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Original Article

Is there a treatment protocol in which platelet-rich plasma is effective?

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ABSTRACT

Aim: We aimed to reveal whether there are prospective suggestions for effective and standard platelet-rich plasma applications.
Methods: We searched for clinical trials and traced all the references of incorporated documents.
Results: In literature, there was no study indicating which disease is treated by which mechanism of action, how much dose and content are prepared and applied, when the treatment is applied and how many cures are applied.
Conclusion: Guides introducing which concentrations of PRP are used for which diseases are to be prepared immediately by a committee which is comprised of primarily orthopedists, clinical pharmacologists and toxicologists.

1. Introduction

Health-related quality of life is decreasing year by year in the world due to muscle and musculoskeletal injuries.[1] As there have not been promising results in the present studies regarding treatment of these injuries, clinical and social problems come up. Scientists turn towards repair of damaged tissues using biological methods in their studies.[2]

As the number of studies regarding the growth factors in wound healing and their interaction increases, it paves the way for personal and cell-based biological treatments, which can be applied as self-executing and combined. While treating damaged tissues, treatments that can provide rapid tissue repair and functional healing, and treatment modalities that are biological-oriented are targeted. In this respect, PRP constitutes an important step.[3]

PRP has gained popularity, which allows non-pharmaceutic and biologic repair-oriented recovery process by releasing local growth factors in the environment. Besides, it has functions of homeostasis and coagulation. It involves PLT, which abundantly contains cytokine and growth factors that are important in tissue repair and bone mineralization.[4] Furthermore, it contains lots of growth factors – in protein and peptide structure – which play key roles in synthesis of tissue matrix.[5]

As a result, in literature, PRP is reported to affect not only resorption of necrotic tissues but also macrophages which accelerate tissue healing, mesenchymal stem cells and osteoblasts. It is also reported to cause release of bioactive proteins.[6] For this very reason, it is applied in all fields of medicine including orthopaedic surgery in tissue damage treatments.[7,8]

PRP's use has been increasing in many cases such as primarily osteoarthritis,[9] proximal hamstring,[10] Achilles,[11] patellar tendinopathies,[12] talar osteochondral lesions,[13] rotator cuff damage repair,[14] lateral epicondylitis treatment,[15] relieving pains following arthroplasty[8] and contributing to bone healing of tibia in distraction osteogenesis.[8]

However, as there are contradictory results reported in some cases in literature where PRP was applied, there are some questions regarding the efficiency of PRP treatment.[16–18] Some

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manuscripts indicated that PRPs with different contents are superior in clinical treatment.[19] There was no well-proven study in which PLT and leucocyte (WBC) rates were separately discussed in PLT concentrations in PRP content. There was not enough evidence regarding how much WBS is responsible from curative or negative effect in clinics.[20,21]

Recently, the use of PRP has gained popularity and has become a treatment option all by itself.[22] There are some questions to be answered such as to whom, when, how and by which mechanism it is to be applied. It is important that a standardization be processed in consequence of basic and clinical studies as using different commercial kits might result in different results, which leads some issues concerning the evaluation of clinic studies.

The purpose of the present study is to determine the different applications of PRP in clinics after reviewing the literature and set forth whether there are suggestions for efficient and standard PRP application.

2. Materials and methods

2.1. Search strategy

The databases of the US National Library of Medicine National Institutes of Health, Embase, OVID and the Cochrane Library, as well as the references within the retrieved articles were searched to find all relevant orthopaedic injury and PRP clinical trial studies from 1954 to June 4, 2016, without any language restrictions. The following keywords were used in the search: "platelet-rich plasma", "PRP", "OA", "proximal hamstring", "achilles tendinopathy", "patellar tendinopathy", "talar osteochondral lesions", "rotator cuff", "lateral epicondylitis" and/or PRP.

The percentage distribution of articles by year was recorded, and the evidence level was determined according to Lijmer et al.[23,24] Bibliographies thought to be missed during the database research were examined again. Unpublished grey literature, including articles, comments, letters, editorials, protocols, guides, meta-analyses and collections were not included. The most highly cited articles were defined and re-examined in order to avoid double entries.

2.2. Eligibility criteria

Double-blind placebo-controlled randomized clinical trials or researches of level I were included to our study. All studies not containing the above information were excluded. The study inclusion process is summarized in Fig. 1.

2.3. Data collection and evaluation

The authors selected the included studies independently and, in order to minimize selection bias, the studies were revised by all authors. In the event of conflicting results, the final decision was taken by authors, who have greater experience regarding PRP preparations design. Finally, the senior authors were consulted and the topics were revised, if necessary.

2.4. Statistical analysis

It was found that the obtained data are not based upon the fact that they were collected from the sources that had probability distribution function. Therefore, non-pragmatic statistical methods were used. Nonetheless, given the lack of common findings, statistical analyses could not be performed and complementary statistical methods were applied instead. Microsoft Office Excel (2010) was used and the results were shown as mean ± standard deviation or frequency (%).

3. Results

A total of 13,248 studies were found to have potential for inclusion. The number of articles published per year is shown in Fig. 2. Following revision of the full text, 10 articles were finally included (Table 1), all of which were comparative, randomized controlled clinical trials.[8–14,25–27]

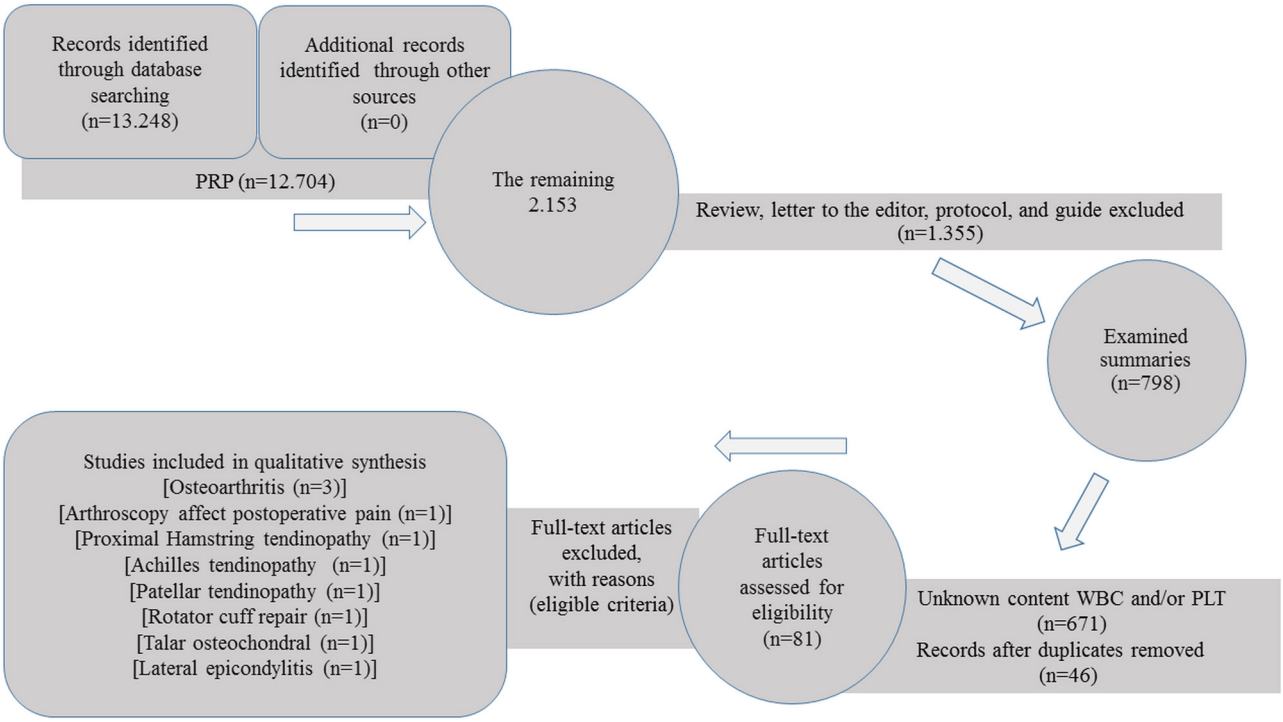

Fig. 1. The follow chart of literatures identification.

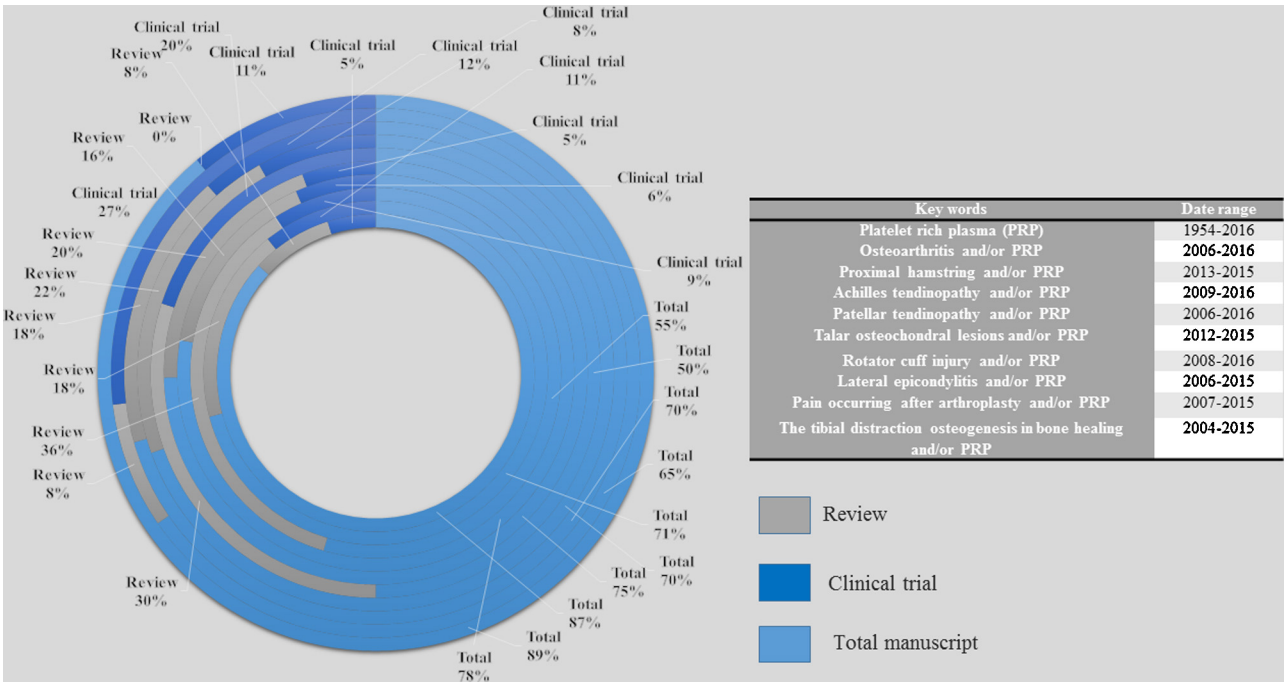

Fig. 2. The frequency of the studies of PRP by years.

Prior to the application, dose response curves of WBC/PLT in PRP were made. When the literature is reviewed, no study has been found purporting the effective dose to be used in treatments. Besides, we observed whether PRP content was tested by pharmaco-molecular analyses. In literature, there was no study indicating which disease is treated by which mechanism of action, how much dose and content are prepared and applied, when the treatment is applied and how many cures are applied.

4. Discussion

Upon reviewing the literature, we could not find a standardized production monograph as different companies use different commercial kits. In addition, there was no precise information regarding in which circumstances PRP was obtained and maximum or minimum revolutions per minute/centrifugal force/capacity of the centrifuges which were used. Consensus that was reached in these studies on obtaining PRP was the number, speed and time of centrifuge. Last but not the least, another thing that was agreed was the thickness of needle to be used during blood intake from donors and after obtaining PRP. The reason for this was as diameter of needles decreases, growth factors in PLT might affect the release time. It was reported that PLT might lead to premature activation, so 21-gauge injector nozzles should be used during autologous blood intake and PRP application.[28,29]

Apart from these there was no well-proven study in which activation of PRP was mentioned and whether it was activated or not. There were some preclinical[30] and clinical[31,32] studies, but there was no consensus on a protocol.

In literature, it was reported that early immature activation and growth factors release during the preparation of PRP pose other problems. It was emphasized that when a double spin centrifuge method was used, PLT concentration rate was 10% of the total blood taken from cases. However, more blood intake was needed when a single spin centrifuge method was used.[33] Furthermore, it was found that PRPs obtained from autologous plasma were directly used without considering their content, from which disease and at what dose they were obtained.[7–22,34]

However, as it is known, growth factors in PRP are various.[34] It is also known that transforming growth factor beta-1 induces chondrogenic activity[2] and fibroblastic growth factor; bone morphogenetic protein-2, -4, -6 induces osteoblastic stimulation and osteogenesis,[2,35] bone morphogenetic protein-12 induces tenogenic activity.[36] Nonetheless, without knowing the concentration of growth factors in PRP content and compound, they are applied. Although bone morphogenetic proteins are highly concentrated, they can be applied in cartilage damages. Similarly, though transformer growth factor in PRP has high incidence, it can be used in bone healing.

Recent well-proved studies have reported that half-life of growth factors is maximum 2 min.[37–40] It was emphasized that when growth factors – *with protein and peptide structure* – in PRP obtained from human were applied after that period, bioactivity decreased and even vanished.[37–40]

When the studies in which PRP was obtained from autologous blood in clinics were considered, it was found that it was prepared from 10 to 20 min.[7–21] Nevertheless, it takes time to apply it to cases, which makes its bioactive and effective treatment results controversy.

In a study, treatments of PRP and hyaluronic acid (HA) were compared in cases with cartilage defect and osteochondropathy ($n = 109$) with the purpose of evaluating the effectiveness of PRP, which contains high incidence of growth factor. 55 cases were applied HA whereas 54 were applied PRP and followed for 12 months. Clinical evaluations were made by means of International Knee Documentation Committee (IKDC), EuroQol (EQ) – Visual Analogue Scale (VAS), Tegner Lysholm Knee Scoring Scale (TLKS), and Knee injury and Osteoarthritis Outcome Score (KOOS), circumference measurement of knee and goniometric measurement of the knee range. As a result, it was observed that cases in both groups had recovery, but cases with PRP applied early-stage defects had better recovery. On the other hand, it was emphasized that the recovery in midlife early-stage osteoarthritis was not superior to the HA applied cases contrary to the literature.[25]

Dragoo et al. injected PRP to cases ($n = 23$) with patellar tendinopathy by means of ultrasonography. Whereas 10 cases

Table 1
Characteristics of the included studies.

		Are WBC/PLT values in PRP content formed following dose response curve?	Is PRP content tested at a molecular level?	Are there any explanations about which diseases are treated by which mechanism of action?	Are there any explanations about what dosage is prepared in what content, when and how many cures are used?
Filardo G et al.[25] (2012)	Platelet-rich plasma vs hyaluronic acid to treat knee degenerative pathology: study design and preliminary results of a randomized controlled trial.	–	–	–	–
Dragoo JL et al.[12] (2014)	Platelet-rich plasma as a treatment for patellar tendinopathy: a double-blind, randomized controlled trial.	–	–	–	–
Malavolta EA et al.[14] (2014)	Platelet-rich plasma in rotator cuff repair: a prospective randomized study.	–	–	–	–
Filardo G et al.[11] (2014)	Platelet-rich plasma injections for the treatment of refractory Achilles tendinopathy: results at 4 years.	–	–	–	–
Duif C et al.[8] (2015)	Does intraoperative application of leukocyte-poor platelet-rich plasma during arthroscopy for knee degeneration affect postoperative pain, function and quality of life? A 12-month randomized controlled double-blind trial.	–	–	–	–
Gobbi A et al.[9] (2015)	The effects of repeated intra-articular PRP injections on clinical outcomes of early osteoarthritis of the knee.	–	–	–	–
Davenport KL et al.[10] (2015)	Ultrasound-guided intratendinous injections with platelet-rich plasma or autologous whole blood for treatment of proximal hamstring tendinopathy: a double-blind randomized controlled trial.	–	–	–	–
Filardo G et al.[26] (2015)	Platelet-rich plasma intra-articular knee injections show no superiority vs viscosupplementation: a randomized controlled trial.	–	–	–	–
Görmeli G et al.[13] (2015)	Clinical effects of platelet-rich plasma and hyaluronic acid as an additional therapy for talar osteochondral lesions treated with microfracture surgery: a prospective randomized clinical trial	–	–	–	–
Montalvan B et al.[27] (2016)	Inefficacy of ultrasound-guided local injections of autologous conditioned plasma for recentepicondylitis: results of a double-blind placebo-controlled randomized clinical trial with one-year follow-up.	–	–	–	–

were injected PRP, 13 cases were applied dry needling. During 3rd, 6th, 9th and 12th weeks, they carried out clinical evaluation using Victorian Institute of Sports Assessment (VISA) scoring. In addition, during 12th and 26th weeks, they obtained secondary data using VAS, TLKS and Short Form (SF-12) questionnaire. After 12 weeks, they reported that they observed an increase by 5.2 in dry needling application in VISA scoring. Nevertheless, they reported that the increase was by 25.4 in PRP applied group. Following 26th week, they observed that the increase in dry needling group was by 33.2 whereas it was by 28.9 in PRP group. On the other hand, they emphasized that although there was not an increase in PRP group in the 12th week, there was not a significant difference between the groups in the 26th week.[12]

Dohan Ehrenfest et al., in their manuscript, informed that PLT concentrations – through topical and infiltrating routes – gained popularity primarily in sports medicine and orthopaedic surgery. Nonetheless, although they presented treatment perspective, they reported that the studies in literature were confusing. They classified PRP products into 4 main groups in order to benefit from them in advanced stage researches; pure platelet-rich plasma (P-PRP), such as the PRGF-Endoret technique; leukocyte- and platelet-rich plasma (LPRP), such as Biomet GPS system; pure

platelet-rich fibrin (P-PRF), such as Fibrinet; leukocyte- and platelet-rich fibrin (L-PRF), such as Intra-Spin L-PRF. They underlined the fact that this classification should be improved and reported that there are discussions on cell content, storage conditions and/or activation.[20]

Malavolta et al. studied effectiveness of PRP injections used in rotator cuff repair. They included 54 cases and divided them into two groups. Complete supraspinatus tears with retraction of less than 3 cm were subjected to arthroscopic single-row repair; at the end of the surgical procedure, liquid PRP prepared by apheresis was given to the patients in the PRP group with autologous thrombin. In clinical evaluations, University of California at Los Angeles (UCLA) and Constant scales, VAS and magnetic resonance imaging were used during the 3rd, 6th, 12th and 24th months after the surgery. They indicated that when they compared the cases in two groups in terms of 24-month follow-up, UCLA score increased from 13.63 in the PRP group while it increased from 13.93 to 32.44. In addition, they stated that Constant score increased from 47.37 to 85.12 in the control group whereas it increased from 46.96 to 84.78. As for VAS score, it decreased from 7 to 1.15 in the control group whereas it decreased from 6.67 to 0.96. They also reported that 1 case had recurrent complete tear and 4 cases had partial tear in the control group, whereas 2 cases had partial tear in the PRP group.[14]

In another study, PRP application results, which include high leucocyte were researched in cases with chronic achilles tendinopathy ($n = 27$), which resulted from decreasing physical performance. In this study, cases were applied PRP 3 times every other 2 weeks consequently. Blazina, VISA, EQ-VAS and TLKS were used to evaluate cases before and during the 2nd, 6th and 54-month follow-up. As a result, they concluded that the results of PRP injection were promising and stabilized when they had been evaluated in the mid phase.[11]

Duif et al. used leukocyte-poor platelet-rich plasma (LP-PRP) to evaluate effects that occurred during the arthroscopic treatment of knee lesions, which have detrimental effects concerning pain, function and quality of life. To this end, they applied randomized and double blind study in cases with knee arthroscopy ($n = 58$). The control group was comprised of 34 cases whereas the workgroup was comprised of 24 cases. They injected LP-PRP intra-articular to cases in workgroup during arthroscopy process. The function of knee was evaluated during the first application, 6th and 12th weeks in terms of quality of life. At the end of 12th month, they reported that 91% of the cases in the group where LP-PRP was applied during the 6th month had less pain, yet there was no significant difference across the groups at end of the 12th month in terms of quality of life. They concluded that during 6–12 months post-op period, intra-operative LP-PRP applied group was superior to other groups as far as pain relief and functionality of knee was concerned.[8]

Gobbi et al. investigated to find out whether intra-articular applied PRP at cyclic dose to cases with early stage osteoarthritis would affect treatment results, so they followed 113 knees of 93 cases for approximately 2 years. They applied PRP at the end of the first year. The cases before the injection and during the 12th, 18th and 24th months after the injection were assessed by KOOS, VAS, Tegner and Marx activity scales systems. They informed that all scored show that there was a significant improvement when compared to the pre-treatment. On the other hand, they emphasized that in the group where PRP was applied during the 18th month as the second cycle, there was a significant improvement in other parameters except for KOOS and Tegner scores. They concluded that a regression occurred at the end of the 2nd year; still, it was not statistically significant.[9]

Görmeli et al. compared HA and PRP applicants in osteochondral ankle injuries, which affect talus dome and lead to athletic disability, so they included arthroscopic micro-fracture cases ($n = 40$) in their study. They injected PRP to 13 cases, HA to 14 cases and the rest of the cases were injected saline. All cases were evaluated by American Orthopedics Foot and Ankle Society (AOFAS) functional scale and VAS scores before and after 15.3 months (in average) the application. They underlined that all cases showed an increase in AOFAS scores, and a decrease in VAS scores. They also maintained that AOFAS score was significantly higher in PRP than saline and HA groups. They stated that AOFAS score was higher in HA group than saline group. As for VAS scoring, they maintained that there was a significant decrease in PRP group than saline and HA groups. They concluded that PRP should be used primarily in talar osteochondral damages as the results of PRP group was better.[13]

Davenport et al. compared PRP and total blood applications in cases with chronic hamstring tendinopathy. Before the application and during the 2nd, 6th, 12th week and the 6th month, they measured pain and function outcomes via the Modified Harris Hip Score (MHHS), Hip Outcome Scores for activities of daily living (ADL) and sport-specific function, and International Hip Outcome Tool 33 (IHOT-33) scoring. They reported that before 12th week, total blood results were better than PRP applications where PRP application results were better at the end of the 6th week. The cases in PRP group showed significant increase in ADL and IHOT-33 scoring during 6th month when compared to pre-injection, yet it was not the case in total blood applications.[10]

In another manuscript, PRP and HA injected groups were compared in cases with osteoarthritis ($n = 192$). The cases were injected PRP and HA for the purpose of delay of surgery. They included cases who had unilateral knee pain for at least four months and with Kellgren-Lawrence score of 0–3 at radiographs or MRI evidence of degenerative chondropathy. The cases were applied PRP and HA injections every other week and 3 times in total. They used IKDC subjective score, KOOS, EQ-VAS and TLKS while evaluating the cases. Following PRP application, they reported that swelling and pain occurred in the injection area. They reported that IKDC score increased from 52 to 66 in PRP group whereas it increased from 49 to 64 in HA group. They emphasized that PRP and HA groups did not gain an advantage over each other.[26]

Montalvan et al. studied intratendinous effect of PRP in cases who had epicondylitis for less than 3 months. They included cases who had PRP and saline injections which was accompanied by ultrasonography four weeks apart. Cases were evaluated by blind study criteria. Following the isometric spasm of extensor carpi radialis and extensor digitorum communis muscles, the cases were assessed by VAS and Roles Mandsley scoring during 1st, 3rd, 6th and 12th months. As a result, two groups had promising results; still, there was no difference between two groups after 6 months.[27]

Given the low number of studies and the conflicting results between these, performing a meta-analysis of the results and deciding which of these results should be disregarded or included is a difficult task. Nevertheless, it is important for such data to be compiled and compared.[24]

The studies included in the present review did not provide common data and therefore homogeneity or heterogeneity tests could not be performed. As a result of this study, it can be concluded that the hypothesis that there is only one real underlying effect that to be predicted cannot be justified statistically. For this reason, the graph drawing of the data regarding sample quantity or variation reciprocal could not be realized. In the present study, all the articles were retrospectively designed and most had small sample sizes subject to systematic and random bias.

5. Conclusion

There is a need to establish worldwide multicentre study set-ups in which volunteers from all races take part. These set-ups include

dose response curves of WBC ratio in PRP content. Afterwards, they should be tested as pre-clinic and clinic at molecular level. It is important to clarify which treatment is received, which mechanism of action is used and which disease is treated. Some questions concerning application of treatment on which cases, amount of doses, duration and number of cures are to be answered. Through the results of these studies, guides introducing which concentrations of PRP are used for which diseases are to be prepared immediately by a committee which is comprised of primarily orthopaedists, clinical pharmacologists and toxicologists.

Conflicts of interest

The authors have none to declare.

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