) International Knee Documentation Committee score. (B) Visual analog scale pain score. *$P < .05$, between-group comparison.

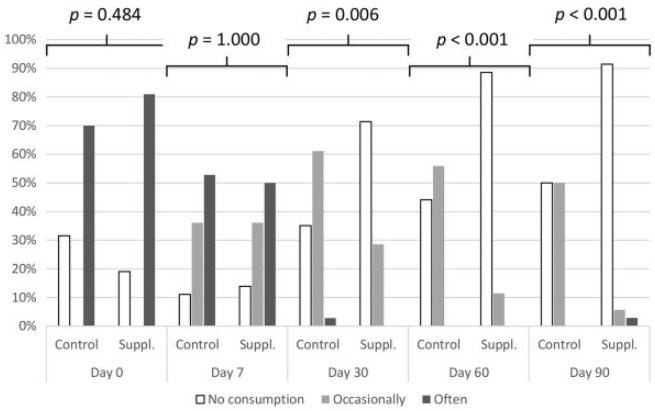

Figure 3. Analgesic consumption during the follow-up in the control and supplemented groups.

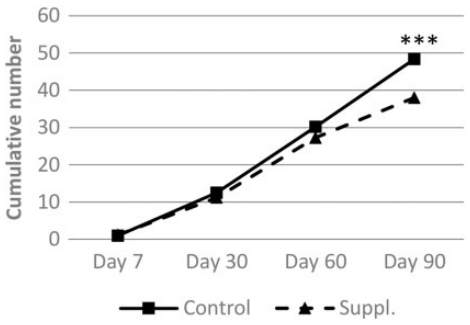

Figure 4. Number of rehabilitation sessions needed for recovery in the control and supplemented groups. ***$P < .001$, between-group differences.

reported 7 AEs 4 in the supplemented group and 3 in the control group. All AEs were considered mild and were attributed to the rehabilitation protocol.

DISCUSSION

In this open-label, unblinded, parallel-group, multicenter, randomized clinical trial in patients who underwent

TABLE 2
Graft Maturation According to the Blinded Magnetic Resonance Imaging Examination[a]

	Grade 1	Grade 2	Grade 3	Grade 4
Control group (n = 34)	8 (23.5)	13 (38.2)	12 (35.5)	1 (2.9)
Supplemented group (n = 34)	2 (5.9)	11 (32.4)	20 (58.8)	1 (2.9)

[a]Grafts were classified according to hypointensity percentage (associated with maturation): grade 1, <25%; grade 2, 25%-50%; grade 3, 50%–75%; and grade 4, 75%–100%. Values are expressed as n (%).

surgical reconstruction after ACL injury, both PT only (control group) and PT combined with a dietary supplement containing PRP, collagen, HA, CS, and vitamin C (supplemented group) were associated with similar improvements in pain and function during the 90-day follow-up period. At the end of the follow-up period (ie, 90 days) patients in both groups showed a significant improvement in pain and knee function, with no significant differences between groups; analgesic consumption was similar between groups for the first 30 days of treatment. At 60-day follow-up, the supplemented group had superior outcomes in terms of function, the need for analgesics, maturation of the graft, number of PT sessions, and patient-reported efficacy and tolerability.

Both groups were well balanced in terms of demographic and clinical characteristics, including the functional and pain scales and the consumption of analgesic drugs at treatment start. Baseline VAS and IKDC scores suggested that the study sample had a moderate severity of injury at the beginning of treatment. The IKDC score has not been used often in previous studies assessing the efficacy of adjuvant treatments for ACL recovery, precluding comparisons; however, the severity of pain in the study patients (overall VAS score at baseline was 5.0) was slightly higher than that reported by other authors, with their scores ranging from 2.3 to 3.5.[18,20,53] This difference could be due to the greater proportion of acute-subacute ACL patients undergoing surgery in our centers (86%), who tend to be more symptomatic than patients with chronic ACL, who report only instability.

As expected, patients in both groups experienced a transitory worsening of symptoms during the first week after surgery. Regarding pain, the transient worsening reached a significant threshold in the control group but not in the supplemented group. Worsening of symptoms during the first week following surgery has been reported in other studies investigating recovery after ACL reconstruction.[18,20,23,30] After the first week of treatment, the VAS score decreased progressively in both intervention groups, showing scores significantly lower than baseline at day 30 and following visits. At the end of treatment, the VAS score had decreased by 3.9 cm in the supplemented group and 3.3 cm in the control group. Although no statistical differences were found between the groups, a clinical trend toward less pain (as assessed by VAS) was found in the supplemented group throughout the course of the study. In addition, analgesic consumption was significantly lower in the supplemented group, indirectly indicating a good clinical effect in controlling pain. At the last visit, 91% of patients in the supplemented group did not require analgesics at all, whereas 50% of patients in the control group were still taking analgesics. This observation is consistent with the proven efficacy of PRP and HA in reducing pain when used either alone or in combination.[9,25,42] Overall, the therapeutic effects of PRP are well accepted in sports medicine in general[5,16,28] and in the context of ACL reconstruction in particular.[4,15] In this regard, the combination of both products may have had an additive and boosting effect.[42] Patients with chondral injury were excluded from this study, thus ruling out the impact of chondroprotective effects of both PRP and HA on pain.

Knee function, assessed by the IKDC score, showed a progressive improvement in the first week after ACL reconstruction. The improvement in IKDC score from baseline was statistically significant at postoperative days 30, 60, and 90. The comparative analysis between groups revealed a significant improvement at 60 days for the supplemented group compared with the control group. We believe this could be because of better pain control and the enhancing effect of the nutritional supplement in the biological process of the ACL reconstruction. According to this hypothesis, at the 60-day assessment, the supplemented group could have achieved better range of motion and more easily tolerated the initial strengthening exercises. However, this difference was no longer seen at 90 days because many patients were likely to achieve optimum graft maturation by 90 days. Differences in PT delivery between centers may have introduced variability among study participants, albeit in a similar way in both study groups.

After a thorough review of the scientific literature, we found only 1 study was found that investigated the influence of a similar supplement on the IKDC score. In that study, Eraslan and Ulkar[14] compared the efficacy of GS versus placebo and found no differences in the IKDC score between groups at 14 weeks after ACL reconstruction. Importantly, their approach differed from ours in the use of a single-ingredient supplement and the start of supplementation 6 weeks after the surgical procedure. Moreover, treatment with GS lasted 2 months. In view of the obtained results, we believe that the use of an isolated supplement, instead of a combination, and the delay in beginning treatment might have underpowered its clinical effect.

PT based on rehabilitation exercises has shown—by itself—high efficacy in recovery after ACL reconstruction,[23,31,52] thus leading some investigators to study significant differences when comparing the efficacy of PT only and that of combined treatments. Accordingly, previous studies investigating the efficacy of adjuvant treatments with PT or comparing the efficacy of different types of exercises have found few differences or even conflicting results.[32,34,47,53]

In the current study, the number of supervised rehabilitation sessions needed, determined at the physician's discretion based on the achievement of rehabilitation goals, was significantly less in the supplemented group. Overall,

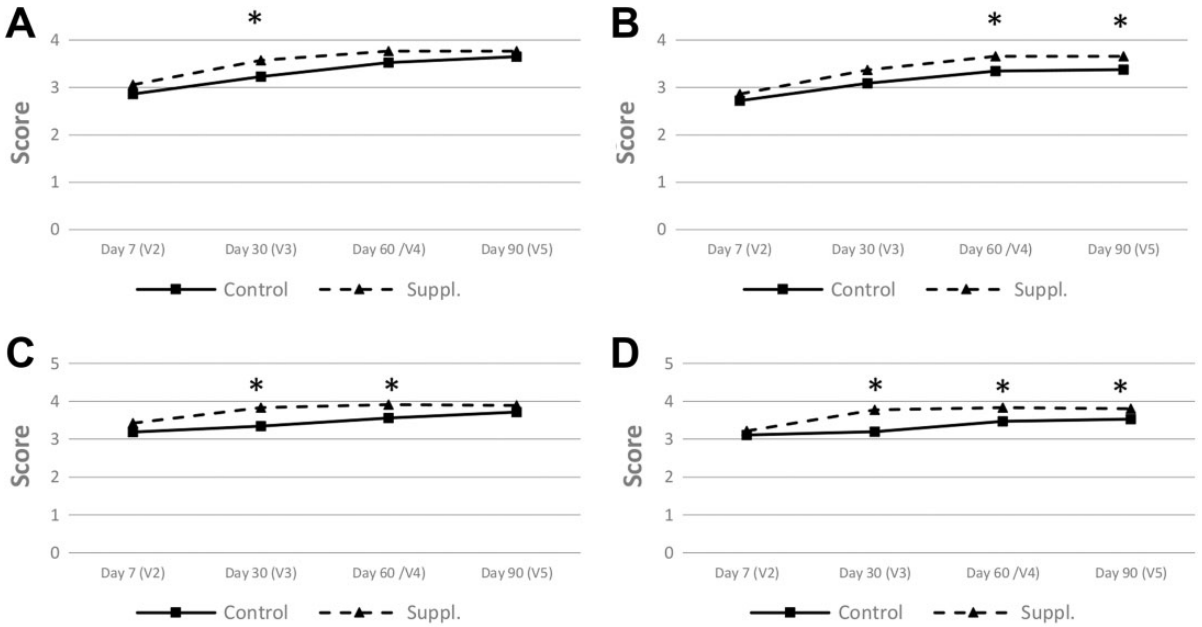

Figure 5. Perception of efficacy and tolerability in the control and supplemented groups throughout all follow-up visits (V2-V5). Perception of efficacy by (A) physicians and (B) patients. Perception of tolerability by (C) physicians and (D) patients. *$P < .05$, between-group differences.

these patients required an average of 10 fewer rehabilitation sessions than those in the control group. This observation might be associated with the good clinical and biological effect of the nutritional supplement on pain and function over time. Typically, the rehabilitation protocol allows spacing of supervised sessions as patients achieve the goals of the various phases. In this study, significant differences were observed at 90 days, when patients who had achieved full range of motion focused on proprioception and strengthening. Interestingly, we found a trend toward significance since the 60-day visit. The beneficial effect of the nutritional supplement on pain and swelling during the first 6-week phase may have led to the improvement of these patients over time compared with the control group, such that the supplemented group required fewer PT sessions.

To assess the biological effect of the nutritional supplement on graft maturation, MRI examinations were performed at baseline and at the final follow-up. ACL parasagittal cuts were performed in T1-weighted sequences[38] and analyzed by a blinded musculoskeletal radiologist to improve the quality of the study. The examinations revealed a significantly higher percentage of patients with advanced maturation (grades 3 and 4) in the supplemented group (61.7%) compared with the control group (38.4%).

These results are comparable with those of different studies using injected PRP to accelerate ACL ligamentization.[36,38] Although promising, the use of PRP is still controversial in terms of maturation. One prior study found a trend toward significance at 3 months and definite differences at 6 months or 1 year.[15] The use of oral PRP in combination with CH in the current study may have enhanced healing and accelerated the natural course of ligamentization of hamstring grafts compared with injected PRP. Because collagen is involved in the ligamentization phase,[13] adding active collagen peptides to the preparation may lead to better results. This approach is important because hamstring autografts are used frequently and they tend to mature more slowly than patellar tendon grafts.[3,19]

Injectable HA has been widely used as intra-articular viscosupplementation, with positive results and minor local adverse effects. Preliminary studies comparing oral HA versus viscosupplementation in the treatment of early osteoarthritis reported similar effects of both treatments regarding pain reduction and functional improvement.[39] Additionally, the combined use of oral and intra-articular therapies has shown a boosting effect in terms of pain relief and reduced analgesic consumption.[37] This approach may open the door to the combined use of oral and intra-articular PRP.

In line with the consistent trend observed in indirect measures of efficacy, patients and physicians in the current study perceived that treatment with PT and dietary supplement was more effective than treatment with PT only, although differences were not significant at all visits. As with the IKDC assessment, significant differences in the subjective perception of efficacy tended to emerge later in the follow-up period.

The safety and tolerability of orally administered chondroprotective agents such as HA, CH, and CS have been widely reported in the literature.[10,22,43,44] However, the concentration and composition of the various nutritional supplements available as adjuvants for musculoskeletal

conditions may differ. Furthermore, the plasma protein included in the study formulation was a novel ingredient, and although registry studies have proven its overall safety,[1] data regarding its use in clinical practice are scarce. Consistent with safety results observed in previous trials, after 90 days of daily administration of the nutritional supplement in the present study, all AEs reported were mild, and none were deemed to be associated with the product. Additionally, treatment tolerability, assessed by the subjective perception of patients and physicians, showed a significantly greater tolerability of the supplemented group at various visits.

These findings should be interpreted taking into account some limitations of the study. First, owing to our national regulations for nutritional support, which do not allow the study of nutritional supplements as drugs (only as adjuvants), the study was open-label, was unblinded to physicians, therapists, and patients, and did not include a placebo for the control group. This is the main limiting factor of the study. The investigators considered conducting the clinical study in another country, but this option entailed a limitation as well, because at the time the product was authorized in only a few countries. This design increases the risk of a placebo effect in the supplemented group, not only for the patient- and physician-reported outcomes but also for the duration of postoperative rehabilitation. In this regard, the MRI results, obtained via a blinded assessment, were of great relevance for supporting the treatment efficacy. Second, the sample size might be a limitation of the study. Although the sample size was estimated by use of a power analysis, a larger sample might have allowed us to find more statistically significant differences between groups in the main efficacy scores at all time points. Nevertheless, we did find significant differences at the 60-day follow-up for IKDC score and at the 90-day final follow-up for maturation of the graft, analgesic consumption, rehabilitation sessions, and efficacy evaluated by the patient. Third, most of the assessments were patient-reported and, therefore, are associated with an increased risk of bias. Despite these limitations, our results showed internal consistency, as improvements were observed—to a greater or lesser extent—in the various descriptors of the therapy outcome.

CONCLUSION

Our results indicate that both interventions are associated with similar pain reduction and functional recovery after arthroscopic reconstruction of the ACL with hamstring autografts. The differences observed between the study groups in direct and indirect measures of efficacy suggest that patients who undergo ACL reconstruction may benefit from adjuvant treatment with the nutritional supplement in terms of function at 2 months, the need for analgesics, maturation of the graft, number of PT sessions, and patient-reported efficacy and tolerability. In addition, our results show that the use of the nutritional supplement daily for 3 months is safe, without any AEs due to the product. Considering the noninvasive nature of oral supplementation, our results have important clinical implications in terms of possible faster recovery and earlier return to play without treatment-associated safety concerns.

To the best of our knowledge, this is the first study of this nutritional supplement in a clinical setting and the first to show clinical and biological improvements associated with nutritional supplementation after ACL reconstruction as a postoperative adjuvant. Nevertheless, further investigations with this product and with larger study samples with placebo controls should be conducted to confirm these improvements.

REFERENCES

1. Agut J, Fernández C, Cos J, Ocana L, Genove E. Platelet-rich plasma compositions. European patent 2628484 A1. August 21, 2013.
2. American Orthopaedic Society for Sports Medicine. IKDC Forms. Formularios del International Knee Documentation Committee—IKDC para la Evaluación de la Rodilla. https://www.sportsmed.org/AOSSMIMIS/members/downloads/research/IKDCSpanish.pdf. Published 2000.
3. Amiel D, Ing D, Kleiner JB, Akeson WH. The natural history of the anterior cruciate ligament autograft of patellar tendon origin. *Am J Sports Med*. 1986;14(6):449-462.
4. Andriolo L, Di Matteo B, Kon E, Filardo G, Venieri G, Marcacci M. PRP augmentation for ACL reconstruction. *Biomed Res Int*. 2015;2015:1-15.
5. Balasubramaniam U, Dissanayake R, Annabell L. Efficacy of platelet-rich plasma injections in pain associated with chronic tendinopathy: a systematic review. *Phys Sportsmed*. 2015;43(3):253-261.
6. Barker T, Leonard SW, Hansen J, et al. Vitamin E and C supplementation does not ameliorate muscle dysfunction after anterior cruciate ligament surgery. *Free Radic Biol Med*. 2009;47(11):1611-1618.
7. Barker T, Leonard SW, Trawick RH, Walker JA, Traber MG. Antioxidant supplementation lowers circulating IGF-1 but not F(2)-isoprostanes immediately following anterior cruciate ligament surgery. *Redox Rep*. 2009;14(5):221-226.
8. Bellamy N, Carette S, Ford PM, et al. Osteoarthritis antirheumatic drug trials. III. Setting the delta for clinical trials—results of a consensus development (Delphi) exercise. *J Rheumatol*. 1992;19:451-457.
9. Bennell KL, Hunter DJ, Paterson KL. Platelet-rich plasma for the management of hip and knee osteoarthritis. *Curr Rheumatol Rep*. 2017;19(5):24.
10. Bruyère O, Zegels B, Leonori L, et al. Effect of collagen hydrolysate in articular pain: a 6-month randomized, double-blind, placebo controlled study. *Complement Ther Med*. 2012;20(3):124-130.
11. Collins NJ, Misra D, Felson DT, Crossley KM, Roos EM. Measures of knee function: International Knee Documentation Committee (IKDC) Subjective Knee Evaluation Form, Knee injury and Osteoarthritis Outcome Score (KOOS), Knee injury and Osteoarthritis Outcome Score Physical Function Short Form (KOOS-PS), Knee Outcome Survey Activities of Daily Living Scale (KOS-ADL), Lysholm Knee Scoring Scale, Oxford Knee Score (OKS), Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC), Activity Rating Scale (ARS), and Tegner Activity Score (TAS). *Arthritis Care Res (Hoboken)*. 2011;63(suppl 11):S208-S228.
12. Daniels SP, van der List JP, Kazam JJ, DiFelice GS. Arthroscopic primary repair of the anterior cruciate ligament: what the radiologist needs to know. *Skeletal Radiol*. 2018;47(5):619-629.
13. Ekdahl M, Wang JH-C, Ronga M, Fu FH. Graft healing in anterior cruciate ligament reconstruction. *Knee Surg Sports Traumatol Arthrosc*. 2008;16(10):935-947.
14. Eraslan A, Ulkar B. Glucosamine supplementation after anterior cruciate ligament reconstruction in athletes: a randomized placebo-controlled trial. *Res Sports Med*. 2015;23(1):14-26.
15. Figueroa D, Figueroa F, Calvo R, Vaisman A, Ahumada X, Arellano S. Platelet-rich plasma use in anterior cruciate ligament surgery: systematic review of the literature. *Arthroscopy*. 2015;31(5):981-988.

16. Fitzpatrick J, Bulsara M, Zheng MH. The effectiveness of platelet-rich plasma in the treatment of tendinopathy: a meta-analysis of randomized controlled clinical trials. *Am J Sports Med*. 2017;45(1):226-233.
17. Fleming BC, Spindler KP, Palmer M, Magarian E, Murray MM. Collagen-platelet composites improve the biomechanical properties of healing ACL grafts in a porcine model. *Am J Sports Med*. 2009; 37(8):1554-1563.
18. Gerber JP, Marcus RL, Dibble LE, Greis PE, Burks RT, Lastayo PC. Safety, feasibility, and efficacy of negative work exercise via eccentric muscle activity following anterior cruciate ligament reconstruction. *J Orthop Sports Phys Ther*. 2007;37(1):10-18.
19. Goradia VK, Rochat MC, Kida M, Grana WA. Natural history of a hamstring tendon autograft used for anterior cruciate ligament reconstruction in a sheep model. *Am J Sports Med*. 2000;28(1):40-46.
20. Hiemstra LA, Heard SM, Sasyniuk TM, Buchko GL, Reed JG, Monteleone BJ. Knee immobilization for pain control after a hamstring tendon anterior cruciate ligament reconstruction: a randomized clinical trial. *Am J Sports Med*. 2008;37(1):56-64.
21. Holm L, Esmarck B, Mizuno M, et al. The effect of protein and carbohydrate supplementation on strength training outcome of rehabilitation on ACL patients. *J Orthop Res*. 2006;24(11):2114-2123.
22. Kalman DS, Heimer M, Valdeon A, Schwartz H, Sheldon E. Effect of a natural extract of chicken combs with a high content of hyaluronic acid (Hyal-Joint®) on pain relief and quality of life in subjects with knee osteoarthritis: a pilot randomized double-blind placebo-controlled trial. *Nutr J*. 2008;7(1):3.
23. Kruse LM, Gray B, Wright RW. Rehabilitation after anterior cruciate ligament reconstruction: a systematic review. *J Bone Joint Surg Am*. 2012;94(19):1737-1748.
24. Laboute E, France J, Trouve P, Puig PL, Boireau M, Blanchard A. Rehabilitation and leucine supplementation as possible contributors to an athlete's muscle strength in the reathletization phase following anterior cruciate ligament surgery. *Ann Phys Rehabil Med*. 2013;56(2): 102-112.
25. Laudy ABM, Bakker EWP, Rekers M, Moen MH. Efficacy of platelet-rich plasma injections in osteoarthritis of the knee: a systematic review and meta-analysis. *Br J Sports Med*. 2015;49(10):657-672.
26. Laver L, Marom N, Dnyanesh L, Mei-Dan O, Espregueira-Mendes J, Gobbi A. PRP for degenerative cartilage disease. *Cartilage*. 2016;8(4): 341-364.
27. Lee M, Sung DJ, Lee J, et al. Enhanced knee joint function due to accelerated rehabilitation exercise after anterior cruciate ligament reconstruction surgery in Korean male high school soccer players. *J Exerc Rehabil*. 2016;12(1):29-36.
28. Lopez-Vidriero E, Goulding KA, Simon DA, Sanchez M, Johnson DH. The use of platelet-rich plasma in arthroscopy and sports medicine: optimizing the healing environment. *Arthroscopy*. 2010;26(2): 269-278.
29. Lopez-Vidriero E, Hugh Johnson D. Evolving concepts in tunnel placement. *Sports Med Arthrosc*. 2009;17(4):210-216.
30. Mayr HO, Hochrein A, Hein W, Hube R, Bernstein A. Rehabilitation results following anterior cruciate ligament reconstruction using a hard brace compared to a fluid-filled soft brace. *Knee*. 2010;17(2):119-126.
31. Meuffels DE, Favejee MM, Vissers MM, Heijboer MP, Reijman M, Verhaar JAN. Ten year follow-up study comparing conservative versus operative treatment of anterior cruciate ligament ruptures: a matched-pair analysis of high level athletes. *Br J Sports Med*. 2009; 43(5):347-351.
32. Mirzatolooei F, Alamdari MT, Khalkhali HR. The impact of platelet-rich plasma on the prevention of tunnel widening in anterior cruciate ligament reconstruction using quadrupled autologous hamstring tendon. *Bone Joint J*. 2013;95(1):65-69.
33. Möller I, Pérez M, Monfort J, et al. Effectiveness of chondroitin sulphate in patients with concomitant knee osteoarthritis and psoriasis: a randomized, double-blind, placebo-controlled study. *Osteoarthritis Cartilage*. 2010;18(suppl 1):S32-S40.
34. Moraes VY, Lenza M, Tamaoki MJ, Faloppa F, Belloti JC. Platelet-rich therapies for musculoskeletal soft tissue injuries. *Cochrane Database Syst Rev*. 2013;(12):CD010071.
35. Oliveira MZ, Albano MB, Namba MM, et al. Effect of hyaluronic acids as chondroprotective in experimental model of osteoarthrosis. *Rev Bras Ortop*. 2014;49(1):62-68.
36. Orrego M, Larrain C, Rosales J, et al. Effects of platelet concentrate and a bone plug on the healing of hamstring tendons in a bone tunnel. *Arthroscopy*. 2008;24(12):1373-1380.
37. Panuccio E, Memeo A, Richetta S. Evaluation of the combined treatment of oral viscosupplementation with hyaluronic acid intra-articular injection on symptomatic knee osteoarthritis [in Italian]. *Clin Ter*. 2015; 166(5):e321-e326.
38. Radice F, Yánez R, Gutiérrez V, Rosales J, Pinedo M, Coda S. Comparison of magnetic resonance imaging findings in anterior cruciate ligament grafts with and without autologous platelet-derived growth factors. *Arthroscopy*. 2010;26(1):50-57.
39. Ricci M, Micheloni GM, Berti M, et al. Clinical comparison of oral administration and viscosupplementation of hyaluronic acid (HA) in early knee osteoarthritis. *Musculoskelet Surg*. 2017;101(1):45-49.
40. Sánchez M, Anitua E, Azofra J, Prado R, Muruzabal F, Andia I. Ligamentization of tendon grafts treated with an endogenous preparation rich in growth factors: gross morphology and histology. *Arthroscopy*. 2010;26(4):470-480.
41. Sánchez M, Anitua E, Lopez-Vidriero E, Andía I. The future: optimizing the healing environment in anterior cruciate ligament reconstruction. *Sports Med Arthrosc*. 2010;18(1):48-53.
42. Saturveithan C, Premganesh G, Fakhrizzaki S, et al. Intra-articular hyaluronic acid (HA) and platelet rich plasma (PRP) injection versus hyaluronic acid (HA) injection alone in patients with grade III and IV knee osteoarthritis (OA): a retrospective study on functional outcome. *Malays Orthop J*. 2016;10(2):35-40.
43. Sawitzke AD, Shi H, Finco MF, et al. Clinical efficacy and safety over two years use of glucosamine, chondroitin sulfate, their combination, celecoxib or placebo taken to treat osteoarthritis of the knee: a GAIT report. *Ann Rheum Dis*. 2011;69(8):1459-1464.
44. Schauss AG, Stenehjem J, Park J, Endres JR, Clewell A. BioCell Collagen™. *J Agric Food Chem*. 2012;60(16):4096-4101.
45. Schauss AG, Stenehjem J, Park J, Endres JR, Clewell A. Effect of the novel low molecular weight hydrolyzed chicken sternal cartilage extract, BioCell Collagen, on improving osteoarthritis-related symptoms: a randomized, double-blind, placebo-controlled trial. *J Agric Food Chem*. 2012;60(16):4096-4101.
46. Schindler OS. Surgery for anterior cruciate ligament deficiency: a historical perspective. *Knee Surg Sports Traumatol Arthrosc*. 2012; 20(1):5-47.
47. Silva A, Sampaio R. Anatomic ACL reconstruction: does the platelet-rich plasma accelerate tendon healing? *Knee Surg Sports Traumatol Arthrosc*. 2009;17(6):676-682.
48. Sonoda M, Morikawa T, Tsuchiya K, Moriya H. Correlation between knee laxity and graft appearance on magnetic resonance imaging after double-bundle hamstring graft anterior cruciate ligament reconstruction. *Am J Sports Med*. 2007;35(6):936-942.
49. Sun SF, Hsu CW, Hwang CW, et al. Hyaluronate improves pain, physical function and balance in the geriatric osteoarthritic knee: a 6-month follow-up study using clinical tests. *Osteoarthritis Cartilage*. 2006;14(7):696-701.
50. Terencio MC, Ferrándiz ML, Carceller MC, et al. Chondroprotective effects of the combination chondroitin sulfate-glucosamine in a model of osteoarthritis induced by anterior cruciate ligament transection in ovariectomised rats. *Biomed Pharmacother*. 2016;79:120-128.
51. Tyler TF, Nicholas SJ, Hershman EB, Glace BW, Mullaney MJ, McHugh MP. The effect of creatine supplementation on strength recovery after anterior cruciate ligament (ACL) reconstruction. *Am J Sports Med*. 2004;32(2):383-388.
52. Vaishya R, Agarwal AK, Ingole S, Vijay V. Current trends in anterior cruciate ligament reconstruction: a review. *Cureus*. 2015;7(11):e378.
53. Valentí Nin JR, Mora Gasque G, Valentí Azcárate A, Aquerreta Beola JD, Hernandez Gonzalez M. Has platelet-rich plasma any role in anterior cruciate ligament allograft healing? *Arthroscopy*. 2009;25(11): 1206-1213.