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Factors Affecting Platelet Concentration in Platelet Concentrates from Canine Blood Donors

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Background: Physiologic factors in dogs that might contribute to enhanced platelet yield in platelet concentrates (PCs) are largely unknown.

Objective: To determine whether individual differences in weight, age, preprocessing blood chemistry, and CBC variables predict the final platelet concentrations in PCs. Our hypotheses were (1) increased lipemic indices would be positively associated with increased platelet concentrations in PCs and (2) increased preprocessing platelet concentrations would be associated with higher platelet concentrations in the PCs.

Animals: All blood donation records of dogs from February 2, 2009 through April 1, 2015 at the University of California—Davis Veterinary Blood Bank were examined with 104 cases included in this study.

Methods: In this retrospective study, data were collected from medical records of canine blood donors. Records were reviewed for internal consistency and accuracy and subjects were included in the study if donor screening and donation occurred on the same day and a viable PC was obtained. Univariate and multivariable regressions were used to test the impact that each variable had on the final platelet concentration in PCs.

Results: Final platelet concentration in PCs was positively associated with the predonation CBC platelet values ($P < .001$), lipemic index ($P = .01$), and phosphorous levels ($P = .001$). Collectively these 3 variables explained 29% of the variance in platelet concentrations in PCs.

Conclusions and clinical importance: Future prospective studies are required to determine if canine blood donations from dogs with lipemia yield PCs with higher platelet concentrations without negatively affecting other blood components.

Key words: Dog; Lipemic index; Thrombocyte; Transfusion.

Recent growth in veterinary transfusion treatment due to client demand and intensive treatment of critical illness requires that blood banks refine their collection and component production protocols to meet these clinical concerns. The increase in demand for platelets and other specific blood products is likely to continue as clinical practice becomes increasingly specialized. Blood components are harvested from a single donation and used to address specific hematologic conditions. These blood products are a valued, finite resource that must be processed to optimize their use.

Veterinary blood banks screen donors for common blood-borne infectious diseases.[1] However, analogous screening procedures are not used to identify factors that might predict high cellular yield at the time of donation. No studies have directly examined physiologic factors that might affect the final cellular concentration in donated samples.

Abbreviations:

ALT	alanine transaminase
AST	aspartate transaminase
BUN	blood urea nitrogen
FWB	fresh whole blood
GGT	gamma-glutamyl transferase
Hct	hematocrit
Hgb	hemoglobin
MCHC	mean cell hemoglobin concentrations
MCV	mean cell volume
PCs	platelet concentrates

Individual variation in blood components can be inferred from prior studies. Breed-related differences and physiologic variations in measured blood variables exist. For example, Greyhounds and other sighthounds have higher hematocrit (Hct), hemoglobin (Hgb) concentration, mean cell volume (MCV), mean cell hemoglobin concentrations (MCHC), and lower platelet concentrations when compared to non-sighthounds.[2–6] Some Cavalier King Charles Spaniels have macrothrombocytopenia relative to other breeds.[7] In addition, pregnancy and puppyhood are often associated with physiologic anemia.[8,9]

Fresh whole blood (FWB) is the most widely available blood product in veterinary medicine.[10] However, given the low platelet concentration per unit, it is less effective for treating primary hemostatic disorders (e.g thrombocytopenia or thrombocytopathy) compared to PCs. For example, 10 mL/kg of FWB will generally increase a platelet count by 10,000/μL, whereas 1

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platelet unit per 10 kg will increase platelet counts by up to 40,000/μL.[11] As such, a dog with thrombocytopenia or thrombocytopathy might ideally receive PCs.

A previous study investigated the effects of 2 processing methods on platelet concentrations in PCs. This investigation found significant physiologic differences in platelet numbers, which persisted independent of the processing method.[12] In addition, anecdotal clinical observations at the University of California—Davis Veterinary Blood Bank suggest that samples with gross lipemia might be associated with increased platelet concentrations in PCs. Prior studies show that factors such as the presence of fragmented red blood cells and nucleated cells, bacteria and fungi might artificially increase platelet counts. In addition, when platelets are counted by optical rather than electronic impedance methods, the presence of high concentrations of lipids might lead to spuriously high platelet counts.[13–15] Nonetheless, in humans prior investigations in which platelets were identified in platelet-rich plasma by electron microscopy have shown that lipemia leads to increases in platelet concentrations.[16]

The current retrospective study sought to identify physiologic factors that might contribute to increased platelet concentrations in PCs obtained from healthy canine blood donors in a community-based blood donor program. We tested the hypotheses that increased lipemic indices are positively associated with platelet concentrations in PCs and that high preprocessing platelet concentrations lead to higher platelet concentrations in PCs. Further, we explored possible associations between weight, age, chemistry panel, and CBC values, and final platelet concentrations in PCs.

Materials and Methods

This investigation retrospectively evaluated associations between predonation physiologic factors and postprocessing platelet concentrations in PCs. Data were obtained by review of medical records from all canine blood donors at the University of California—Davis Veterinary Blood Bank from February 2, 2009, to April 1, 2015. Before donation, animals were screened by the University of California—Davis Veterinary Blood Bank donor protocol.[12] There is no specific protocol regarding predonation fasting. These animals were cared for according to the principles

Table 1. Distribution of independent variables measured from preprocessed FWB donations in 104 canine donors.

Variable (Unit)	Mean Value	Standard Deviation	Minimum Value	Maximum Value
Weight (kg)	36.2	7.9	26	64
Age (year)	5.3	1.8	2.0	9.0
Platelet count (1,000/μL)	228.3	76.9	67	742
Hemoglobin (gm/dL)	17.1	1.6	13.1	21.2
Hematocrit (%)	49.8	4.8	36.8	63.4
Mean Platelet Volume (fl)	12.4	3.2	7.4	37.9
White Blood Cell Count (1,000/μL)	7.6	2.4	3.8	18.7
Red Blood Cell Count (M/μL)	7.2	0.7	5.19	9.2
Eosinophil Count (1,000/μL)	0.47	0.41	0	2.8
Neutrophil Count (1,000/μL)	5.0	2.1	1.8	14.8
Lymphocyte Count (1,000/μL)	1.7	0.6	0.6	4.5
Monocyte Count (1,000/μL)	0.35	0.31	0.1	2.5
Basophil Count (1,000/μL)	0.31	0.35	0	0.2
Anion Gap (mmol/L)	17	2	13	26
Sodium (mmol/L)	146	2	142	150
Potassium (mmol/L)	4.1	0.3	3.4	5.2
Chloride (mmol/L)	111	2	106	115
Bicarbonate (mmol/L)	22	2	19	27
Phosphorus (mg/dL)	3.6	0.6	1.6	4.9
Calcium (mg/dL)	10.5	0.4	9.4	11.5
BUN (mg/dL)	18	5	7	38
Magnesium (mg/dL)	2.1	0.2	1.8	2.7
Creatinine (mg/dL)	1.1	0.2	0.7	1.8
Glucose (mg/dL)	95	12	57	120
Total protein (g/dL)	6.2	0.4	5.3	7.4
Albumin (g/dL)	3.8	0.3	2.9	4.3
Globulin (g/dL)	2.4	0.4	1.8	3.8
ALT (IU/L)	51	68	20	552
AST (IU/L)	29	9	5	81
Creatine kinase (IU/L)	139	59	67	367
Alkaline phosphatase (IU/L)	52	52	13	308
GGT (IU/L)	3	2	0	11
Cholesterol (mg/dL)	232.3	59.0	127	407
Hemolysis Index	33	35	3	194
Lipemic Index	31.1	37.3	0	199

set forth by institutional animal care and use committee of the University of California—Davis. Blood components were created from the FWB donations following standard University of California—Davis Veterinary Blood Bank procedures to produce PCs via the platelet-rich plasma protocol. In this protocol, CBCs were obtained with a hematology analyzer[a] , which employs optical detection methods. All PCs were standardized to a volume between 50 and 60 mL.[12]

Dogs were excluded from this study if they did not undergo screening on the same day as donation or the platelet concentrate was not considered viable. Nonviable samples were contaminated with red blood cells (>0.1 $\times$ 10^6 cells/μL) or bacteria (based on aerobic and anaerobic bacterial cultures).

Donor data were recorded by a commercially available spreadsheet program[b] and reviewed for internal consistency and accuracy. In addition to donor age and weight, chemistry panel, and CBC variables (listed in Table 1) were evaluated for their effect on platelet concentration in PCs.

Descriptive statistics, including the mean, range, and standard deviations, were calculated for each variable. Histograms of the data were examined visually to assess for normality. Linear regression was used to identify the association between each variable and the final platelet concentration in PCs. Subsequently, variables that were not normally distributed based on a Shapiro–Wilk test and that were to be included in the multivariable linear regression were transformed by taking their log or square root. This transformation was based on Tukey's ladder of transformations test to determine which transformation would lead to a normal distribution of the data. Multivariable linear regression was then done on independent variables with a P value <.2 on the univariate linear regression. The relationship between age and phosphorous concentration was examined by linear regression. All statistics were done by a commercially available software program.[c] Statistical significance was set at $P \leq .05$.

Results

A total of 104 dogs were included in the study; 103 had complete data sets. A single donor was missing a Hct value. The mean weight of all dogs was 36.16 kg (SD $\pm$ 7.85, range 26–64). The mean age of all dogs was 5.29 years (SD $\pm$ 1.84, range 2.04–9.03). There

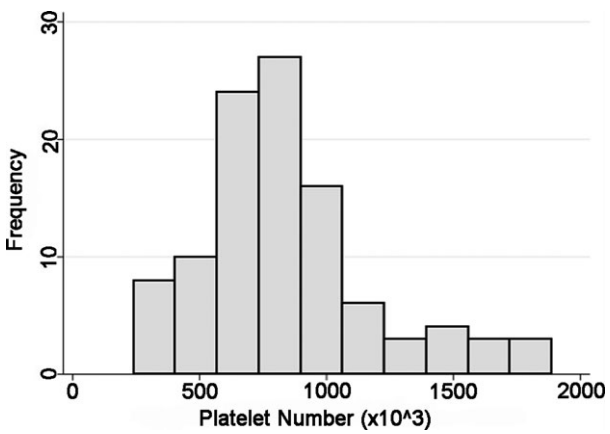

Fig 1. Histogram of resulting platelet concentration in platelet concentrates from 104 dogs.

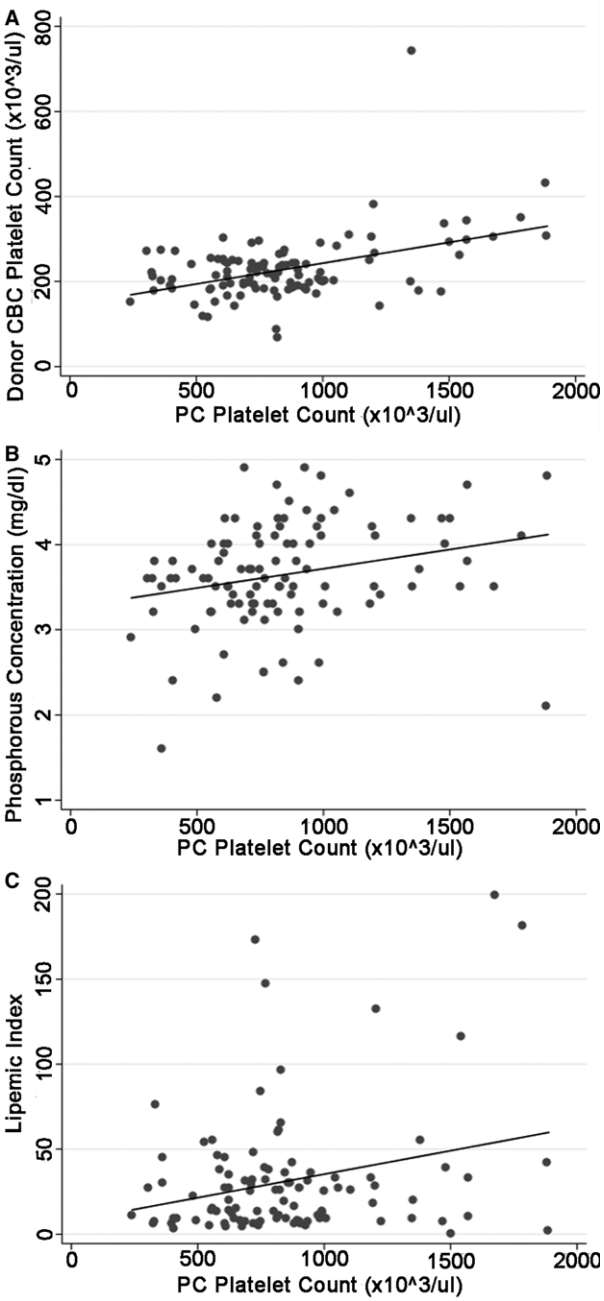

Fig 2. (**A**) Scatter plot and resulting regression line showing initial donor platelet concentrations and the final platelet concentration in PCs in 104 canine blood donors. This association was statistically significant at $P < .001$, $r^2 = 0.20$ on univariate analysis and remained significant on multivariable analysis at $P < .001$. (**B**) Scatter plot and resulting regression line showing phosphorous concentrations and the final platelet concentration in PCs. This association was statistically significant at $P = .008$, $r^2 = 0.06$ on univariate analysis and remained significant on multivariable analysis at $P = .001$. (**C**) Scatter plot and resulting regression line showing lipemic index and the final platelet concentration in PCs. This association was statistically significant at $P = .008$, $r^2 = 0.07$ on univariate analysis and remained significant on multivariable analysis at $P = .01$.

were 37 mixed breed dogs, 14 Labrador Retrievers, 13 German Shepherd Dogs, 8 Weimaraner, 7 Pit Bull Terrier, 3 Doberman Pinscher, 2 each of Boxer dog, Canaan hound, Flat-coated Retriever, Golden Retriever, and Rottweiler, and one each of an Akita, Australian Shepherd dog, Bouvier des Flanders, Bull Mastiff, Bulldog, Catahoula hound, Chesapeake Bay Retriever, Collie, Coonhound, German Wirehaired Pointer, Great Dane and Rhodesian Ridgeback. There were 52 neutered males, 43 spayed females, 5 intact females, and 4 intact males. Table 1 presents the mean, standard deviation, and range of each of the independent variables. Figure 1 shows the distribution of the platelet concentration in PCs. These data ranged from 240 to 1889 (mean = 849.97, SD = 351.52) platelets/μL.

Univariate analysis showed that the following variables were positively associated with PC concentration: cholesterol ($P = .04$), calcium ($P = .02$), phosphorus ($P = .008$), CBC platelet concentration ($P < .001$), hemolysis index ($P = .03$), creatinine ($P = .03$), potassium ($P = .07$), and the lipemic index ($P = .008$). No association was found for WBC and lipemic index ($P = .97$) or between Hgb and lipemic index ($P = .16$).

Multivariable analysis showed that the following variables remained significantly positively associated with PC concentration: CBC platelet concentration ($P < .001$), lipemic index ($P = .01$), and phosphorous concentration ($P = .001$). Age and phosphorous concentrations were not related ($P = .18$). The multivariate model based on these 3 variables explained 29% of the variance in PCs, and each of these 3 measures made independent contributions to platelet concentration in PCs. The relationship between each of these 3 significant variables and platelet concentrations in PC is shown in Figure 2.

Discussion

Of the 35 variables evaluated, only the preprocessing blood platelet concentration, phosphorous concentration, and lipemic index were significantly positively associated with platelet concentrations in the resulting PCs. No other variable was significantly related to platelet concentrations in PCs. This study could not assess the potential effects of breed or sex due to the small number of subjects in some groups.

The observation that pre- and postprocessing platelet counts have a strong positive association is not surprising when complete and careful processing is used to process FWB donations into PCs. This observation is compatible with a prior study showing that individual differences in platelet concentration persist in PCs.[12]

By contrast, the underlying mechanisms explaining the positive correlation between phosphorous levels and platelet concentrations in PCs are unknown. A previous study showed that phosphorous levels increase with age.[17] However, in the current study the subjects' age was unrelated to platelet concentration in PCs and there was no association between age and phosphorous concentration, perhaps because of the limited range of the age of the subjects used in this study. Another study revealed that thrombocytosis results in falsely increased serum potassium concentrations and phosphorus concentrations as they are released from the cells during coagulation.[18] This phenomenon is unlikely to explain the increase in phosphorous levels as there was no concurrent increase in potassium. Thus, at present the relationship between phosphorus levels and platelet concentrations in PCs remains unclear.

The link between lipemic index and platelet concentration in PCs may be due to the effect that lipemia has on platelet sedimentation properties during sample processing. Previous work has shown lipemia might promote the movement of platelets into the plasma during the centrifugation process by altering the structure and buoyancy of platelets.[16] Thus, lipemia might result in a higher percentage of platelets in the PCs.

This study showed that the preprocessing platelet concentration, phosphorous concentration, and lipemic index correlated positively with final platelet concentrations in PCs. A minor limitation in the current study is that final PC volumes are standardized to a volume ranging between 50 and 60 mL, which may influence the platelet concentration in PCs. A potentially larger limitation of the current study is that lipemia may artificially inflate the apparent concentrations of platelets, WBC, and hemoglobin when measured by optical method analysis.[13–15] However, lipemia was associated only with platelet concentration and not with either WBC or hemoglobin concentrations. The observation of a specific association between lipemia and platelet concentration suggests that the effect of lipemia should be considered in designing effective canine blood donation programs. In addition, future investigations might examine the effects of altering lipemic index by comparing the platelet concentration in the PCs of fasted and postprandial blood donors. The current investigation might also be extended by determining whether postprandial donations negatively impact the yield of other blood components and whether the in vivo functionality of these blood products is maintained. Future studies using larger sample sizes and relying on manual or electrical impedance procedures to measure platelets may identify additional screening variables for successful platelet donor selection.

Footnotes

[a] ADVIA 120 Hematology System, Siemens Healthineers, Erlangen, Germany
[b] Excel 2016, Microsoft Corporation, Redmond, WA
[c] Stata Statistical Software: Release 14, StataCorp. LP. College Station, TX

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Off-label Antimicrobial Declaration: The authors declare no off-label use of antimicrobials.

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