

THERAPEUTIC POTENTIAL OF PLATELET-RICH PLASMA (PRP) IN DOG OSTEOARTICULAR DISORDERS

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Abstract

Platelet-Rich Plasma (PRP) therapy, derived from autologous canine blood, enhances tissue repair and modulates inflammation by concentrating platelets and growth factors. This study involved 20 dogs of various ages, breeds and gender, with joint traumatic injuries, such as coxofemoral luxation, patellar luxation, cruciate ligament rupture and degenerative joint disease (DJD), diagnosed through radiological exams. PRP was prepared by extracting blood into tubes containing separation gel and anticoagulant, followed by high-speed centrifugation. The PRP was injected into affected joints under general anaesthesia and administered according to each patient's specific treatment protocol. Periodic evaluations at 15, 30, 60 and 90 days, as well as at 6 months and 1 year have demonstrated excellent recovery without complications, highlighting the healing potency of PRP for both traumatic and degenerative conditions. The improvement was observed in 60% of cases after one administration, with 50% (n=10) of non-surgical and 10% (n=2) of surgical patients. Additionally, 10% of non-surgical and 30% (n=6) of surgical patients required two PRP administrations. The study demonstrated the safeness of treatment, even for unresponsive patients to conventional anti-inflammatory therapies.

Key words: Platelet-Rich Plasma, degenerative joint disease, inflammation, healing.

INTRODUCTION

Degenerative conditions of synovial joints encompass multiple conditions, which involve an interaction between biological and mechanical factors affecting the articular cartilage, subchondral bone, and synovium, being either monoarticular or polyarticular (Da Costa, 2010). Primary degenerative joint disease of the synovial joint is diagnosed when no predisposing factor exists, being more often observed in the category of geriatric dogs. In such cases, aging process of joint may be accelerated. Postmortem examination reveals the aging and fibrosis of cartilage as incidental findings as a result of mild degenerative changes of the articular surface (Smith et al., 2012). Secondary degenerative joint disease of the synovial joints is associated with abnormalities in the primary joint or in supporting structures, leading to premature degeneration of the cartilage. Any condition that directly causes the degeneration of the articular cartilage creates instability or results in abnormal directional force that may predispose to degeneration of articular cartilage (Osland et al., 2011).

Osteoarthritis is characterized by an imbalance between the synthesis and degradation of the components of articular cartilage, resulting in the breakdown of the articular cartilage (Liu et al., 2023).

Osteoarthritis has historically been classified based on the analysis of synovial fluid, comparing the levels of inflammatory proteins across various types of arthritis. Some studies have demonstrated that the synovial fluid in osteoarthritis (considered non-inflammatory) contains significantly fewer inflammatory proteins compared to the synovial fluid found in rheumatoid arthritis or septic arthritis (Enomoto et al., 2019).

Among the various conditions affecting the joints, gout is a well-known pathology in humans, but it can also be seen in dogs, though much more rarely. Gout is characterized by the deposition of urate crystals in the joints and periarticular tissues, leading to inflammation and severe pain (Soare et al., 2022).

Articular cartilage is avascular and lacks nerve endings, with limited capacity for self-regeneration when articular conditions develop (Zaragoza et al., 2015).

Platelet-rich plasma (PRP) is an emerging treatment that offers a safe solution by stimulating a natural repair process, containing a high concentration of platelets and growth factors such as IGF-1 (Insulin-Like Growth Factor), TGF-Beta (Transforming Growth Factor Beta), VEGF (Vascular Endothelial Growth Factor), EGF (Epidermal Growth Factor) and PDGF (Platelet-Derived Growth Factor) (Cook et al., 2015). PRP is an autologous blood product that provides critical haemostatic effect when tissue injury occurs. Thus, platelets are among the first blood cells to migrate to the affected area, releasing beta growth factor, which initiates the healing process (Farghali et al., 2021).

These growth factors promote the formation of new blood vessels, trigger mitosis, activate macrophages and stimulate collagen production from fibroblasts (Iacopetti et al., 2020). Direct administration of PRP can support tissue healing with improved quality and speed, without the risk of disease transmission or autoimmune reactions (Lopez et al., 2019).

The aim of this research is to present a relatively new, safe and natural approach to treating joint disorders of various types. The advantages of PRP therapy include its ability to be administered as often as needed for optimal results, the increased concentration of growth factors and the acceleration of the healing process. The treatment is performed under anaesthesia to ensure accurate and precise administration, with studies reporting no side effects (Da Costa, 2010).

MATERIALS AND METHODS

The study was conducted on 20 dogs, including both genders, ranging in age from 1 to 16 years and representing a variety of breeds: Golden Retriever, Pekingese (n=2), Pomeranian, German Shepherd, Beagle, Romanian Raven Shepherd, Yorkshire Terrier (n=2), small-sized mixed breed, American Staffordshire Terrier, Staffordshire Bull Terrier, Labrador Retriever (n=2), Maltese (n=2), Rottweiler, medium-sized mixed breed, and West Highland White Terrier (n=2). Of the 20 dogs, 85% (n=17) had joint conditions of both traumatic and degenerative origin, while only 15% (n=3) dogs presented

solely with degenerative osteoarticular conditions.

During the study, 60% (n=12) received a single PRP administration, of which 50% (n=10) were non-surgical and 10% (n=2) were surgical patients, while 40% (n=8) underwent two administrations, with 10% being non-surgical patients and 30% (n=6) being surgical patients. For an accurate diagnosis, canine gait analysis, which involved visual and subjective observation of the dogs from multiple angles at both the walk and trot on a flat surface to detect lameness. Additionally, the Canine Osteoarthritis Staging Tool (COAST) was utilized as standardized system for assessing the severity of osteoarthritis. Radiographic imaging and clinical examination were also conducted to provide a complete assessment of joint health. Periodic evaluations were conducted at 15, 30, 60 and 90 days, as well as at 6 and 12 months, using the canine gait analysis and the COAST system to assess for signs of lameness or pain. These assessments aimed to determine whether a single PRP dose was sufficient or if a second dose was necessary for optimal therapeutic outcome.

PRP preparation

Blood collection has been performed with a G21 green butterfly needle with adapter and a kit with a 10 ml PRP New Life ACDA tube. The PRP tubes are pre-labelled, red-capped tubes. Each tube has a pre-calibrated vacuum for the collection of 10 ml of blood. Inside each tube is an inert polymer-based separating gel and an anticoagulant essential for the separation of platelet-enriched plasma (injectable fluid) from the red blood cell concentrate, which is achieved through centrifugation.

For the preparation phase, two stages are followed:

Stage 1 - Preparation of collected blood for centrifugation: After collecting the blood, the tubes are gently inverted 3-4 times to prevent microclot formation. The tube is then placed into the centrifuge with an angle rotor, set to 4000 rpm for 7 minutes.

Stage 2 - Preparing the patient for intra-articular administration of Platelet-Rich Plasma under anaesthesia. Sedation of the patient was achieved using Medetomidine and Butorphanol, with Propofol for maintaining the anaesthesia.

All the collected samples undergo the same preparation protocol for obtaining PRP. The preparation of PRP involves collecting autologous venous blood using the Buffy Coat Method (consists of blood extraction, collection and high-speed centrifugation until it separates into three layers: platelet-poor plasma (PPP), platelet-rich plasma (PRP) and red blood cells (Wang et al., 2024). To obtain a high concentration of platelets, the excess platelet-poor plasma (PPP) is gently aspirated from top to bottom, avoiding contact with the platelet at the bottom of the tube. The aspirated volume represents approximately 50% of the total plasma obtained after centrifugation. The tube containing the remaining liquid and the platelet pellet in the leukoplatic layer is gently rotated until the platelet-rich plasma (PRP) is obtained. For each dog a 5ml syringe is used to extract the PRP. The difference between PPP and PRP is visibly noticeable, with PRP appearing darker due to the higher concentration of platelets.

Once the patient is anesthetized, they are connected to a pulse oximeter. The affected limb is positioned to allow for the precise insertion of a needle attached to an empty syringe into the joint capsule. After the needle is inserted, gentle aspiration is performed to confirm correct placement. The empty syringe is then detached and replaced with one containing PRP for administration.

The final volume of PRP was determined by the amount recovered after centrifugation, which varied for each dog. The administered dose ranged between 1 and 3 ml, depending on individual differences. Several factors influenced the PRP yield, including haematocrit levels, baseline platelet concentration, physiological state, hydration status and technical variations during processing. These variables explain why some patients produced a higher PRP volume while others yielded less, despite the same initial blood collection volume.

RESULTS AND DISCUSSIONS

After the first administration, 60% (n=12) of the dogs showed significant improvement, with progressive pain reduction, increased use of the affected limb, and improved joint flexibility during palpation, flexion, and extension. However, 40% (n=8) required a second PRP

injection due to suboptimal recovery observed during follow-up evaluations. Among them, 30% (n=6) were post-operative cases, while 10% (n=2) were non-operated.

Among the operated dogs (n=8), six of them required a second PRP administration, indicating a bigger need for additional treatment due to persistent pain and lameness. Conversely, in the non-operated group (n=12), 10 dogs responded well to a single administration, while only two required a second administration.

Dogs diagnosed with purely degenerative joint conditions 15% (n=3) required only a single PRP administration. In contrast, dogs presenting with both traumatic and degenerative conditions were more variable in their response: 45% (n=9) required one injection, while 40% (n=8) needed two administrations for optimal recovery.

At 15 days post-treatment, 60% (n=12) of the dogs that received a single PRP injection showed complete clinical improvement, displaying no pain, lameness, or gait abnormalities. Their condition remained stable at 30, 60, and 90 days, as well as at 6 and 12 months, with no recurrence of symptoms.

Conversely, the 40% (n=8) that required a second PRP dose still exhibited pain and lameness at the 15-day evaluation. By 30 days, their symptoms remained unchanged, leading to the decision to administer a booster PRP injection. Following the repeated dose, all 8 dogs demonstrated significant improvement, with no clinical signs of lameness, pain, or discomfort at their 15-day follow-up. The radiographs taken before and after the PRP therapy show healing post-surgery (Figure 1).

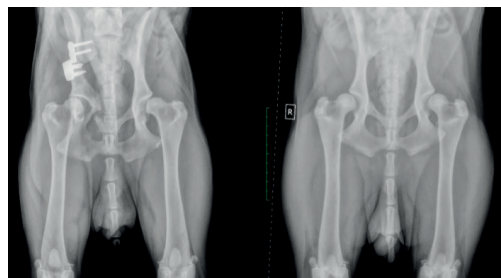


Figure 1. Internal plate fixation in iliac fractures with concurrent mild coxo-femoral subluxation after surgery. 60 days follow-up radiograph imaging demonstrates complete healing after the second PRP administration, with the joint showing no further signs of damage or abnormality

Additionally, in a separate case where no surgical intervention was performed, comparative radiographs (Figure 2) reveal stabilization of degenerative changes such as periarticular osteophyte formation, joint space narrowing and remodelling of the femoral condyles and tibial plateaus.

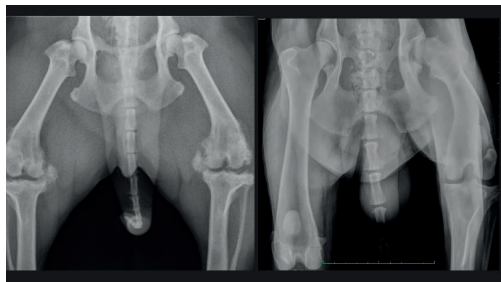


Figure 2. 90 days follow-up radiograph imaging demonstrates notable improvements including preservation of joint space width, absence of further osteophyte development and reduction in periarticular soft tissue density, suggesting decreased inflammation and healing after one PRP administration

Continued evaluations at 30, 60, 90 days, 6 months, and 1 year confirmed full recovery, with no further recurrence.

All 20 dogs responded positively to PRP therapy, with no cases of treatment failure or recurrence of symptoms during the 1-year follow-up period.

Dogs that received two PRP administrations were predominantly post-surgical cases (30%), while 10% of post-operative dogs required only one injection due to faster recovery.

PRP therapy was administered to 15% of dogs with degenerative conditions only and 85% of dogs with both degenerative and traumatic joint diseases. The results suggest that PRP is effective in both groups, with a higher need for a subsequent administration in post-surgical and trauma-related cases.

No adverse reactions were observed following PRP injection, neither after the first nor the last administration.

Temporary discomfort, lasting from a few hours up to a maximum of 24 hours, was observed only in post-surgical dogs ($n=8$), while no cases of local inflammation or pain were reported after PRP administration in any of the patients.

The 60% success rate after a single administration aligns with previous reports in

veterinary medicine, confirming PRP effectiveness as a non-pharmacological alternative to long-term NSAID therapy. However, most existing studies focus on experimental models or human applications, making this investigation an important step toward validating PRP in clinical veterinary practice (Anderson et al., 2021).

A key observation was that 30% of post-surgical dogs required two PRP injections to achieve full recovery. This suggests that a single PRP administration may be not sufficient to counteract the post-surgical inflammatory response, necessitating booster administrations to optimize tissue regeneration.

The severity of the inflammatory process, the complexity of the intervention and its extent can influence the number of PRP administration. However, in cases involving trauma-associated osteoarthritis, a significant proportion (40%) required two injections to reach full functional recovery.

This may indicate that traumatic joint injuries induce a more complex inflammatory response, requiring more aggressive regenerative stimulation compared to chronic degenerative conditions.

One of the most significant findings was the absence of adverse effects, reinforcing PRP safety profile as a regenerative treatment. Compared to NSAIDs or corticosteroids, which can have systemic side effects, PRP offers a biological, low-risk alternative.

Post-injection discomfort was observed only in surgical cases, most likely due to underlying tissue trauma rather than PRP-related inflammation. This supports previous evidence that PRP is well tolerated and does not induce significant inflammatory reactions.

Small sample size ($n=20$) limits general applicability and the absence of a control group (e.g., untreated dogs or NSAID-treated cases) prevents direct comparison of PRP efficacy against conventional therapies.

Variability in breeds, sizes, and ages may introduce response differences that were not fully controlled.

The results of this study suggest that PRP could become a adequate therapeutic option for managing canine osteoarthritis and joint trauma, reducing the reliance on chronic NSAID use and minimizing drug-related side effects.

PRP has been previously used by various authors in combination with other therapies, demonstrating positive results in the management of osteoarticular conditions. Studies integrating PRP with anti-inflammatory drugs, physical therapy, or other regenerative treatments have reported improved pain relief and functional recovery, suggesting that multimodal approaches can enhance therapeutic outcomes.

However, our study took a different approach by assessing PRP's effects as a standalone treatment, without the influence of additional therapies. Sergio López et al. administered Carprofen as a rescue analgesic at least once during the first week post-treatment. The inclusion of an NSAID in their study may have influenced the evaluation of PRP's true efficacy, as the analgesic effect of Carprofen could have masked or altered the natural progression of recovery.

In our study, the absence of adjunctive medication allowed for a more direct assessment of PRP regenerative potential. The results indicated a clear and progressive improvement in joint function and pain relief, with some patients requiring an additional dose for optimal recovery. Importantly, no adverse effects were observed, and no additional pain management was necessary during the evaluation period.

The findings indicate that PRP alone can be an effective treatment for osteoarticular conditions, reducing the need for supplementary analgesia. However, further research directly comparing PRP with and without NSAID co-administration is needed to determine the most effective clinical protocol.

Additionally, some researchers have opted for repeated PRP administrations at fixed intervals regardless of clinical response. For example, Xue et al. applied PRP in three consecutive doses over a set period, whereas in our study, a second administration was only performed if the initial response was insufficient. Despite these differing protocols, both approaches led to similar long-term improvements in joint function, indicating that while multiple doses may be beneficial in some cases, they are not always necessary.

In our study, we used the buffy coat method for preparing platelet-rich plasma (PRP), a technique that isolates the platelets from the

blood by separating the buffy coat layer after centrifugation. This method is known for its simplicity and efficiency in obtaining a high concentration of platelets. Other studies, however, have used different PRP preparation methods, such as the single-spin method (Goodale et al., 2023), double-spin method (Shin et al., 2017), and gel separation techniques (Dalğın et al., 2020), which involve more complex processes but are aimed at achieving a higher platelet yield or different plasma characteristics.

For instance, Shin et al., used a double-spin technique to separate the platelets from plasma, which resulted in a higher concentration of growth factors compared to the buffy coat method. However, their findings indicated that both methods were effective in promoting healing and reducing pain in musculoskeletal conditions, although they noted slight variations in platelet concentrations.

Similarly, Dalğın et al., utilized the gel separation method to prepare PRP, achieving a higher volume of plasma enriched with platelets. Despite these differences in preparation, the clinical outcomes for both methods were comparable, with improvements in pain reduction and functional recovery in patients.

Although the methods used by others differ from the buffy coat method in terms of platelet concentration and preparation complexity, our study shows that the buffy coat method is equally effective in producing satisfactory clinical results for musculoskeletal conditions. This suggests that even simpler techniques can be a viable and cost-effective option for PRP preparation without compromising the therapeutic efficacy, as seen in the positive outcomes reported in both our study and in studies employing more advanced PRP preparation methods.

CONCLUSIONS

This study demonstrates that PRP is an effective and reliable treatment for osteoarticular conditions in dogs. The results showed significant improvements in pain reduction and mobility, with no adverse effects observed. Despite the simplicity of the buffy coat method compared to other more complex PRP preparation techniques, the clinical outcomes

were similar, indicating its potential as a cost-effective alternative. Further studies are needed to confirm the long-term benefits and refine treatment protocols for osteoarticular pathologies.

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