Foot and Ankle Surgery 26 (2020) 750–754

Contents lists available at ScienceDirect

Foot and Ankle Surgery

journal homepage: www.elsevier.com/locate/fas

Treatment of lateral ankle sprain with platelet-rich plasma: A randomized clinical study

Juancarlos Blanco-Rivera, Jorge Elizondo-Rodríguez, Mario Simental-Mendía, Félix Vilchez-Cavazos, Víctor M. Peña-Martínez, Carlos Acosta-Olivo*

Department of Orthopedics and Traumatology, University Hospital "Dr. José Eleuterio González, Universidad Autónoma de Nuevo León, Monterrey, Nuevo León, Mexico

ARTICLE INFO

Article history:
Received 13 April 2018
Received in revised form 9 July 2019
Accepted 20 September 2019

Keywords:
Anterior talofibular ligament
Lateral ankle sprain
Rigid immobilization
Platelet-Rich plasma

ABSTRACT

Background: We aimed to clinically evaluate the effect of platelet-rich plasma (PRP) therapy in patients with acute lateral ankle sprain treated with rigid immobilization.
Methods: Patients with first-time grade II lateral ankle sprain clinically diagnosed were evaluated (n = 21). A rigid immobilization was placed in all patients for ten days; previously, an application of PRP over the anterior talofibular ligament was performed in patients from the experimental group. The Visual Analogue Scale, the American Orthopedic Foot and Ankle Score, and the Foot and Ankle Disability Index were applied at 3, 5, 8 and 24 weeks of follow-up period.
Results: The experimental group presented the highest reduction in pain and better functional scores than the control group at 8 weeks. At the end of follow-up period the results of both groups were similar.
Conclusions: A similar evolution was observed in patients treated with rigid immobilization with or without PRP after 24 weeks.
Trial registration: Clinical Trials.gov with ID NCT02609308.

1. Introduction

The lateral ankle sprains are one of the most common orthopedic injuries, representing up to 85% of ankle injuries and accounting for approximately 40% of sports injuries [1–3]. The most commonly injured structure is the anterior talofibular ligament (ATFL), followed subsequently by the calcaneofibular ligament (CFL) and the posterior talofibular ligament [2,4]. The diagnosis of this lesion is mainly clinical and is usually supplemented by imaging studies, such as X-rays in two positions, mainly to rule out fractures or associated injuries [5,6]. Van Dijk et al. found that with a well-guided clinical examination and a 48-h delay to wait for the lesion to be well delimited may provide a sensitivity and specificity of 84–96% [7].

Ankle sprains are classified clinically based on the severity of the injury and are usually divided into three grades: grade I, in which the ligaments are slightly stretched, without any macroscopic ruptures or joint instability; grade II (moderate), in which the ligaments are partially ruptured, with moderate pain and inflammation, and the patient presents with difficulty supporting himself; and grade III, in which the ligaments are completely ruptured, with severe pain, edema and hematoma, and where there is inability to function with instability [3,8]. Most lateral ankle sprains of any degree can be handled orthopedically, using a plaster cast below the knee or a semirigid support. This treatment causes significant reductions in the symptoms and the disability caused by the injury. In addition, there is greater benefit with immobilization for short periods of time (10 days) than with extended periods of immobilization (for three or more weeks), as extended immobilization may have deleterious effects on muscles, ligaments and joints [3,8–10]. Moreover, early mobilization helps to resolve the symptomatology associated with a lateral ankle sprain and, with body weight support, allows a faster recovery in the mobility of the ankle. Therefore, this treatment strategy contributes to a more rapid incorporation into daily life activities or sports, without affecting the mechanical stability in the long term [3,10].

For the last decade, platelet rich plasma (PRP) has been a widely used treatment in orthopedics and several reports have focused on soft tissue lesions [11–14]. PRP therapy involves an autologous

Abbreviations: PRP, Platelet-rich plasma; VAS, Visual analogue scale; AOFAS, American Orthopedic Foot and Ankle Score; FADI, Foot and Ankle Disability Index; ATFL, Anterior talofibular ligament; CFL, Calcaneofibular ligament; PDGF, Platelet-derived growth factor; VEGF, Vascular endothelial growth factor; TGF-β, Transforming growth factor-β; bFGF, Basic fibroblast growth factor; DASH, Disabilities of the Shoulder, Arm and Hand; KJOC, Kerlan-Jobe Orthopedic Clinic Shoulder and Elbow.

* Corresponding author at: Ave. Francisco I. Madero y Ave. Dr. José Eleuterio González S/N, Mitras Centro, 64480, Monterrey, Nuevo León, Mexico.
E-mail addresses: dr.carlosacosta@gmail.com, carlos.acostalv@uanl.edu.mx (C. Acosta-Olivo).

https://doi.org/10.1016/j.fas.2019.09.004
1268-7731/

infiltration of a high concentration of platelets at the site of the lesion [15]. Platelets are known to deliver growth factors and other recruiting proteins as part of a wound's healing process [16]. Some of the growth factors that have been described to be stored in platelets and that are also involved in the regeneration of ligamentous lesions include platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), transforming growth factor-β (TGF-β) and basic fibroblast growth factor (bFGF). These factors have been found in greater amounts in PRP and induce cell proliferation [17,18]. It has been reported that the application in vitro of PRP promotes positive effects in cultured tenocytes, increasing proliferation and cell migration in the anterior cruciate ligament and promoting its revascularization and reinnervation [18]. Animal studies have shown opposite PRP effects in medial collateral ligament injuries of the knee, where no differences were observed in the maturation values of PRP-treated ligaments [19]. However, a clinical study in ulnar collateral ligament injury showed that PRP is an effective option for these patients, with a success rate of approximately 90% [14]. Based on these contradictory results, in this study we focused in evaluated the clinical effect of PRP in patients with acute lateral ankle sprain treated with rigid immobilization for ten days measured with foot and ankle scales of function and pain.

2. Methods

2.1. Patients

This is a control randomized clinical trial with patients who attending to the emergency unit of our institution. We selected subjects with first-time lateral ankle sprains of no more than 48 h, grade II (incomplete ligament rupture with moderate functional disability, moderate edema and pain, mild to moderate ecchymosis, hypersensitivity to involved structures, light loss of mobility and function, weight-bearing pain and ambulation), mild to moderate instability, according to the classification of the American College of Foot and Ankle Surgeons [8], and were 18–60 years of age and gender indistinct. Patients with other associated pathologies (knee and ankle dysfunctions, osteoarthritis, rheumatoid arthritis, ankylosing spondylitis, diabetes or neurological abnormalities such as mental retardation and psychiatric disorders), pregnant or had previous ankle surgeries or treatments were excluded. Patients who agreed to be included in our study signed a letter of voluntary informed consent for their participation.

2.2. Study design

Patients were randomly divided into two groups (Fig. 1) according to a randomization plan generated from an online tool (randomization.com). The control group was placed in a short plaster cast, below the knee, with the foot in neutral position. Patients received the indication of weight bearing as soon as the pain allowed. In the same way, a short plaster cast was used in the experimental group, with the foot in neutral position. Previously, in this group, 5 mL of PRP was applied to the ATFL. In both groups of patients, the plaster cast was removed after ten days of treatment, and after the removal of the cast, all patients received the same rehabilitation protocol [20]. All patients were evaluated at baseline to determine their level of pain according to the Visual Analogue Scale (VAS). All the patients were evaluated at 3, 5, 8 and 24 weeks in the outpatient clinic, with the American Orthopedic Foot and Ankle Score (AOFAS) [21,22] and the Foot and Ankle Disability Index (FADI), in addition to the VAS.

2.3. Preparation and application of the PRP

A total of 30 mL of whole blood from the baseline or antecubital vein of the upper limb was collected in sterile, vacuum-closedtubes with 0.129 M sodium citrate as anticoagulant (BD Vacutainer;

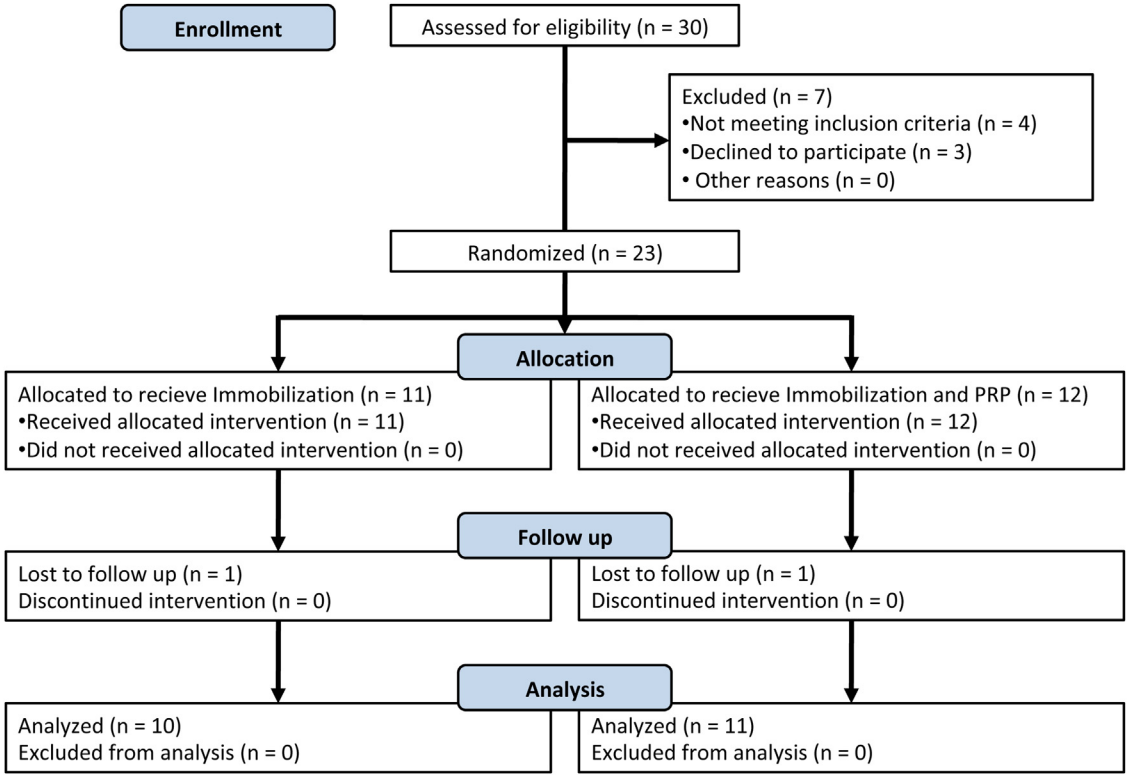

Fig. 1. Flow diagram of treatment.

Becton, Dickinson and Company, Franklin Lakes, NJ, USA). The PRP was prepared as mentioned elsewhere [23]. Briefly, the samples were initially centrifuged for 5 min at 1800 rpm (Heraeus Megafuge 1.0R; Thermo Electron Corporation, Ashville NC, USA) to separate the cell fraction corresponding to erythrocytes and leukocytes. The top plasma layer and the buffy coat were collected from each tube; all samples were collected in a 50-mL sterile conical polypropylene tube (Corning, NY) to perform a second centrifugation step for 3 min at 3200 rpm. The supernatant was removed from plasma or platelet-poor plasma, leaving a volume of 5 mL, which was considered the PRP. The 5 mL of PRP obtained was transferred to a sterile, vacuum-closed tube without anticoagulant (BD Vacutainer; Becton, Dickinson and Company, Franklin Lakes, NJ, USA). Samples were handled in a sterile environment within a Class II A2 biosecurity cabinet (Logic 3,440,801; Labconco, Fort Scott KC, USA). Prior to the application of PRP, platelet activation was induced by adding 0.15 mL of 10% calcium gluconate per mL of PRP. Asepsia of the application site was performed, after which sterile fields were placed, leaving the work area free. The 5 mL of PRP was injected under the lateral malleolus by introducing the needle superficially without invading the joint capsule and following the ATFL path. Once applied, a sterile patch was placed over the area, and the short plaster cast was then placed.

2.4. Statistical analysis

Based on a previous study, using a formula for the comparison of means with a power of 90% and a confidence of 95%, with a bilateral α value of 1.96, at least seven patients per group were required to make the study statistically significant [24]. Calculation the non-parametric Chi-square test and Fisher's exact test were used to investigate differences between groups. To analyze the comparisons of the quantitative variables between both groups of patients, Student's T distribution test was used. Data were analyzed using IBM SPSS Statistics software version 24.0 for Windows, where a value of $p \leq 0.05$ was considered statistically significant. Data are presented as the mean values ± standard deviations.

3. Results

In total, 23 patients who met the inclusion criteria of the study and who complied with the follow-up were analyzed; two patients were lost for the final evaluation. Ten patients were part of the control group, while the remaining eleven patients were included in the experimental group. The mean age of the patients included in the study was 26.71 ± 16.12 (range 18–54) years. We did not find differences between the groups in terms of age, gender and location of the sprain, nor did we see differences between the groups regarding their body mass index (Table 1).

3.1. Visual analogue scale (VAS)

In the initial patient evaluation, both groups had prominent levels of pain, with no differences between the groups. However, since their first evaluation, and during the study time, patients who were part of the experimental group had significantly greater pain reductions compared with the control group, but at the end of evaluation comparable results were observed in both groups (Table 2).

Table 1
Demographics by treatment group.

Variable	Control	PRP	p Value
Patients (n)	10	11	
Age (years)	25.5 ± 15.4	27.9 ± 12.1	0.567
Gender (female)	6 (60.0%)	4 (36.3%)	0.589
BMI	25.5 ± 3.8	26.8 ± 3.5	0.399
Affected ankle (left)	8 (80.0%)	4 (36.3%)	1.000

PRP, platelet rich plasma; BMI, Body mass index. Data are presented as mean ± standard deviation (SD), frequency and percentage ($p < 0.05$).

3.2. American orthopedic foot and ankle score (AOFAS)

Both groups of patients improved throughout the study according to the values obtained in the AOFAS. However, when comparing the two groups, we found that the improvement in patients treated with PRP was significantly higher than in patients who were only immobilized. This improvement in AOFAS values was maintained during the follow-up period, however in the final evaluation the result was similar in both groups without difference (Table 3).

3.3. Foot and ankle disability index (FADI)

For both study groups, FADI values indicated similar levels of improvement over time. Despite this similarity, patients who received PRP treatment showed a significant improvement over control patients at the 8-week of follow-up evaluation. Nevertheless, both groups showed comparable results by the 24 weeks (Table 4).

4. Discussion

Traditionally, lateral ankle sprains have been treated with immobilization by splints or short plaster casts (below the knee) for periods ranging from 10 days to 3 weeks, followed by a period of physical rehabilitation to enable the patient to reincorporate him or herself normally into daily life activities or sports. The use of PRP for the treatment of ankle sprains has been primarily described to treat high-performance athletes. In the study by Laver et al. [24], 16

Table 2
Post-treatment clinical results of the Visual Analogue Scale (VAS) score.

Follow-up (weeks)	Control	PRP	p Value
Baseline	8.0 ± 1.2	7.5 ± 1.9	0.532
Three	5.8 ± 0.6	3.9 ± 0.8	<0.0001
Five	4.0 ± 0.5	2.3 ± 0.5	<0.0001
Eight	1.4 ± 0.5	0.3 ± 0.5	<0.0001
Final	0.2 ± 0.4	0.1 ± 0.3	0.493

PRP, platelet-rich plasma. Data are presented as mean ± standard deviation (SD) ($p < 0.05$).

Table 3
Post-treatment clinical results of the American Orthopedic of Foot and Ankle Score (AOFAS).

Follow-up (weeks)	Control	PRP	p Value
Three	82.1 ± 3.7	86.5 ± 3.0	0.007
Five	87.7 ± 1.5	89.5 ± 1.8	0.026
Eight	89.8 ± 0.6	98.2 ± 4.0	<0.0001
Final	97.8 ± 2.6	98.5 ± 3.4	0.613

PRP, platelet-rich plasma. Data are presented as mean ± standard deviation (SD) ($p < 0.05$).

Table 4
Post-treatment clinical results of the Foot and Ankle Disability Index (FADI).

Follow-up (weeks)	Control	PRP	p Value
Three	117.1 ± 14.4	122.0 ± 8.8	0.142
Five	124.6 ± 8.7	127.2 ± 8.7	0.987
Eight	129.5 ± 4.0	133.1 ± 1.0	0.0003
24	135.3 ± 1.0	135.4 ± 1.0	0.884

PRP, platelet-rich plasma. Data are presented as mean ± standard deviation (SD) ($p < 0.05$).

patients with syndesmosis sprains were included and randomized, patients were treated with two PRP applications, at the beginning of the injury and on the seventh day and were then immobilized without weight support for 11 days; the patients in the control group were only immobilized, and both groups of patients then started a rehabilitation program. The patients were assessed at 3 weeks to determine the stability of syndesmosis. It was determined that patients in the PRP group had a shorter period of incorporation into their sports activities; in addition, they presented less pain than the control group. In another study, a series of cases of 11 young patients with lateral grade III ankle sprains who were treated with PRP in the lateral ankle complex with immobilization were reported. During the 6-week follow-up, it was determined that the recovery period of these patients was approximately 5.18 weeks, while 90% of them returned to sports activities 6 weeks after their injuries [25].

In a report of sprains in the general population, it was reported the intervention of 37 patients divided into two groups and blinded for the treatment received. One group was treated with PRP and immobilization, while the other group was treated with saline solution and immobilization. The groups were evaluated using VAS and the Lower Extremity Functional Scale. No differences were reported between treatment groups during the study time, which was 30 days [26]. The use of PRP in ligamentous lesions has been described before, in this report they evaluated 34 athletes with partial ruptures of ulnar collateral ligaments with failure to undergo nonsurgical treatment for at least two months. The athletes were treated with a single injection of PRP guided by ultrasound plus physical therapy and were evaluated with the Disabilities of the Shoulder, Arm and Hand (DASH) and Kerlan-Jobe Orthopedic Clinic Shoulder and Elbow (KJOC) assessments. With an average follow-up of 70 weeks (11–117 weeks), 88% (30 of 34) of the patients returned to the same level of symptom-free competition. There were significant improvements in the initial values of the DASH and KJOC assessments; only one patient had persistent failure of the ulnar collateral ligament and required ligament reconstruction [14].

The economic cost of the use of PRP, that in many occasions is not covered by insurance, it is an important aspect to consider, the value of one application it is between $500 to $2500 USD [27], depending of distributing company and institutional relationships, these points can be avoided with the use of an “in-office” preparation, and obtain a PRP of excellent quality.

Our study consisted of evaluating the patient from the clinical point of view, which considers the level of pain and functional capacity, through pain (VAS) and ankle function (AOFAS and FADI) assessments. We were able to observe that all of the treated patients improved in their pain symptomatology and in the values of the functional scales; however, patients who received PRP therapy showed the best results, which were significant when compared with the immobilization group in their short-term assessment. These findings demonstrate that PRP therapy allows a patient with ankle sprain to resolve the pain faster, allowing faster reincorporation into their sports activities and daily lives. As far as we are aware, this study reports the longest follow-up in patients with lateral ankle sprain treated with PRP. This allowed us to see the improvements of the patients at the end of their evaluation in the FADI results (8 weeks of the study); in the previous evaluations, the results between groups were similar to each other. All patients included in our study returned to their levels of activity prior to their injuries at the end of follow-up, we did not observe any complications.

There are several limitations in our study that should be acknowledge. First, the diagnosis of lateral ankle sprain was based on a clinical classification, which may vary depending on the observer. Second, the three functional scales that were considered are based merely on the patient’s point of view and can yield subjective results (AOFAS, FADI, VAS). Third, we didn’t use an ultrasound scan to classify the lateral ankle sprain, to perform the application of the PRP, or to assess morphologically the affected area before and after the treatment. Finally, even though power calculation was performed, the number of patients included was small. All our patients were incorporated to sports and daily living activities at the end of the study, though the 24 weeks is considered a short follow-up. The inclusion of a placebo group may have contributed to confirm the improvements seen. All the aforementioned aspects should be noticed for future projects. However, within the strengths of this study, we can highlight that we performed a randomized controlled clinical trial; additionally, all of our patients completed their follow-up examinations. The preparation of a PRP “in office” allows that to greatly reduce the cost of PRP treatment.

5. Conclusions

We can conclude that the use of PRP therapy as an adjuvant for the treatment of lateral ankle sprains allows the patient to report less pain during his recovery time and a better functionality outcome when compared with immobilization only. A larger study, including a placebo group would be necessary to confirm these findings.

Funding

This research was funded with resources of our own department.

Ethics approval and consent to participate

This research was performed in accordance with the Declaration of Helsinki. The Research Ethics Committee of our institution approved this study with the number OR15-008.

Declaration of interests

None.

Acknowledgement

Not applicable.

References

[1] Clanton TO, McGarvey W. Athletic injuries to the soft tissues of the foot and ankle. Surg Foot Ankle 2007;1425–63 8th ed. St Louis: Mosby.
[2] Molloy A, Selvan D. Ligamentous injuries of the foot and ankle. DeLee Drez's Orthop Sport Med. 4th ed. Philadelphia: Elsevier Saunders; 2015. p. 1392–407.
[3] Petersen W, Rembitzki IV, Koppenburg AG, Ellermann A, Liebau C, Brüggemann GP, et al. Treatment of acute ankle ligament injuries: a systematic review. Arch Orthop Trauma Surg 2013;133:1129–41.
[4] Roos KG, Kerr ZY, Mauntel TC, Djoko A, Dompier TP, Wickstrom EA. The epidemiology of lateral ligament complex ankle sprains in national collegiate athletic association sports. Am J Sports Med 2016;201–9.
[5] Bachmann LM, Kolb E, Koller MT, Steurer J, ter Riet G. Accuracy of Ottawa ankle rules to exclude fractures of the ankle and mid-foot: systematic review. BMJ 2003;326: 417–417.
[6] Omey ML, Micheli LJ. Foot and ankle problems in the young athlete. Med Sci Sports Exerc 1999;31:S470–86.
[7] van Dijk CN, Lim LS, Bossuyt PM, Marti RK. Physical examination is sufficient for the diagnosis of sprained ankles. J Bone Joint Surg Br 1996;78:958–62.
[8] Wolfe MW, Uhl TL, Mattacola CG, McCluskey LC. Management of ankle sprains. Am Fam Physician. United States 2001;63:93–104.
[9] Weinstein ML. An ankle protocol for second-degree ankle sprains. Mil Med. United States 1993;158:771–4.
[10] McGovern RP, Martin RL. Managing ankle ligament sprains and tears: current opinion. Open Access J Sport Med 2016;7:33–42.

[11] Jo CH, Shin JS, Lee YG, Shin WH, Kim H, Lee SY, et al. Platelet-rich plasma for arthroscopic repair of large to massive rotator cuff tears. Am J Sports Med 2013;41:2240–8.
[12] Lebiedziński R, Synder M, Buchcic P, Polguj M, Grzegorzewski A, Sibiński M. A randomized study of autologous conditioned plasma and steroid injections in the treatment of lateral epicondylitis. Int Orthop 2015;39:2199–203.
[13] Bubnov R, Yevseenko V, Semeniv I. Ultrasound guided injections of platelets rich plasma for muscle injury in professional athletes. Comparative study. Med Ultrason 2013;15:101–5.
[14] Podesta L, Crow SA, Volkmer D, Bert T, Yocum LA. Treatment of partial ulnar collateral ligament tears in the elbow with platelet-rich plasma. Am J Sports Med 2013;41:1689–94.
[15] Hall MP, Band Pa, Meislin RJ, Jazrawi LM, Cardone Da. Platelet-rich plasma: current concepts and application in sports medicine. J Am Acad Orthop Surg 2009;17:602–8.
[16] Creaney L, Hamilton B. Growth factor delivery methods in the management of sports injuries: the state of play. Br J Sports Med 2008;42:314–20.
[17] Geaney LE, Arciero RA, Deberardino TM, Mazzocca AD. The effects of platelet-rich plasma on tendon and ligament: basic science and clinical application. Oper Tech Sports Med 2011;19:160–4.
[18] Salamanna F, Veronesi F, Maglio M, Della Bella E, Sartori M, Fini M. New and emerging strategies in platelet-rich plasma application in musculoskeletal regenerative procedures: general overview on still open questions and outlook. Biomed Res Int 2015;2015:846045.
[19] Amar E, Snir N, Sher O, Brosh T, Khashan M, Salai M, et al. Platelet-rich plasma did not improve early healing of medial collateral ligament in rats. Arch Orthop Trauma Surg 2015;135:1571–7.
[20] Mattacola CG, Dwyer MK. Rehabilitation of the ankle after acute sprain or chronic instability. J Athl Train 2002;413–29.
[21] Kitaoka HB, Alexander IJ, Adelaar RS, Nunley JA, Myerson MS, Sanders M. Clinical rating systems for the ankle-hindfoot, midfoot, hallux, and lesser toes. Foot Ankle Int 1994;15:349–53.
[22] Ibrahim T, Beiri A, Azzabi M, Best AJ, Taylor GJ, Menon DK. Reliability and validity of the subjective component of the American Orthopaedic Foot and Ankle Society clinical rating scales. J Foot Ankle Surg 2007;46:65–74.
[23] Simental-Mendía M, Vílchez-Cavazos JF, Peña-Martínez VM, Said-Fernández S, Lara-Arias J, Martínez-Rodríguez HG. Leukocyte-poor platelet-rich plasma is more effective than the conventional therapy with acetaminophen for the treatment of early knee osteoarthritis. Arch Orthop Trauma Surg 2016;136:1723–32.
[24] Laver L, Carmont MR, McConkey MO, Palmanovich E, Yaacobi E, Mann G, et al. Plasma rich in growth factors (PRGF) as a treatment for high ankle sprain in elite athletes: a randomized control trial. Knee Surg Sports Traumatol Arthrosc 2015;23:3383–92.
[25] Frei R, Biosca FE, Handl M, Trc T. Conservative treatment using plasma rich in growth factors (PRGF) for injury to the ligamentous complex of the ankle. Acta Chir Orthop Traumatol Cech 2008;75:28–33.
[26] Rowden A, Dominici P, D'Orazio J, Manur R, Deitch K, Simpson S, et al. Double-blind, randomized, placebo-controlled study evaluating the use of platelet-rich plasma therapy (PRP) for acute ankle sprains in the emergency department. J Emerg Med 2015;49:546–51.
[27] Jones IA, Togashi RC, Vagsness Jr CTV. The economics and regulation of PRP in the evolving field of orthopedic biologics. Curr Rev Musc Med 2018;11:558–65.