Canine Autologous Conditioned Plasma Using the Arthrex ACP® System: A Brief Review of the Evidence

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Cellular composition

Four peer-reviewed, published studies and 2 unpublished, university-performed studies have documented the cellular composition of autologous conditioned plasma prepared using the Arthrex ACP® system in canines. The results of these studies are listed and are consistent in many facets. In all studies the leukocyte concentrations in the ACP were reduced in comparison to the corresponding whole blood sample. Similarly, these values were negligible in the 4 studies that reported the erythrocyte concentration.

The studies were also relatively consistent with regard to the platelet concentration. In 5 of the 6 investigations there was a mild to modest increase in platelet concentration with (on average) 1 to 2.5 times the platelet concentration in the ACP when compared to that in the corresponding whole blood sample. It is worth noting that the 2 unpublished studies by Gines et al (2016) and Shmalberg et al (2015) that were supported by Arthrex Vet Systems did not report the highest platelet concentrations; rather both of these studies reported intermediate values of platelet concentration. The greatest increases in platelet concentration were in the studies published in the *Journal of Orthopaedic Research* and the *Journal of Knee Surgery*.[1,2]

Only 1 of the 6 studies reported a decrease in platelet concentration in ACP in comparison to the baseline whole blood sample; the study reported that the platelet concentration in the platelet-rich plasma (PRP) was negligible.[3] This result is highly inconsistent with the 5 other studies. There are a few possible reasons for the discrepancy between this study and the other 5 studies that showed a 1 to 2.5× increase in platelet concentration. These reasons include platelet clumping and/or technical error in preparation.

Most veterinarians know that platelet clumping may cause erroneous automated platelet counts. When a complete blood count is performed on a whole blood sample as part of routine clinical practice and thrombocytopenia is noted, a blood smear should be reviewed under a microscope to evaluate the sample for platelet clumping. This process is performed any time an outside, contract laboratory (such as Antech Diagnostics) is used to perform routine bloodwork. Accordingly, there is usually a comment on the corresponding results page confirming that review of the slide was performed and commenting on whether platelet clumping was seen and whether the platelet concentration appeared decreased or adequate. In the aforementioned study[3] that reported that platelet concentration in the Arthrex ACP sample was negligible, the samples were assessed on an in-house hematology analyzer and there is no specification in the methods that a blood slide was reviewed. Consequently, it is feasible that the platelets in the Arthrex ACP were clumped and not counted by a hematology analyzer as platelets. This scenario is feasible when one considers a few other aspects of PRP preparation and platelet counting. Most notably, citrate-based anticoagulants have been shown to result in greater platelet aggregation and artificially lower platelet counts when compared to blood samples that are anticoagulated with EDTA.[4] This problem is most relevant in conjunction with hematology analyzers that use light diffraction technology rather than impedance technology.[4] Acid citrate dextrose-A (ACD-A), a citrate-based anticoagulant, and a hematology analyzer that utilizes light diffraction technology were used in the aforementioned study.[3]

Although errors in platelet counting might contribute to the anomalous results of this study,[3] it is more likely that the unusual results obtained are attributable to the ACP preparation technique. The Arthrex ACP technique is a single-spin manual preparation protocol that is simple in concept. Anticoagulated blood is centrifuged and the cellular components are distributed into layers based on their size and specific gravity. As most veterinarians

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know, the red blood cells are densest and pellet in the bottom of the tube or syringe and the overlying plasma resides on top. A buffy coat that contains the leukocytes sits at the interface between the red blood cells and the plasma. The platelets reside in the most bottom portion of the plasma, adjacent to and extending into the buffy coat. The more superficial plasma is platelet-poor and is referred to as platelet-poor plasma (PPP). During preparation of ACP, the user withdraws the plasma starting from the most superficial layer and working progressively closer to the deep, platelet-rich layer and the buffy coat. Optimal collection involves removal of the plasma immediately adjacent to the buffy coat and the superficial portion of the buffy coat. Consequently, if one does not withdraw enough of the deep layer of plasma and into the buffy coat, one will essentially only collect overlying PPP. The error in this situation is not one of the system or protocol, but rather is technical. The individual drawing the ACP into the second syringe of the Arthrex ACP double-syringe system is failing to withdraw the deep layer of plasma and some of the buffy coat. This is the simplest and easiest explanation as to how the results from one study[3] can be so dramatically different from those of 5 other studies,[1,2,5-7] which are all relatively consistent with each other.

Table 1. Results of 6 studies assessing the cellular composition of canine ACP®.

Year/Author(s)	No. of Dogs	Platelet Concentration	WBC Concentration	RBC Concentration	Platelet:WBC
Stief et al 2011	20	1.1×	Negligible	Negligible (0)	Not reported
Cook et al 2015	6	2.5×	"Leukoreduced"	Not reported	296 (180-400)
Bozynski et al 2015	9	2.4×	"Leukoreduced"	Not reported	280 (174-400)
Shmalberg et al 2015	21	1.5×	0.15×	Negligible (0)	Mean of 370
	21	1.6×	0.39×	Hematocrit of 4.4%	Mean of 147.6
Gines et al 2016	4	1.4×	Negligible	Not reported	Not reported
	4	1.7×	Negligible	Not reported	Not reported
Carr et al, 2015	10	0.09×	0.11×	0.02×	Not reported

Optimal platelet concentration

Clinicians commonly question what the optimal platelet concentration is in PRP. The answer to this question remains unknown. However, users are cautioned against assuming that more platelets are better. Rather, clinicians should be aware of a couple possible factors that are potentially relevant, one of which is PRP volume. Although there is great emphasis on platelet concentration, the more relevant and quantifiable characteristic may be platelet number. As an example, use of 5 mL of PRP with a platelet concentration of 200,000 platelets/µL provides the same number of platelets as use of 2 mL of PRP that has a concentration of 1 million platelets/µL. Consequently, users should always consider the platelet concentration in conjunction with the associated volume.

Ultimately, reviewing data on the platelet concentration in different PRPs is not the best founded method for assessing clinical efficacy. Rather, the most clinically relevant data are those that assess the efficacy of products in live animals, rather than those that merely specify the cellular composition of the samples.

In Vivo Evidence of Efficacy

Three peer-reviewed, published studies report on the in vivo efficacy of ACP in dogs. The first study assessing canine ACP compared the use of a single intra-articular injection of ACP in comparison to a single intra-articular injection of hyaluronic acid plus corticosteroid in dogs with elbow osteoarthritis.[8] The study was prospective and double blinded with neither the owners nor the assessing veterinarian knowing which treatment a given patient received. Outcome measures included subjective assessment of lameness by the assessing veterinarian and use of a validated owner-based outcome assessment. Significant improvements were identified in both treatment groups based on owner and veterinarian assessments. Some improvements were greater in the ACP treatment group, and no adverse reactions were recorded in either treatment group, consistent with product safety. Although these results are positive, the study did not include force plate data and so the conclusions about efficacy that can be drawn are limited.

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The aforementioned studies were followed by 2, rigorous studies done in research hounds. One study induced osteoarthritis in the stifle of 12 dogs by arthroscopic, partial cranial cruciate ligament (CCL) transection and complete medial meniscal release to induce osteoarthritis. Dogs were randomized to receive 5 injections of either saline or ACP over the next 8 weeks. Numerous benefits of ACP were seen. Comfortable range of motion was better maintained in the treatment group. Based on objective, kinetic pressure mat data, weightbearing was significantly better (by about 10%-15%) at weeks 5, 12, and 18 following treatment when compared to the saline control group. Blinded histologic scoring of the CCL was also significantly greater in the treatment group.

The same group performed another in vivo study in which they sought to compare the short-term benefits of a single injection of ACP, use of an oral nonsteroidal anti-inflammatory drug (carprofen), or arthroscopic lavage for the treatment of experimentally induced CCL injury (synovial debridement or partial transection). Both the ACP and arthroscopic lavage groups had significantly less lameness, pain, and effusion than the dogs receiving carprofen and had greater comfortable range of motion and greater subjectively assessed function.

Summary

Numerous studies have evaluated canine ACP, both with regard to the cellular composition and clinical efficacy in vivo. The great majority of studies are consistent in showing a mild to moderate increase in platelet concentration in ACP with a reduction in leukocytes. The conclusions from the in vivo data are somewhat limited by the use of research dogs rather than dogs with naturally occurring disease in 2 studies, and the lack of objective, force plate data in 2 studies. However, all 3 in vivo studies were prospective, randomized, and controlled with valid outcome measures reported and all 3 reported positive outcomes with the use of ACP.[1,2,8] In total, these data represent the most thorough evaluation of any canine platelet-rich plasma.

References

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Disclosure: Dr. Franklin is a consultant for Arthrex Vet Systems.

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