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Title

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Permalink

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Journal

Veterinary clinical pathology, 43(4)

ISSN

0275-6382

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Publication Date

2014-12-01

DOI

10.1111/vcp.12195

Peer reviewed

BRIEF COMMUNICATION

Comparison of the platelet-rich plasma and buffy coat protocols for preparation of canine platelet concentrates

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Key Words

Aggregometry, dog, platelet centrifugation, platelet function, platelet storage lesion

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DOI:10.1111/vcp.12195

Background: Platelet (PLT) concentrates (PC) can be produced via the buffy coat (BC) or platelet-rich plasma (PRP) protocols. The 2 methods have not been compared with canine blood.

Objectives: The aims of the study were to compare the PLT, WBC, and RBC concentrations, in vitro PLT function, and markers of platelet storage lesion (PSL) in canine PC generated by 2 different protocols, and determine microbial growth throughout storage.

Methods: PC from 8 healthy donor dogs were produced using 2 standard protocols, PRP and BC. PLT, WBC, and RBC counts, optical aggregometry assays, and PSL markers (pH, $p\text{CO}_2$, HCO_3 , lactate and glucose concentrations, and LDH activity) were determined on storage days 0, 1, 3, 5, and 7. Aerobic and anaerobic bacterial cultures were also performed.

Results: Mean PLT counts were comparable between protocols and remained stable throughout storage up to day 7, while median WBC and RBC counts on day 0 were significantly higher in the BC-PC group (17,800 WBCs/ μL ; 195,000 RBCs/ μL) than in the PRP-PC group (200 WBCs/ μL ; 10,000 RBCs/ μL) ($P = .012$). In PRP-PC aggregometry, the median slope and amplitude in response to γ -thrombin and convulxin (+ADP) were significantly decreased, and virtually absent in BC-PC during storage. PSL markers (lactate, LDH activity) were higher in BC-PC. Aerobic bacterial growth was observed in 2 PRP-PC and 1 BC-PC.

Conclusions: This in vitro study suggests that PRP-PC had lesser WBC and RBC contamination and superior PLT function compared with BC-PC. In vivo studies are required to address safety and efficacy of PRP-PC.

Introduction

There is growing use of platelet (PLT) transfusions in veterinary therapy. Platelet concentrates (PC) are used over whole blood to prevent transfusion-associated circulatory overload, polycythemia, and alloimmunization against RBCs.

Platelets are sensitive cells that can be irreversibly activated during processing and storage, thereby losing their ability to adhere to damaged endothelium. PLT damage during storage is referred to as the PLT storage lesion (PSL). Changes can be induced by shear stress¹, contact with charged surfaces, changes in temperature², and decreased oxygen availability.³ Additionally, contamination with WBCs and RBCs, activated

clotting factors, cellular debris, and proteolytic enzymes all can contribute to PSL. Routine evaluation of PLT function and PSL markers in PCs is essential, as it determines suitable protocols and acceptable storage times for transfusion of functional PLTs with reasonable posttransfusion survival times.⁴

PC can be produced by centrifugation or apheresis. The most commonly used centrifugation method for human PC in North America is the platelet-rich plasma (PRP) protocol, while European countries favor the buffy coat (BC) technique.⁵ The 2 methods differ in the order and force of centrifugation. Conflicting data exist as to which preparation method is superior for maintenance of PLT function in PC. Aggregometry, flow cytometric determination of markers of PLT activation, and Platelet Function Analysis-100 (PFA-100)

studies all demonstrated that both protocols result in PLT activation^{5–9}; however, there are currently no published veterinary studies directly comparing these 2 methods. The aims of this study were to compare the PLT, WBC, and RBC concentrations, in vitro PLT function, and markers of platelet storage lesion (PSL) in canine PC generated by 2 different protocols, and determine microbial growth throughout storage.

Eight healthy (based on current medical history, normal physical examination, CBC, and chemistry panel within the previous 1–9 months) client-owned dogs of various breeds (3 Pit Bulls, one each Labrador mix, Labrador Retriever, Boxer, Bullmastiff, and Catahoula) were enrolled. The experimental protocol was approved by the institutional animal care and use committee of the University of California–Davis. All dogs fulfilled the blood donor enrollment criteria of the University of California–Davis Veterinary Medical Teaching Hospital: 1–8 years old, body weight > 20 kg, no previous pregnancy, current on flea, tick, and heart worm preventive medicines, and normal von-Willebrand Factor activity. Donors were screened yearly for *A phagocytophilum*, *E canis*, *A platys*, *R rickettsii*, *B burgdorferi*, *Leishmania* sp., *Babesia* sp., *M haemocanis*, *M haemominutum*, *Bartonella*, *Brucella canis*, and *Dirofilaria immitis*.

All procedures were performed at the University of California–Davis Veterinary Blood Bank by 2 trained and experienced staff members. From each dog, both types of PC were produced with at least 6 weeks between donations, with PRP being the first method. A CBC was performed prior to each blood collection (AC•T diff analyzer, Beckman-Coulter, Miami, FL, USA).

After standard aseptic preparation of the skin, a jugular venipuncture was performed using a 16-Gauge needle. The first 5–10 mL of blood were collected into the diversion arm of the collection system before 450 mL of whole blood were collected into the polyvinyl chloride (PVC) sodium citrate-phosphate-dextrose-containing bag (Teruflex; Terumo, Tokyo, Japan). Blood was held for a maximum of 2 h at room temperature after collection. PC preparation followed UC Davis Blood Bank protocols.

For PRP-PC, whole blood was centrifuged (Sorvall RC 12 BP; Kendro Laboratory Products, Stortford, UK) at 1000g at 24°C for 5 min, followed by a 1 min 45 s deceleration time. The PRP was isolated in a satellite bag (Storage bag XT612; Terumo), which was then detached from the collection bag, welded (TCD tube welder; Genesis BPS, Hackensack, NJ, USA) to a 300 mL transfer bag (Transfer Bag T-300; Terumo, Tokyo, Japan), and spun (2000g, 24°C) for 10 min

with a 2 min deceleration time. Most of the supernatant was expressed into the transfer bag, leaving 50–60 mL of plasma in the bag (Storage bag XT612; Terumo) along with the PLT pellet.

For BC-PC, the whole blood unit was centrifuged at 3000g, at 24°C, for 11 min with a 2 min deceleration time. All but approximately 30 mL of the supernatant was transferred to a satellite bag and the remaining plasma, BC, and approximately 20 mL of the upper portion of RBCs were transferred to another satellite bag. The latter was isolated, welded to a 300 mL transfer bag, and centrifuged at 1000g, 24°C for 5 min, followed by a 1 min 45 s deceleration time. The supernatant was removed.

After both centrifugation protocols, the PC was kept for 1 h at room temperature and then gently massaged to resuspend the PLTs in the remaining plasma, and subsequently was stored at 22°C (PC 100i platelet incubator; Helmer, Noblesville, IN, USA) with gentle agitation (PF 15i platelet agitator, Noblesville, IN). WBC, RBC, and PLT counts, optical aggregometry assays, and PSL markers were determined on samples from each PC on days 0, 1, 3, 5, and 7, and microbiology cultures were set up on days 1, 5, and 7 (Figure 1). Prior to each puncture for sample collection the PC port was scrubbed 3 times with chlorhexidine and rinsed with 70% isopropyl alcohol, the alcohol was allowed to dry prior to sampling.

RBC, WBC, and PLT counts (AC•T diff analyzer) were obtained on days 0, 1, 3, 5, and 7. For aggregometry and depending on the initial PLT count, samples were diluted in platelet-poor plasma (PPP) or concentrated by serial centrifugation (Micromax; Thermo Scientific, Asheville, NC, USA) at 10,000g for 30 s to achieve a final concentration of approximately 250,000 PLTs/μL ($\pm$ 80,000). Aggregometry results were excluded from the study when the PLT concentration was not within this range. Aggregometry was performed with a temperature-controlled aggregometer (Chronolog Corporation Optical Aggregometer model 490, Havertown, PA, USA) after induction using ADP (Chronolog Corporation; final concentration 20 μM), Convulxin (Haematologic Technologies, Essex Junction, VT, USA; final concentration 20 nM), and human γ -thrombin (Thrombin, Sigma Chemical Co, St. Louis, MO, USA; final concentration 100 nM), either alone or in combination. Software (Chronolog Corporation) analysis was used to calculate amplitude and slope.

For PSL-marker analysis, samples were collected anaerobically. pCO₂, pH, and glucose and lactate concentration (ABL 700, Radiometer Brønshøj, Denmark) were measured within 5 min of sample collection from

the PC, HCO_3^- was calculated from pH and $p\text{CO}_2$, and LDH activity was determined on an automated chemistry analyzer (Cobas c 501; Roche Diagnostics, Indianapolis, IN, USA). When measured values were above or below the analytic range of the instrument, the upper or lower end of that range was used for statistical analysis, respectively.

Approximately 0.7 mL of each PC were submitted for aerobic (Blood agar plate and tryptase soy broth—Hardy diagnostics, Santa Maria, CA, USA) and anaerobic bacterial culture (Brucella agar plate—Anaerobe systems, Morgan Hill, CA, USA).

Normality was assessed by visual inspection of normal probability plots and the Shapiro–Wilk W test for normal data. When normality was accepted, pairwise comparisons were made using the Student's t -test. Correlations were assessed with Pearson's correlation coefficient. When normality was rejected, pairwise comparisons were made using the Wilcoxon signed rank test. Repeated ANOVA was used to evaluate changes in PLT concentration and markers of PSL during storage time for each dog. Interactions between PLT concentration process and time were also evaluated in the models. For models in which time was a significant factor, pairwise comparisons between individual time points were made using paired t -test. Data for aggregometry were not normal, even with standard transformations; therefore, median values for amplitude and slope were compared over time with

the Kruskal–Wallis (KW) ANOVA for each agonist and PLT concentration process. For KW-ANOVA models in which time was significant, pairwise comparisons were made using the KW test. Statistical significance was set at $P \leq .05$. Statistical analyses were performed using commercial software (Stata, version 10, Statacorp, College Station, TX, USA).

The mean PLT concentration in the group of 8 healthy dogs prior to blood donation was comparable and within the reference interval. There was no statistical difference ($P = .87$) for the PLT concentration in PRP-PC or BC-PC on day 0 (PRP-PC $343,000 \pm 223,641$ PLTs/ μL , $2.1 \times 10^{10} \pm 1.3 \times 10^{10}$ PLTs/unit; BC-PC $360,750 \pm 199,926$ PLTs/ μL , $2.2 \times 10^{10} \pm 1.2 \times 10^{10}$ PLTs/unit) (Table 1). Likewise, there was no significant change of mean PLT concentration ($P = .87$) during storage up to 7 days for either method while controlling for potential effects of individual dogs. In conclusion, there was no significant interaction between the PLT concentration method and storage time ($P = .06$) (Table 1). Altogether, none of the protocols produced PC with PLT counts matching the recommended human American Association of Blood Banks (AABB) standard of $> 5.5 \times 10^{10}$ PLTs/unit.¹⁰

In contrast, the median WBC and RBC concentrations on day 0 were significantly higher in the BC group (17,800 WBCs/ μL , range 800–53,500 WBCs/ μL ; 195,000 RBCs/ μL , range 20–660,000 RBCs/ μL ,

Figure 1. Study design for comparison of platelet-rich plasma (PRP) and buffy coat (BC) protocols for preparation of canine platelet concentrates (PC). PCs were tested up to day 7 after preparation for WBC, RBC, and platelets counts, aggregometry, and variables indicating platelet storage lesion, and for bacterial contamination. $p\text{CO}_2$ indicates partial pressure of CO_2 .

Table 1. Platelet (PLT) concentrations and biochemical markers of platelet storage lesion (mean $\pm$ standard deviation [SD]) measured in platelet concentrates (PC) prepared by the platelet-rich plasma (PRP) and the buffy coat (BC) protocol, on days 0, 1, 3, 5, and 7 of storage.

Day	PLT Concentrations			pH		$p\text{CO}_2^*$ (mmHg)		HCO_3^- (mmol/L)		Lactate**† (mmol/L)		Glucose**† (mg/dL)		LDH**† (unit/mL)	
	PRP	BC		PRP	BC	PRP	BC	PRP	BC	PRP	BC	PRP	BC	PRP	BC
0				7.21 $\pm$ 0.05	7.17 $\pm$ 0.10	39 $\pm$ 5	39 $\pm$ 8	15 $\pm$ 1	13 $\pm$ 1	2 $\pm$ 0.3	3 $\pm$ 0.5	532 $\pm$ 29	558 $\pm$ 14	42 $\pm$ 12	133 $\pm$ 76
1	352,250 $\pm$ 234,794	323,375 $\pm$ 214,043		7.31 $\pm$ 0.13	7.05 $\pm$ 0.25	28 $\pm$ 7	40 $\pm$ 16	14 $\pm$ 1	10 $\pm$ 2	3 $\pm$ 1	8 $\pm$ 4	526 $\pm$ 25	510 $\pm$ 46	49 $\pm$ 18	221 $\pm$ 127
3	334,125 $\pm$ 218,241	320,625 $\pm$ 237,024		7.36 $\pm$ 0.23	7.06 $\pm$ 0.36	20 $\pm$ 6	21 $\pm$ 7	11 $\pm$ 3	6 $\pm$ 3	6 $\pm$ 3	13 $\pm$ 6	515 $\pm$ 27	461 $\pm$ 66	60 $\pm$ 18	271 $\pm$ 84
5	350,500 $\pm$ 223,740	298,250 $\pm$ 189,672		7.30 $\pm$ 0.3	6.83 $\pm$ 0.53	16 $\pm$ 4	10 $\pm$ 3	8 $\pm$ 4	3 $\pm$ 4 (n = 7)†	9 $\pm$ 5	18 $\pm$ 8	498 $\pm$ 41	383 $\pm$ 119	92 $\pm$ 41	528 $\pm$ 497
7	333,000 $\pm$ 223,669	322,875 $\pm$ 201,878		7.17 $\pm$ 0.41	6.62 $\pm$ 0.47	12 $\pm$ 3	10 $\pm$ 4	6 $\pm$ 4	4 $\pm$ 5 (n = 3)†	12 $\pm$ 6	24 $\pm$ 12	466 $\pm$ 40	345 $\pm$ 140	131 $\pm$ 49	1027 $\pm$ 812

*Statistically significant change ($P < .001$) over storage time while adjusting for process and dog.†Statistically significant difference ($P < .05$) between PRP-PC and BC-PC.‡pH and $p\text{CO}_2$ were out of range; therefore, no HCO_3^- values available for some samples.

respectively) than in the PRP group (200 WBCs/ μL , range 0–700 WBCs/ μL ; 100,000 RBCs/ μL , range 0–40,000 RBCs/ μL) ($P = .012$ for both variables).

In aggregometry, convulxin alone failed to induce PLT aggregation in the first 3 PRP-PCs studied on days 0 and 1; therefore, they were excluded from statistical analysis. On the following days, ADP (at a final concentration of 10 μM) was added to convulxin (final concentration of 20 nM) to induce aggregation in the BC-PCs from those 3 dogs. For the PRP-PCs of the remaining 5 dogs and the BC-PCs of all dogs, the modified aggregometry protocol was used starting on day 0.

There were no significant aggregation changes during storage time for the PRP method using ADP as the agonist ($P_{\text{slope}} = .489$ and $P_{\text{amplitude}} = .873$). However, median slope and amplitude decreased significantly over time for PRP-PC induced with human γ -thrombin ($P_{\text{slope}} = .002$ and $P_{\text{amplitude}} = .001$) and convulxin + ADP ($P_{\text{slope}} = .002$ and $P_{\text{amplitude}} = .004$) (Figures 2 and 3). In PRP-PC activated with human γ -thrombin, there was a statistically highly significant decrease in slope and amplitude when comparing day 0 with days 3, 5, and 7 in PRP-PC ($P_{\text{slope}} = .011$, .002, and .001; $P_{\text{amplitude}} = .002$, .003, and .001, respectively). A similar effect was observed with convulxin + ADP stimulation ($P_{\text{slope}} = .007$ and .007; $P_{\text{amplitude}} = .011$ and .007, respectively). In BC-PC, the PLT aggregability was minimal to absent.

The analysis of markers for PSL revealed evidence of PLT activation with significantly higher lactate concentrations and LDH activities, and lower HCO_3^- and glucose concentrations in BC-PC (Table 1).

All anaerobic cultures were negative. In 2 PRP-PC, *Corynebacterium* sp. was identified on days 3, 5, and 7, and *Bacillus* sp. on day 0, respectively. One BC-PC was positive for a *Bacillus* sp. on day 3.

Independent of storage time, BC-PC PLTs demonstrated minimal aggregation in response to the agonists used. The relatively large number of WBCs in this preparation could be a significant contributing factor, as in vitro studies with human PLT have shown reduced PLT aggregation in the presence of WBCs, most likely due to stimulation and aggregation during processing and storage.¹¹ As RBC can directly enhance PLT aggregation by thromboxane¹² and ADP release¹³, RBC contamination also may have contributed to PLT activation during processing, dampening the aggregation response afterward.

BC-PCs had significantly higher lactate concentration and LDH activity, and lower HCO_3^- and glucose concentrations, which is suggestive of PSL.

Figure 2. Comparison of median slope of canine platelet (PLT) aggregation curves for PLT concentrates prepared from 8 healthy dogs by the platelet-rich plasma (PRP) protocol. Agonist: ADP (20 μ M), human γ thrombin (100 nM), or convulxin (20 nM) + ADP (10 μ M). *Significant difference ($P < .05$) with Kruskal–Wallis ANOVA between preparation methods. +Significant difference ($P < .05$) compared to day 0 with pairwise comparison.

Figure 3. Comparison of median amplitude of canine platelet (PLT) aggregation curves for PLT concentrates prepared from 8 healthy dogs by the platelet-rich plasma (PRP) protocol. Agonist: ADP (20 μ M), human γ thrombin (100 nM), or convulxin (20 nM) + ADP (10 μ M). *Significant difference ($P < .05$) with Kruskal–Wallis ANOVA between preparation methods. +Significant difference ($P < .05$) compared to day 0 with pairwise comparison.

As PLT metabolism relies on glucose and oxygen, substrate depletion may have been exacerbated by RBC and WBC contamination. If the oxygen supply is insufficient, PLTs may switch to anaerobic glycolysis, generating lactic acid, which contributes to a decrease in pH. Also, bacteria can compete with PLT for substrates, including glucose and oxygen. In addition, bacterial endotoxins have been shown to induce PLT aggregation¹⁴, further reducing contaminated PC quality. This appeared to be the case in one PRP-PC that had consistent bacterial growth in 3 consecutive samples, either due to contamination during sampling or processing, on days 0 and 1. One BC-PC had bacterial growth on one day only, suggesting plate contamination in the microbiology laboratory.

Our study has several limitations. No PC reached $\geq 5.5 \times 10^{10}$ PLTs/unit, matching the AABB standards.¹⁰ In addition, no additional methods were used to assess PLT function, such as flow cytometry,

although aggregometry remains the gold standard. Finally, extensive variability in PLT aggregometry data for the PRP-PC suggests either high inter-individual variability or technical issues, compromising a meaningful statistical evaluation.

Our study demonstrated that in the absence of leukoreduction, PRP-PC were superior to BC-PC in terms of inducible platelet aggregation and PSL, which might well have been related to the higher numbers of WBC and RBC. Future studies of PC should include leukoreduction filters.¹⁵ In addition, future *in vivo* studies should also evaluate PLT recovery, and half-life and *ex vivo* function of transfused PLT.

Acknowledgments

The authors thank the University of California–Davis veterinary blood bank for the preparation of the PLT concentrates. This project was supported by the Center for Companion

Animal Health, school of veterinary medicine, University of California–Davis and by the Canine Health Foundation. The contents of this publication are solely the responsibility of the authors and do not necessarily represent the views of the Foundation.

Disclosure: The authors have indicated that they have no affiliations or financial involvement with any organization or entity with a financial interest in, or in financial competition with, the subject matter or materials discussed in this article.

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