Lower centrifugation speed and time are positively associated with platelet concentration in a canine autologous conditioned plasma system

Margaret B. Goodale, DVM1; Holly A. Phelps, DVM, DACVS1; Jennifer A. Barnhard, MS1; Abigail B. Shoben, PhD2; Matthew W. Brunke, DVM, DACVSMR1*

1Veterinary Surgical Centers, Vienna, VA
2Division of Biostatistics, College of Public Health, The Ohio State University, Columbus, OH
*Corresponding author: Dr. Goodale (meggoodale@gmail.com)

Received May 3, 2023
Accepted July 12, 2023
doi.org/10.2460/javma.23.04.0218

OBJECTIVE
To evaluate the effect of variable centrifugation protocols on the cellular composition of the final product of a canine autologous conditioned plasma double-syringe system.

ANIMALS
30 client-owned healthy adult medium- to large-breed (17- to 45-kg) dogs.

METHODS
35 mL of anticoagulated whole blood from each subject was aliquoted into 3 samples: a baseline and 2 double syringes. The syringes were processed for platelet-rich plasma (PRP). Each double syringe was randomly assigned to 1 of 5 groups, which varied in centrifugation settings between 580 and 1,304 X g and 5 and 10 minutes. CBC analysis was performed on each of the samples to determine cellular composition. A mixed-effect linear model was fit to the data.

RESULTS
60 PRP samples and 30 whole blood samples were analyzed. Manufacturer settings generated a platelet fold change > 1 but did not increase concentration to the extent expected. When comparing speed alone, increased centrifugation force was associated with lower platelet fold change. When comparing time alone, increased centrifugation time was also associated with lower platelet fold change and lower leukocyte concentration.

CLINICAL RELEVANCE
Autologous conditioned plasma double syringes require a low volume of initial whole blood, making them preferable for canine PRP in clinical settings. This study aimed to evaluate the effect of the centrifugation protocol on the final product cellular composition in dogs and add to the available data on protocols to maximize platelet yield in PRP. Due to inherent individual variability, this study emphasized the importance of evaluating biological samples prior to administration to predict and improve patient outcomes.

Keywords: platelet-rich plasma, canine, centrifugation protocol, autologous conditioned plasma, biologics

Platelet-rich plasma (PRP) continues to increase in popularity as a biologic regenerative therapy option and is applied to an ever-expanding array of conditions across numerous species.1,2 PRP originated in human medicine and was then adopted in veterinary medicine, initially with equine patients.3-8 It is now increasingly common in canine medicine for the treatment of soft tissue injuries, wound healing, bone healing, the treatment of tendon and ligament injuries, and patients experiencing clinical signs of osteoarthritis.9-11 The benefit of PRP administration is attributed to the high concentration of growth factors contained within the alpha granules released when platelets are activated. These alpha granules release a variety of growth factors, including but not limited to platelet-derived growth factor, insulin-like growth factor, transforming growth factor-β1 and β2, vascular endothelial growth factor, basic fibroblastic growth factor, and epidermal growth factor.12-18 These growth factors have been shown to mitigate inflammation, initiate anabolic processes, and regenerate tissue.17,18 In contrast, other components of PRP, particularly leukocytes, are thought to release proinflammatory cytokines that can exacerbate or perpetuate inflammation.
and potentially impede tissue healing. As shown by Sundman et al, growth factor and catabolic cytokine concentrations are influenced by the cellular composition of PRP. Therefore, the cell distribution created through the use of commercial PRP systems will likely ultimately impact patients’ clinical outcomes.

The therapeutic use of PRP has faced substantial criticism over the years due to gaps in the understanding of the best methods for generating a consistent, effective final product. The systems used to generate PRP vary by blood volume, use of anticoagulant, number of spins, and centrifugation speed, resulting in drastically different cell distributions. The variation in clinical outcomes is further compounded by administration variables such as dose volume, timing of treatment, and redosing intervals. Because of these variables, some studies have concluded that there is no clinical benefit of PRP. However, these conclusions can often be traced to the generation of PRP that is inconsistent in quality. The makeup of an ideal PRP product (concentration of platelets, neutrophils, macrophages, total leukocytes, and erythrocytes) remains unknown. Extensive clinical trials are necessary to determine the ideal composition of an ideal canine PRP product. This study examined a specific widely used commercial system to optimize variables in the canine patient for that specific system.

The objective of this study was to evaluate the cellular composition of the final product of a double-syringe system when subjected to different centrifugation speeds and times. This system requires 13.5 mL of whole blood, substantially less than any other system on the market, making it ideal for small-animal patients. It is also straightforward to use, with relatively short spin times (5 to 10 minutes), still generating a clinically useful volume of PRP. Through quantifying the platelet concentrations and fold changes achieved and assessing leukocyte and erythrocyte concentrations or reductions in the PRP products, an optimal protocol for PRP generation with this system can be established. This study aimed to answer a necessary question along the path to generating consistent and effective PRP in canine patients. We hypothesized that manufacturer direction settings would produce PRP with platelet concentrations greater than that of whole blood. We further hypothesized that increasing centrifugation force and time would negatively impact the concentration of platelets.

**Methods**

This ex vivo experimental study was performed from July to December 2022 at a single private practice referral center. Thirty healthy adult dogs were recruited on a volunteer basis to participate in this study. These dogs were either employee-owned pets or patients of the sports medicine service undergoing elective orthopedic procedures. Owner consent for participation in the study was obtained. All animals were evaluated to be free of medical problems as determined by physical examination (performed by a veterinary surgical resident), clinical history, and CBC analysis. All procedures were directly overseen by a licensed veterinarian.

The system used in this study was the Arthrex autologous conditioned plasma (ACP) double-syringe system. The blood collection for PRP preparation was performed following the manufacturer’s instructions. The Arthrex system consists of a single centrifugation method using 13.5 mL of whole blood to produce PRP.

Thirty-one milliliters of whole blood was aseptically collected from the jugular or cephalic vein of each patient. The whole blood was aspirated into a 35-mL syringe, while rocking the syringe carefully to allow for mixing of the blood with 4 mL of anticoagulant citrate dextrose solution (Solution A; ACD-A). The anticoagulant-to-blood ratio was maintained from manufacturer directions. Whole blood was evaluated with a CBC analysis for baseline hematologic analysis (1 to 2 mL used/CBC analysis), and the remainder was processed for PRP.

Fifteen milliliters of the anticoagulated blood was transferred into 2 double syringes. Each double syringe was randomly assigned to 1 of 5 groups, which varied by centrifugation force and time (Table 1).

<table>
<thead>
<tr>
<th>Group</th>
<th>RCF (X g)</th>
<th>Time (min)</th>
<th>No. of syringes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>906</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>2</td>
<td>906</td>
<td>10</td>
<td>11</td>
</tr>
<tr>
<td>3</td>
<td>580</td>
<td>5</td>
<td>12</td>
</tr>
<tr>
<td>4</td>
<td>580</td>
<td>10</td>
<td>12</td>
</tr>
<tr>
<td>5</td>
<td>1,304</td>
<td>5</td>
<td>11</td>
</tr>
</tbody>
</table>

**RCF** = Relative centrifugal force.

These syringes were then placed into the centrifuge (Rotofix 32; Andreas Hettich GmbH and Co KG) with appropriate counterbalances, without the brake engaged, at the assigned speed and time. The group centrifugation speeds and times were based on those established at the authors’ institution.

After the 1-step centrifugation process, double syringes were removed, with care taken to keep them in the upright position so as to not disturb the plasma layer. The total volume of PRP generated was measured and recorded. PRP was then transferred into the inner syringe until the tip of the smaller syringe reached the transition layer and a flash was obtained. The smaller syringe was removed and agitated to distribute platelets uniformly in solution. An aliquot of each PRP was placed in a lavender-top (EDTA) tube and then routinely shipped with the whole blood sample to a commercial laboratory (IDEXX Laboratories Inc). The results were returned electronically within 24 to 48 hours of submission.
Statistical analysis

Baseline descriptive statistics were calculated for all measured variables. Linear mixed models were used to estimate differences from baseline by group, with a random effect of each dog to account for correlated observation from the same animal. Linear mixed models were also used to estimate the independent effects of speed and time on the final product. These models were run separately for each measured variable, and models in which the final value was the outcome were adjusted for the baseline value of that variable. P values (P < .005 considered significant) are presented unadjusted for multiple comparisons; the a priori primary comparison was the effect of speed and time on mean platelets in the final product.

Results

A total of 90 CBCs were performed on 60 PRP and 30 peripheral whole blood samples. The average amount of PRP produced was 3.5 mL (range, 0.5 to 7.5 mL) per double syringe. Data from 2 dogs were excluded from the statistical analysis of platelet fold change due to initial thrombocytopenia (flagged in an automated CBC report and confirmed by a clinical pathologist), raising concern for the patient's systemic health.

Blood was obtained from a total of 30 healthy adult dogs. The following breeds were represented: German Shepherd Dog (n = 6), Pit Bull-type breed (5), Husky or Husky mix (4), Labrador Retriever (3), Weimaraner (2), Border Collie (2), Boxer (1), Goldendoodle (1), Labrador (1), Australian Cattle Dog (1), English Bulldog mix (1), and mixed breed (3). The patients ranged from 1.5 to 12 years of age, with a median age of 5 years. The patients ranged from 17 to 45 kg, with a median weight of 24.75 kg. Sixteen of the study participants were neutered males, 13 were spayed females, and 1 was an intact female. The samples were randomly assigned to groups, and there were no significant differences between the demographics of cohorts in each group.

The only group with statistically significant platelet increase compared to whole blood baseline was group 3, which was prepared using a centrifugation speed of 580 X g for 5 minutes (Figure 1).

Seventy-five percent (9/12 syringes) of PRP generated in group 3 had a fold change > 10%. In contrast, only 18% (2/11 syringes) PRP samples in group 2, which was prepared using a centrifugation speed of 906 X g for 10 minutes, had a fold change > 10% (Figure 2).

The RBC concentration was significantly decreased from baseline in all groups, though there was not a statistically significant difference among the groups. The overall leukocyte concentration was significantly decreased in all groups, except for group 3 (Table 2). Group 3 had a significantly decreased neutrophil concentration but significantly increased lymphocyte and monocyte concentrations, compared to baseline.

When comparing the distribution of blood components at the 3 centrifugation forces (580, 906, and 1,304 X g), while holding the time variable constant, there was a statistically significant difference between platelet concentrations at the lowest speed compared to the 2 higher speeds (Table 3). There were no significant differences between total leukocyte, neutrophil, or monocyte concentrations among the rates. However, when compared to 1,304 X g, 580 X g did yield a significantly lower lymphocyte concentration. When comparing the distribution of blood components at
Table 2—Comparison of leukocyte and Hct concentrations compared to baseline means by group, as shown by the difference from baseline and 95% CI.

<table>
<thead>
<tr>
<th>Group</th>
<th>Total Leukocytes (K/µL)</th>
<th>Neutrophils (K/µL)</th>
<th>Monocytes (K/µL)</th>
<th>Lymphocytes (K/µL)</th>
<th>Hct (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-3.0 (-4.6 to -1.3)*</td>
<td>-3.5 (-4.4 to -2.5)*</td>
<td>-0.01 (-0.14 to 0.13)</td>
<td>0.70 (-0.05 to 1.45)</td>
<td>-35 (-39 to -30)*</td>
</tr>
<tr>
<td>2</td>
<td>-5.6 (-7.2 to -4.0)*</td>
<td>-4.4 (-5.3 to -5.5)*</td>
<td>-0.09 (-0.22 to 0.04)</td>
<td>-0.72 (-1.44 to -0.01)</td>
<td>-40 (-44 to -35)*</td>
</tr>
<tr>
<td>3</td>
<td>-1.9 (-3.4 to -0.4)</td>
<td>-3.3 (-4.2 to -2.5)*</td>
<td>0.24 (0.11 to 0.37)*</td>
<td>1.23 (0.54 to 1.92)*</td>
<td>-33 (-37 to -28)*</td>
</tr>
<tr>
<td>4</td>
<td>-4.0 (-5.5 to -2.5)*</td>
<td>-3.6 (-4.5 to -2.7)*</td>
<td>-0.07 (-0.19 to 0.06)</td>
<td>-0.16 (-0.85 to 0.53)</td>
<td>-33 (-37 to -28)*</td>
</tr>
<tr>
<td>5</td>
<td>-4.3 (-5.9 to -2.8)*</td>
<td>-3.7 (-4.6 to -2.8)*</td>
<td>-0.08 (-0.21 to 0.05)</td>
<td>-0.30 (-1.01 to 0.41)</td>
<td>-36 (-41 to -32)*</td>
</tr>
</tbody>
</table>

*P value < .001.

Table 3—Comparison of blood cell concentrations between speed and time variables, as shown by the difference between the variables and 95% CI. Negative values indicate that the first value is lower than the second, while holding the other variable constant.

<table>
<thead>
<tr>
<th>Centrifugation variables</th>
<th>Platelets (K/µL)</th>
<th>Total Leukocytes (K/µL)</th>
<th>Neutrophils (K/µL)</th>
<th>Monocytes (K/µL)</th>
<th>Lymphocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>RCF (X g)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1,304 vs 906</td>
<td>-40 (-112 to 33)</td>
<td>-1.2 (-3.0 to 0.7)</td>
<td>-0.1 (-1.1 to 0.9)</td>
<td>-0.11 (-0.28 to 0.06)</td>
<td>-1.0 (-1.9 to -0.1)</td>
</tr>
<tr>
<td>1,304 vs 580</td>
<td>-125 (-196 to -55)*</td>
<td>-2.5 (-4.3 to -0.8)</td>
<td>-0.5 (-1.5 to 0.4)</td>
<td>-0.25 (-0.41 to -0.09)</td>
<td>-1.6 (-2.4 to -0.7)*</td>
</tr>
<tr>
<td>906 vs 580</td>
<td>-86 (-139 to -32)*</td>
<td>-1.4 (-2.7 to -0.1)</td>
<td>-0.5 (-1.2 to 0.3)</td>
<td>-0.14 (-0.26 to -0.02)</td>
<td>-0.5 (-1.2 to 0.1)</td>
</tr>
<tr>
<td>Time (min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 vs 5</td>
<td>-97 (-151 to -43)*</td>
<td>-2.5 (-3.8 to -1.1)*</td>
<td>-0.6 (-1.3 to 0.2)</td>
<td>-0.21 (-0.33 to -0.09)*</td>
<td>-1.4 (-2.1 to -0.8)*</td>
</tr>
</tbody>
</table>

See Tables 1 and 2 for key.

Discussion

The results of this study supported the acceptance of both hypotheses 1 and 2. The first hypothesis was that the manufacturer settings would generate a platelet fold change > 1, which was supported by an average result of 1.31 times that of baseline, with 50% of samples in group 1 generating a > 10% increase compared to the baseline platelet count. These results are lower than previously reported, with prior studies reporting a platelet fold change between 1.55 and 2.5 times that of baseline. The second hypothesis was that when comparing variables alone, increasing centrifugation speed or time would be associated with lower platelet fold change, which was supported by analysis of the individual variables. However, none of the protocols tested using the ACP double-syringe system generated final products with a platelet fold change consistent with the general definition of PRP, which has a fold change of approximately 3 to 7 times that of baseline whole blood. A fold change of this magnitude would be unlikely to achieve considering the single soft spin utilized by this system. The systems that generate this type of PRP generally concentrate the platelet layer further through additional spin cycles or by apheresis.

The results of this study supported the acceptance of both hypotheses 1 and 2. The first hypothesis was that the manufacturer settings would generate a platelet fold change > 1, which was supported by an average result of 1.31 times that of baseline, with 50% of samples in group 1 generating a > 10% increase compared to the baseline platelet count. These results are lower than previously reported, with prior studies reporting a platelet fold change between 1.55 and 2.5 times that of baseline. The second hypothesis was that when comparing variables alone, increasing centrifugation speed or time would be associated with lower platelet fold change, which was supported by analysis of the individual variables. However, none of the protocols tested using the ACP double-syringe system generated final products with a platelet fold change consistent with the general definition of PRP, which has a fold change of approximately 3 to 7 times that of baseline whole blood. A fold change of this magnitude would be unlikely to achieve considering the single soft spin utilized by this system. The systems that generate this type of PRP generally concentrate the platelet layer further through additional spin cycles or by apheresis.

Of the different protocols tested in this study, the protocol for group 3 produced PRP with the most consistently increased platelet fold change. Group 3 had the lowest relative centrifugation force and the least time of any of the groups, with 580 X g for 5 minutes. These results may suggest that the manufacturer settings utilize a centrifugation speed that may be higher than optimal. Given the origins of this system in equine patients and the subsequent adoption of the system for canine patients, adjustments to the protocol should be made to tailor the system more narrowly to the specific cellular components of dogs.

The clinical relevance of a lower platelet fold change than the general definition of PRP is unknown, considering previous studies have reported positive outcomes in a model of canine anterior cruciate ligation and meniscal deficiency using this system. Although the PRP products generated with this system in this study did not concentrate canine platelets to the extent reported for other systems, all groups produced a significantly leukocyte-reduced product (Table 2). These findings suggest that the biologic produced using the ACP double-syringe system may provide greater anti-inflammatory effects than systems that produce leukocyte-rich PRP. Specifically, group 3 had decreased neutrophil and lymphocyte populations but also had statistically significantly concentrated monocytes in addition to platelets. This reduction of selective leukocytes may be an additional benefit of the protocol used in group 3, considering IL-1ra originates from monocytes. IL-1ra acts to mitigate the proinflammatory cytokine, IL-1β, which is linked to the progression of osteoarthritis.

One of the major advantages of this system in small animals is the relatively low volume of starting whole blood. The subjects in this study were all ≥ 17 kg, allowing the blood draws performed to be < 2% of the patients’ total blood volume. However, single weekly blood draws of up to 7.5% of total blood volume (6 mL/kg) have been reported to be safe in...
dogs. With this in mind, this double-syringe system could be used in patients as small as 2.5 kg. The drawback of the low volume of starting whole blood is the potential for low volume of PRP generated. The volume obtained in this study ranged from 0.5 to 7 mL, with no significant difference found among groups. This large volume discrepancy is more likely due to the inherent patient variability and hydration status than due to the differences in group protocols. Often in a clinical setting, multiple joints of a patient may be injected in a single session, and the ideal volume to be injected is yet to be determined. Additionally, the volume of the joint spaces of these small patients will likely necessitate a small volume of therapeutic agent, so as not to overly distend an already inflamed joint. Although the volume an individual blood collection may yield is difficult to predict prior to processing, if a volume deficit arises, it may be mitigated by preparation of a second ACP syringe or with the addition of other intra-articular therapies, such as hyaluronic acid.

A range of factors affect the quality of PRP generated by a single individual on 1 occasion. However, in clinical settings, the quality of the PRP product is rarely analyzed for cellular composition. The method of analysis used in this study, applied to a clinical setting, would allow practitioners to gain insight into the outcomes achieved following PRP administration. This study looked to optimize variable parameters within a specific PRP system; this was not a clinical efficacy study. No claims regarding the overall efficacy of PRP therapy in dogs or of the PRP formulations evaluated in this study can be made. Further studies to evaluate the specific cytokine and growth factor concentrations yielded by specific protocols as well as extensive clinical trials are necessary. Due to the inherent patient and user variability associated with biologics, specific factors may influence whether platelets are concentrated in a PRP.

This study demonstrated the importance of evaluating biological samples prior to administration to predict and improve patient outcomes. The future of regenerative medicine in small animals holds incredible promise for treating a plethora of disease processes, yet there is much still to be determined for optimization of this treatment method.

Acknowledgments

Idexx Laboratories provided the CBC analysis for this study. The authors have nothing to declare.

References

doi:10.1038/s41598-019-4657-5


