Original Study

Use of fresh platelet concentrate or lyophilized platelets in thrombocytopenic dogs with clinical signs of hemorrhage: a preliminary trial in 37 dogs

Elizabeth B. Davidow, DVM, DACVECC; Benjamin Brainard, DVM, DACVA, DACVECC;
Linda G. Martin, DVM, MS, DACVECC; Matthew W. Beal, DVM, DACVECC; Arthur Bode, PhD;
Michael J. Ford, PhD; Noel Ramsey, BS, LVT, LATG; Alicia Fagella, DVM, DACVECC
and Ari Jutkowitz, VMD, DACVECC

Abstract

Objective – To examine the safety and feasibility of using lyophilized platelets (LYO) and fresh platelet concentrate (FRESH) in bleeding thrombocytopenic dogs.

Design – Preliminary prospective randomized clinical trial.

Setting – Two private referral centers and 3 university teaching hospitals.

Animals – Thirty-seven dogs with a complaint of hemorrhage associated with thrombocytopenia (platelet count $<70 \times 10^9$/L [70,000/μL], a hematocrit >15%, and that had received neither vincristine nor platelet-containing transfusions within 72 h of enrollment were studied.

Interventions – Animals were randomized to receive LYO or FRESH, dosed according to weight. Physical examination, complete blood counts, and coagulation testing (prothrombin time and activated partial thromboplastin time) were performed at enrollment. Physical examinations were also performed immediately post transfusion, and at 1 and 24 h after transfusion. Complete blood counts were repeated immediately post transfusion and at 24 h. Collected data included bleeding score (BLS), response to transfusion, adverse reactions, hospitalization time, need for additional transfusions, survival to discharge, and 28-d survival.

Measurements and Main Results – Twenty-two dogs received LYO and 15 received FRESH. There was no difference between groups in age, weight, BLS, platelet count, white blood cell count, hematocrit, or presence of melena. There was no difference between groups in transfusion reaction rates, the need for additional transfusions, 24-h BLS, hospitalization time, survival to discharge, or 28-d survival.

Conclusions – Transfusion of LYO was feasible and associated with a low transfusion reaction rate in this limited study of thrombocytopenic canine patients presenting with mild-to-severe hemorrhage. LYO were easy to use and provided storage advantages over FRESH. Further study of this product, including examination of efficacy and platelet life span, is warranted.

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Keywords: bleeding disorders, immune-mediated thrombocytopenia, platelet storage, primary hemostasis, transfusions

Abbreviations

aPTT	activated partial thromboplastin time
BLS	bleeding score

CBC	complete blood count
DIC	disseminated intravascular coagulation
DMSO	dimethyl sulfoxide
HCT	hematocrit
IMT	immune-mediated thrombocytopenia
LYO	lyophilized platelets
FRESH	fresh platelet concentrate
PGE_1	prostaglandin E_1
pRBC	packed red blood cells
PT	prothrombin time
WBC	white blood cell count

Introduction

Thrombocytopenia occurs commonly in small animal patients. Clinical signs of severe thrombocytopenia include petechiae, ecchymoses, and bleeding from mucosal surfaces such as the mouth, nasal cavity, gastrointestinal tract, and urinary tract.[1] Bleeding can range from mild to life threatening. The most common cause for thrombocytopenia in dogs is primary immune-mediated thrombocytopenia (IMT).[2–4] Other causes include drug-induced IMT,[5] infectious diseases such as ehrlichiosis,[6] bone marrow insults from disease or medication, neoplasia,[7] disseminated intravascular coagulation, and blood loss.[8,9]

In people, platelet transfusions are recommended for prophylaxis against hemorrhage in any patient with a platelet count $<10 \times 10^9/L$ [10,000/µL] and in patients who require an invasive procedure and have a platelet count $<50 \times 10^9/L$ [50,000/µL].[10–12] Platelets are recommended therapeutically in any actively bleeding patient with a platelet count $<20 \times 10^9/L$ [20,000/µL].[10,11] Platelet transfusions are normally avoided with IMT due to the rapid platelet destruction but are recommended in the face of life-threatening hemorrhage.[11] Platelet transfusions are also recommended in patients with drug-induced or hereditary impairment of platelet function that require an invasive procedure that might result in hemorrhage.[10,11] Platelets are usually provided to human patients as a fresh platelet concentrate (FRESH), obtained by plateletpheresis. Platelet concentrates must be stored at room temperature under continuous gentle agitation, and have a shelf life of 5 d.[13]

Reactions to platelet transfusions are significant in people with rates of minor febrile reactions of up to 38% and rates of severe reactions of up to 2% reported in some clinical studies.[3] There are limited publications in veterinary medicine examining triggers for platelet transfusion, rates of platelet transfusion reactions, or the therapeutic efficacy of currently available platelet products in actively bleeding patients.[3,4]

Because of the cost and logistics involved in creating and maintaining stores of FRESH, transfusion of platelets in most veterinary institutions is usually limited to those platelets contained in fresh whole blood.[14] A frozen canine platelet concentrate, with apheresed platelets stabilized with 6% dimethyl sulfoxide has been commercially available for several years.[a,15–17] There is some evidence that these platelets are effective, but have significantly less aggregatory activity than fresh platelets.[15,16,18]

A lyophilized canine platelet product (LYO) has recently been developed.[b] Platelets are stabilized using aldehyde crosslinking of membrane proteins and lipids. This process allows for lyophilization and reconstitution with preservation of platelet structure and hemostatic function.[19] The LYO can be stored for up to 24 mo in the refrigerator, and are reconstituted with 0.9% saline immediately prior to use. Research studies on LYO have shown that they bind to collagen, von Willebrand factor, and damaged endothelium, but do not stick to intact endothelium at high shear.[20,21] When activated, the platelets express procoagulant activity and bind fibrinogen and factor VIIa.[22–25] When fluorescently labeled LYO platelets were infused into normal dogs, they could be identified as part of the hemostatic plug of ear puncture wounds made to assess bleeding time.[26] In a study of dogs that experienced thrombocytopenia and platelet dysfunction due to 2 h of recirculation on cardiopulmonary bypass, infusion of canine LYO led to improvement in venous bleeding time as measured by time to clot formation at a small puncture made in the jugular vein; the effect was most pronounced at 20–30 min after infusion and continued for up to 3 h.[19]

The aim of this study was to conduct a preliminary investigation of a commercial LYO product[b] in thrombocytopenic dogs with active hemorrhage. Our hypothesis was that this product would be equivalent in safety to FRESH. In addition, we hypothesized that efficacy would be similar, as measured by time to resolution of bleeding, change in platelet count, need for additional red blood cell transfusions, hospitalization time, or survival in thrombocytopenic dogs that received LYO versus those receiving FRESH.

Materials and Methods

Data were collected from October 2008 to June 2009. The study was approved by the animal care and use committees or medical directors at Animal Blood Resources International[c] and the participating institutions. Clients presenting to the 5 institutions (University of Georgia [A], Michigan State Univerity [B], Dove Lewis

Table 1: Bleeding scores (BLS)

Score	Criteria
0	No gross evidence of bleeding; normal PCV/TP
1	Mild petechiation, normal PCV/TP
2	Moderate petechiation and/or ecchymosis; epistaxis; normal to low normal PCV/TP
3	Evidence of severe bleeding including cutaneous hematoma, GI bleeding, hematuria, mild decrease PCV/TP (>3% below normal range)
4	Severe bleeding including intracavitary, intracranial hemorrhage, GI bleeding, PCV/TP (>5% below normal range) RBC needed
5	Severe bleeding with PCV/TP >10% below normal range requiring ongoing RBC support

RBC, red blood cells; PCV, packed cell volume; TP, total protein.

Table 2: Initial parameter measurements

Category	FRESH	LYO	*P* value
Age (y)	8.07 (2.58)	6.68 (3.06)	0.16
Weight (kg)	54.17 (31.45)	57.31 (32.09)	0.28
Hematocrit (%)	32.3 (13.5)	28.9 (11.7)	0.43
Platelet count ($\times 10^9$/L)	18.53 (16.69)	16.45 (12.61)	0.91
White blood cell count ($\times 10^9$/L)	15.05 (13.70)	21.15 (16.37)	0.08
Temperature (°C)	38.6 (0.94)	38.1 (1.47)	0.11
Heart rate	127/min (27)	115/min (260)	0.17
Bleeding score	3.27 (1.24)	2.98 (1.31)	0.51

Values listed are means (standard deviation in parentheses). FRESH, fresh platelet concentrate; LYO, lyophilized platelets.

Emergency Animal Hospital [C], Auburn University [D], and Animal Critical Care and Emergency Services [E]) during this time with dogs that met the eligibility criteria were offered study enrollment by the clinicians at each institution. Dogs were eligible if they were >12 mo of age, had a platelet count of <70 $\times 10^9$/L [70,000 /μL], had a hematocrit (HCT) of >15%, had evidence of active bleeding with a bleeding score (BLS) greater than or equal to 1 (Table 1), and had a prothromin time (PT) and activated partial thromboplastin time (aPTT) that were within 10% of the normal reference interval. In addition, eligible dogs had received neither vincristine nor platelet-containing blood products within the 72 h preceding admission to the study. All owners provided informed consent prior to patient inclusion into the study.

Preparation of study compounds

FRESH[d] was created via apheresis by proprietary techniques similar to previously described methods in dogs.[14] Acid-citrate-dextrose formula A was used as the anticoagulant. The target product content was 220 $\times 10^9$ platelets using a draw speed of 70 mL/min. Each collection was split into 2 units containing approximately 110 $\times 10^9$ platelets. FRESH was shipped to each institution weekly during the study period using temperature controlled containers[e] conforming to platelet shipping standards for people. Platelets were stored at each institution at room temperature on rockers, not in a platelet incubator, for up to 7 d from the date collected. LYO were prepared using published patented procedures[22] from canine platelets obtained by apheresis.[f] The apheresed platelets were shipped as listed above to another laboratory facility[g] for lyophilization. The platelets were treated with prostaglandin E_1 and washed in a phosphate washing buffer containing 2 mM theophylline to prevent activation during processing. The washed platelets were then resuspended and incubated for 45 min in a buffer solution containing paraformaldehyde at 0.68%. They were subsequently washed with imidazole buffer and saline to remove the paraformaldehyde and resuspended at a concentration of approximately 2 $\times 10^9$/mL in 4% human serum albumin as a bulking agent. Then, the exact cell count in the fixed washed platelet suspension was determined with a Coulter AcT Diff Analyzer[h] and a number of doses of a minimum of 50 $\times 10^9$ total platelets was measured by pipettor into glass LYO bottles with a fill volume of 20–25 mL, then frozen at –70°C. The frozen platelets were then dried in a lyophilizer.[i] The actual measured total platelet counts for the 144 dose bottles produced from the 9 LYO preps made for the clinical trial material ranged from 52 to 57 $\times 10^9$. All the LYO preparations were done in a 6-mo period prior to the start of the clinical trial and were stored at –70°C for 1–3 wk before shipment on 4°C cold packs to the coordinating center[c] for distribution to the participating clinical centers.

Study protocol

Once enrolled, patients were randomized by the coordinating center[c] using a random number table to receive either FRESH or LYO. Data collection was performed by the clinician in charge of the case at each institution. Physical examination, complete blood count (CBC), and coagulation parameters (PT and aPTT) were performed on all patients at time of inclusion into the study to both determine eligibility and for baseline measurements (Table 2). BLS was assessed by the clinician in charge of the case at each institution using the criteria in Table 1. The BLS system was modified from a BLS previously used in children with idiopathic thrombocytopenia purpura and more recently in Greyhounds with postoperative bleeding.[27,28] Patients weighing <7.2 kg were given half unit of product, between 7.2 and 22.7 kg were given 1 unit, between 22.8 and 34 kg were given 1 and half units, and those weighing >34.1 kg were given 2 units. These weight categories approximated a dose of 6.6 $\times 10^9$ fresh

platelets per kg or 3.3×10^9 lyophilized platelets per kg. Actual platelet dose was approximated by dividing the assumed baseline concentration of 50×10^9 platelets per vial for LYO and 100×10^9 platelets per bag for FRESH by the patient's weight. Each vial of LYO was reconstituted in 60 mL of 0.9% sodium chloride and the total dose was administered over 15–20 min. Fresh platelets, each bag containing an average of 70 mL, were administered slowly for the first 15 min and then administered within 2 h. During the administration of the platelet products, patients were monitored closely, as with any transfusion, with frequent assessments (every 5–15 min) for changes in heart rate, respiratory rate, or rectal temperature, as well as for signs of acute allergic reactions (eg, vomiting, facial swelling, pruritis, erythema). An additional physical examination and CBC were performed immediately following completion of the platelet product transfusion. Another physical examination was performed 1 h following the termination of the transfusion. Red blood cell transfusions (pRBC) were given as needed, before or after the platelet transfusion, based on clinician assessment of the patient. Additional platelet-containing transfusions were only performed after the 1 h data collection point. In some cases, because of paucity of product kept at each institution, additional platelet transfusions were of the opposite type from the initial transfused product (LYO or FRESH). A final physical examination, CBC, and clotting profile (PT and aPTT) were repeated again 24 h after the platelet transfusion. BLSs were assessed prior to platelet administration, and again at 24 h. Information was collected about any adverse events seen during the 24-h study period, as well as the number of additional transfusions of red blood cell containing products or platelets that were administered, the number of days the animal was hospitalized, the survival to discharge from the hospital, and the 28-d survival of the subject. If death occurred, the cause of death or reason for euthanasia was documented.

Statistical Methods

All statistical analyses were conducted using an open-source web-based statistical package.[j] Differences between treatment groups in presence or absence of clinical signs, outcome, or other binary traits were tested using Fisher's exact tests of a 2-way contingency tables. Differences between treatment groups in continuous or numeric characteristics (age, BLS, HCT, platelet count, white blood cell [WBC], heart rate) were evaluated using single factor analysis of variance (ANOVA) after visual inspection of the data for approximate normality. Three traits, platelet count, WBC count, and number of days hospitalized were log transformed to improve nor-

mality prior to statistical analysis. Differences between treatment groups in the relationship of the platelet count before and after platelet transfusions and in the BLSs initially and at 24 h were evaluated using the interaction term in a 2-factor ANOVA. Values of $P < 0.05$ were considered significant.

Results

Admission data

Thirty-seven patients were enrolled in the trial. None of the patients had received vincristine or any platelet-containing transfusions in the week prior to admission. Twenty-two of the 37 dogs (60%) received LYO and 15/37 (40%) received FRESH for their initial platelet transfusion. Patients were enrolled from each of the participating institutions – 10 (27%) from institution B, 8 (22%) from institution E, 7 (19%) from institution D, 6 (16%) patients from institution A, and 6 (16%) from institution C. In the LYO group, there were 8 spayed females, 1 intact female, 12 neutered males, and 1 intact male. In the FRESH group, there were 10 spayed females and 5 neutered males. In the study population, there were 7 mixed breeds, 5 Labrador retrievers, 3 Collies, 2 Golden Retrievers, 2 Boxers, and 1 each of 18 other breeds. There were no significant differences in age or weight between the treatment groups (Table 2).

Of the 22 dogs in the LYO group, 14 (63.6%) of the dogs had IMT that was thought to be primary based on exclusion of other common causes such as tick borne diseases and neoplasia using titers, radiographs, and ultrasound as appropriate. An additional 4 (18.2%) dogs had IMT and concurrent evidence of immune-mediated red blood cell destruction, based on the presence of either spherocytes or autoagglutination. Of the remaining dogs, 1 (4.5%) dog was diagnosed with *Ehrlichia canis* by serum antibody titers, 1 (4.5%) dog had Stage 5 B cell lymphosarcoma (diagnosed by immunohistochemistry on peripheral blood), and 1 (4.5%) dog had splenic hemangiosarcoma (diagnosed by histopathology). Of the 15 dogs in the FRESH group, 13 (86.7%) dogs had primary IMT, 1 (6.7%) dog had IMT but also evidence of a possible underlying infection (as manifest by perirenal inflammation and omental inflammation seen on ultrasound), and 1 (6.7%) dog was thrombocytopenic secondary to sepsis from a leaking jejunostomy tube.

Clinical signs were consistent with severe thrombocytopenia. Of the 37 dogs in the group, 33 (89%) had petechiae or ecchymoses, 23 (62%) had evidence of some gastrointestinal hemorrhage with 18 (49%) having melena specifically. In addition, 10 (27%) had ocular hemorrhage, 7 (18.9%) had oral hemorrhage, 5 (13.5%) had epistaxis, and 4 (10.8%) each had hematuria or neurologic signs. Neurologic signs included progressive decline in

Table 3: Bleeding scores initially and at 24 h

GROUP	BLS = 0	BLS = 1	BLS = 2	BLS = 3	BLS = 4	BLS = 5
Initial LYO *n* = 22	0	4	3	8	4	3
Initial FRESH *n* = 15	0	1	3	3	1	3
24-h LYO *n* = 22	2	4	4	5	3	4
24-h FRESH *n* = 14	3	2	2	4	1	2

BLS, bleeding score; LYO, lyophilized platelets; FRESH, fresh platelet concentrate.

mentation in 2 patients, a head tilt in another, and progressive obtundation with head pressing and a seizure in the fourth. There was no significant difference in the incidence of gastrointestinal bleeding, skin bleeding, ocular hemorrhage, oral hemorrhage, epistaxis, hematuria, nor neurologic signs between the LYO and the FRESH group. There was also no significant difference in BLS at admission, platelet count at admission, HCT, or WBC count at admission between the dogs in the LYO or the FRESH groups (Table 2). BLS of dogs in both the LYO and FRESH group ranged from 1 to 5 (Table 3).

Initial responses

Actual individual weights and product platelet count resulted in a mean platelet dose of 3.43×10^9/kg (range of 2.1–6.8×10^9/kg) for the LYO, and 7.75×10^9/kg (range 3.89–22×10^9/kg) for the FRESH. Immediate post-transfusion increments in platelet count were highly variable in both groups and appeared greater with the FRESH platelets but neither treatment gave a uniform increase in count (Figure 1). ANOVA suggested that across all transfusions there was a significant positive correlation ($r = 0.44$, $P = 0.016$) for change in platelet count versus dosage of platelets given, but separate trends between groups could not be discerned with the low number of cases in each separately.

Evidence of active bleeding was unchanged on physical examination immediately post transfusion in 18/22 (81.8%) dogs after receiving LYO. In 3/22 (13.6%) dogs, active bleeding was observed to cease, with epistaxis stopping in 2 and a bleeding skin wound clotting in the third. In 1/22 (4.5%) dog, a new bruise was noted at a venipuncture site. Evidence of bleeding was unchanged on physical examination immediately post transfusion in all dogs receiving FRESH. The HCT was not significantly different from baseline when checked immediately post transfusion in either the LYO (mean 26.5%, versus 28.9% at baseline, $P = 0.27$) or the FRESH group (mean 31.0% versus 32.3% at baseline, $P = 0.48$). Heart rate also remained stable at mean 127 bpm for FRESH and 115 bpm for the LYO group ($P = 0.23$) (Table 4).

Two dogs in the study received platelets immediately prior to a surgical procedure. One dog had a hemoperitoneum secondary to a bleeding splenic mass, a HCT of
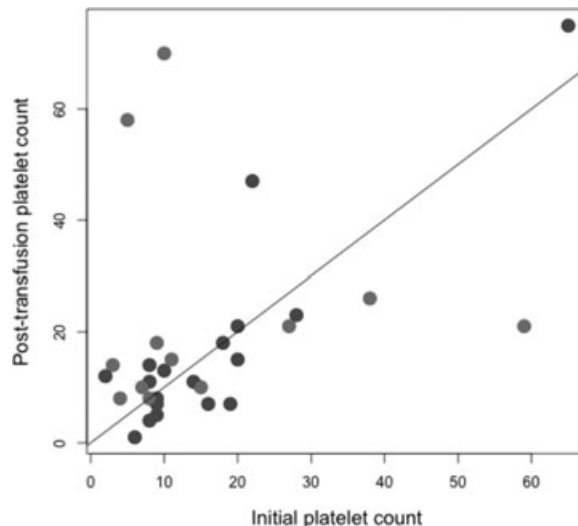
Figure 1: Platelet count following platelet product administration. The line indicates no change in platelet count ($\times 10^3$/µL) with transfusion. Dots to the left of line indicate that the platelet count increased after transfusion, dots to the right indicate that the count decreased despite transfusion. As shown, neither fresh concentrate nor lyophilized platelets were successful consistently in raising the measured count. Red, FRESH (fresh platelet concentrate); Blue, LYO (lyophilized platelets).

30%, and 65×10^9/L [65,000/µL] platelets. LYO platelets were given. The other dog had a septic abdomen due to a slipped jejunostomy tube requiring immediate surgical intervention but had a platelet count of only 18×10^9/L [18,000/µL]. FRESH platelets were given. Neither dog had excessive bleeding in surgery and neither needed pRBC after their procedures.

Table 4: Parameters immediately after transfusion

Category	FRESH	LYO	*P* value
White blood cell count ($\times 10^9$/L)	16.76 (15.97)	19.71(12.20)	0.30
Hematocrit (%)	31.0 (15.37)	26.5 (9.79)	0.34
Platelet count ($\times 10^9$/L)	23.25 (20.02)	16.61(17.81)	0.34
Heart rate	129/min (26)	117/min (30)	0.23

Values listed are means (standard deviation in parentheses). FRESH, fresh platelet concentrate; LYO, lyophilized platelets.

Table 5: Outcome variables

Category	FRESH	LYO	*P* value
Hematocrit at 24 h – mean (SD)	28.1% (13.3%)	25.6% (9.9%)	0.53
Platelet count at 24 h – mean (SD)	32.21×10^9/L (39.79)	20.66×10^9/L (35.73)	0.30
White blood cell count at 24 h – mean (SD)	24.38×10^9/L (12.99)	24.31×10^9/L (14.28)	0.97
Bleeding score at 24 h –mean (SD)	2.32 (1.73)	2.73 (1.63)	0.48
Additional transfusions – RBC and platelets	7/15 (47%)	11/22 (50%)	1
Additional transfusions – RBC only	4/15 (27%)	7/22 (32%)	1
Hospitalization days – mean (range)	3.67 (range 1–8)	4.68 (range 1–16)	0.61
Discharge survival	11/15 (73%)	15/22 (68%)	1
28-d survival	10/15 (67%)	12/22 (55%)	0.51
Discharge IMT only	10/13 (77%)	11/14 (79%)	1
28-d survival IMT only	9/13 (69%)	11/14 (79%)	0.68
Discharge survival – omitting dogs receiving both platelet types	10/12 (83%)	15/21 (71%)	0.68

FRESH, fresh platelet concentrate; LYO, lyophilized platelets; RBC, red blood cells; IMT, immune-mediated thrombocytopenia.

At the 1-h physical examination, 20 dogs (91%) receiving LYO had unchanged physical examinations. In 2 dogs (9%), active bleeding had restarted or worsened. In the FRESH group, 12 dogs (80%) had unchanged physical examination findings, 2 dogs (13%) had new signs of bleeding, and in 1 dog (7%) bleeding was mildly improved. Heart rate was still stable at the 1-h examination with a mean of 122/min in the FRESH group and 115/min in LYO group ($P = 0.54$).

Twenty-four-hour response

Thirty-two percent (7/22) of the dogs that received LYO and 40% (6/15) of the dogs that received FRESH product had a decrease in their BLS 24 h following the initial platelet transfusion. The change in BLS between time periods was not significantly different between the treatment groups ($P = 0.82$). Three dogs (14%) in the LYO group and 1 dog (7%) in the FRESH group did receive additional platelet containing products in this first 24 h. Of these 4 dogs, 1 in the LYO group that then received fresh whole blood had a decrease in the BLS, 2 in the LYO group, 1 receiving more LYO and 1 receiving FRESH had unchanged BLSs, and 1 in the FRESH group that received LYO had an increase in the score. All the dogs in both groups had PT and aPTT values that remained within the reference range at the 24-h time point.

Adverse reactions

Three of the 22 dogs (14%) in the LYO groups had a possible mild transfusion reaction. One dog had a 2°F (1.0°C) rise in rectal temperature, which had returned to normal by the 1-h physical examination, 1 dog had sinus tachycardia (HR 180 beats per min) at the physical examination 1 h after transfusion that resolved with transfusion of pRBC, and 1 dog experienced an episode of emesis. Two of the 15 dogs (13%) in the FRESH group had a mild transfusion reaction, 1 dog developing urticaria and peri-orbital swelling and 1 dog experiencing a single episode of emesis at the start of the transfusion. The number of reactions in each group was not significantly different ($P = 1$). No delayed transfusion reactions were recognized. There were no signs of type III hypersensitivity reactions in any of the patients either during hospitalization or after discharge.

Hospitalization and discharge

There was no significant difference between the groups in the need for additional pRBC or platelet transfusions, hospital time, or hospital discharge rate. In the LYO group, 50% (11/22) of the dogs received additional blood products with 7/22 (32%) receiving pRBC products alone and 4/22 (18%) receiving additional platelets as well as pRBC In the FRESH group, 47% (7/15) received additional blood products with 4 (27%) receiving pRBC alone and 3 (20%) receiving pRBC and platelets. Four dogs of the 37 in the study (11%) received the opposite type of platelet product for 1 of their additional transfusions. There was no change in the significance of the survival results when the data were reanalyzed with these dogs removed. (Table 5) The dosage of platelets, of either LYO or FRESH, was not significantly different between those animals that received additional transfusions and those that did not (LYO, $P = 0.52$ and FRESH, $P = 0.18$). The dogs in the LYO group had a mean hospitalization time of 4.68 d with a range of 1–16 d while the FRESH group had a mean hospitalization time of 3.67 d with a range of 1–8 d. Sixty-eight percent (15/22) of the dogs in the LYO group were discharged while 73% (11/15) of the dogs in the FRESH group were discharged. In contrast to other studies of dogs with thrombocytopenia, neither melena nor overall evidence of gastrointestinal hemorrhage was associated with a decreased chance of

discharge ($P = 1$). Three of the 4 dogs with neurologic signs were discharged from the hospital. The fourth dog was euthanized due to severe neurologic deterioration.

There was no significant difference in 28-d survival between the LYO and FRESH group. In the LYO group, 55% (12/22) were still alive at 28-d post hospital discharge while in the FRESH group, 67% (10/15) were still alive. When only dogs with IMT were examined, 28-d survival in the LYO group was 79% (11/14) and in the FRESH group was 69% (9/13) (Table 5).

Discussion

In this preliminary study, we found that transfusion of LYO was feasible and associated with a low transfusion reaction rate. Efficacy of LYO compared to FRESH could not be ascertained with the number of patients enrolled. The original enrollment goal was 100 patients. However, budget constraints led to early termination of the study and a significant loss of power for determining comparative efficacy. The observational data collected add to the extremely limited veterinary literature on use of platelet transfusions in bleeding patients.

One of the major deterrents to use of platelet transfusions in veterinary medicine has been cost and handling issues with fresh platelets. FRESH may be produced from fresh whole blood using a double spin technique, or may be acquired by plateletpheresis. An apheresis donation will produce a platelet concentrate containing $100–400 \times 10^9$ platelets, 4–6 times the amount in a centrifuged platelet concentrate, with negligible WBC and RBC contamination.[4] Regardless, fresh platelets must be stored in gas-soluble bags at room temperature and must be constantly agitated to remain active. Bacterial contamination is a risk and recommended storage time for human platelets is 5 d.[13] An unanticipated observation of this study was the difficulty and high cost for a single blood bank to provide a constant supply of fresh aphered platelet concentrate to 5 institutions in various parts of the country over a prolonged period. Because of environmental temperature constraints with shipping and the need for an individual to be available to receive and immediately unpack the platelets, there were several instances in which institutions did not have fresh platelets on hand when a study animal was enrolled.

In contrast, the LYO platelets are shipped cold but not frozen in light weight plastic vials that are then refrigerated and are purported to retain viability for up to 24 mo in the refrigerator. Thus, the LYO platelets shipped to the institutions are thought to have remained available for the entire length of the study period. The lack of FRESH at certain times led to a default use of LYO in a few study patients so a higher number of patients received this type of product. This break in randomization, though due uncontrollable factors, is a limitation to this trial.

Only 3 minor possible transfusion reactions (13.8%) were seen in patients receiving LYO, and in only 2 (13.2%) patients receiving FRESH. Only 1 (4.5%) dog receiving LYO had a febrile reaction. The only previous study looking at platelet transfusion reaction rates in dogs examined 5 dogs undergoing bone marrow transplantation that developed severe thrombocytopenia. These 5 dogs received 46 units of platelet concentrate made by centrifugation. There were 8 reactions (17%), 5 febrile, 1 vomiting, 1 uticaria, and 1 anaphylactic reaction.[29] In clinical studies of platelet transfusion in humans, the rate of minor febrile reactions in some studies has been much higher (38%).[3] A 2% rate of severe reactions has also been reported in people given fresh platelets.[3,30] In humans, the risk of reaction increases when the number of WBCs in the platelet unit increases, when storage time increases, and with non-ABO blood type identical transfusions.[30–32] No acute severe reactions nor septic reactions were seen in this study, and there have been no reported severe acute adverse events in previous experimental use of LYO platelets.[33] Apheresis was used to create both types of platelets that leads to less WBC contamination.[14] The fixation process used in the creation of the LYO platelets kills bacteria and viruses added to the suspension so that it is truly pathogen-reduced versus other blood bank products, which could be one explanation for the low rate of febrile reactions.[26] Blood type of the platelet donor and storage time of the FRESH were not recorded. Examination of these variables in relation to reaction rates is an area for further study. In our study, no obvious delayed reactions that could be type III hypersensitivity were identified despite the inclusion of human albumin in the formulation of the LYO. In future preparations, canine albumin could be substituted which may decrease possible risk associated with the small amount of human albumin.

In the current study, we were not able to identify a difference in outcome, as assessed by changes in platelet count, BLS, need for additional transfusions, hospitalization times, or survival, between patients who received LYO versus FRESH. Measuring the efficacy of a platelet transfusion in the clinical setting is challenging; often the platelet count does not increase following transfusion as the delivered platelets are rapidly used for platelet plugs.[34] In the cardiopulmonary bypass canine model study, there was often no significant increase in platelet count despite a measurable correction in the venous bleeding time.[19] In our study, the measured change in platelet count was statistically similar in the LYO and FRESH group but did not consistently rise after a platelet transfusion.

A BLS was used to try to look at both severity of signs and to examine efficacy. However, in practice, the BLS was challenging. Some dogs classified with low BLS due to normal HCT and lack of petechiae actually had gastrointestinal hemorrhage, which has been associated with more severe disease.[35] Because petechiae do not resolve quickly and changes in the quantity of gastrointestinal hemorrhage are difficult to assess in short timeframes, comparison of BLS across short periods of time had limited value in assessing platelet transfusion efficacy and were not assigned immediately or at 1 h. Detailed physical examination forms were more helpful in identifying changes immediately post transfusion and at one hour. BLS at 24 h remained lower in many patients receiving either FRESH or LYO transfusion but some patients had received pRBC transfusions or additional platelets making evaluation of this result difficult. In future studies, measuring the platelet count and observing for active bleeding at time points between 5 min and 6 h would be useful in evaluating efficacy and length of action. A revised BLS with more reliance on points for different areas and types of bleeding would also be useful. In addition, using a larger population of dogs with active external bleeding (eg, epistaxis, oral hemorrhage) might be useful for observing whether the platelets have an effect on cessation of bleeding and the timing of these effects. Other proactive assessments of platelet function (eg, using the PFA-100 platelet function analyzer) may also have been used to provide evidence of efficacy, although these machines are not always accurate in patients with low platelet counts and low HCT.

The need for additional pRBC transfusions was not correlated to the dose of platelets given and was not different between the LYO and FRESH groups despite difference in dosing between the groups. To simplify the protocol, dosing was done based on half unit increments. This scheme led to a large variation in total platelet dosage. The study was also designed so that the dogs that received FRESH received twice the number of platelets as those receiving LYO. The dosage of LYO was based on previous studies examining the dose threshold for improved adhesion in situations of active blood flow[20] and for shortened bleeding times in thrombocytopenic rabbits.[22] Previous studies had also shown a reconstitution yield of 90–100% cell count.[21] The FRESH dosage was set independently and was based on previously published recommendations.[4] When the effect of platelet dosage on the platelet count immediately following transfusion was examined, it was positively correlated. However, close examination of the data showed that the correlation was driven by a very few patients who got high doses of fresh platelets. Platelet dosing for thrombocytopenic people continues to be debated. One apheresed unit of human FRESH contains 300 mL and more than 300×10^9 platelets and current recommended dosing is 50 mL per 10 kg (5×10^9 platelets/kg).[11,13] Human studies have shown that higher platelet doses do lead to higher increases in platelet count.[36–38] However, in a recent study in thrombocytopenic people looking at different dosages of platelets, there was no difference in the incident of bleeding between the high- and the low-dose groups.[38]

Hospitalization time in our study was a median of 3 d for the FRESH group and 3.5 d for the LYO group. A recent retrospective study of 73 dogs with IMT showed similar hospitalization time with a median of 4 d.[35] The majority of veterinary studies examining survival in thrombocytopenic dogs have focused on only those dogs with IMT. In the only small study looking at transfusions for platelet support in 40 dogs with a variety of causes of thrombocytopenia, there was 44% survival to discharge.[4] Survival, defined with differing endpoints, of dogs in published studies on IMT has ranged from 74% to 97%.[35,39,40] In the recent retrospective of 73 dogs with IMT, there was 84% survival to discharge but only 60% in those dogs with melena.[35] Survival of the dogs in our study was comparable to the results in these studies, especially when dogs with thrombocytopenia caused by diseases other than IMT were removed from the data set (Table 5).

The lack of improvement in outcome variables in our dogs receiving platelet transfusions may be due to the unique pathology of IMT, where rapid clearance of platelets, including transfused platelets, may mitigate sustained improvements in platelet count. Another possibility includes the relatively good prognosis for most dogs with IMT; this study was underpowered to detect a significant improvement in survival or hospitalization in a group of dogs that already have a relatively short hospital stay. Further work on platelet transfusions should focus on those dogs with the most life-threatening bleeding and in dogs with other causes of thrombocytopenia.

There were several limitations to this initial trial, in addition to the small sample size. FRESH was not kept in platelet incubators and was kept for up to 7 d. Previous studies of canine platelet concentrate showed preservation of platelet number and lack of bacterial growth during a 7-d period but potential loss of in vitro function.[41] However, some human studies have demonstrated good in vivo activity even at 8 d.[42] Because storage time was not recorded, some of the fresh transfusions might have been older units and could have had less efficacy. The length of time over which the platelets were given was not controlled and could have influenced the appearance of immediate reactions. In addition, entry criteria were set fairly broadly to provide information about use of platelet transfusions in the range

of bleeding thrombocytopenic patients that are seen in practice. RBC transfusion prior to admission to this study was left up to clinician discretion so the degree of anemia was not controlled. However, the risk of bleeding with thrombocytopenia is affected by the degree of anemia.[43–46] A higher packed cell volume leads to movement of platelets toward the endothelium and reduction of shear stress. In addition, RBCs contain mediators that increase platelet activity and also scavenge nitric oxide.[43] Thus, in cases of moderate thrombocytopenia and concurrent anemia, the risk of bleeding may be lessened with pRBC transfusion alone.[44,45] Interestingly, in the only 3 dogs that were observed to have active bleeding cease immediately after platelet transfusion, 2 had a HCT <20%. In future studies, the degree of anemia should be controlled.

The length of time platelets will remain in circulation is influenced by the degree of thrombocytopenia and by the type of platelet processing. A set number of platelets are needed for endothelial maintenance resulting in a higher percentage that is needed and a decrease in platelet circulation time for any product in thrombocytopenic conditions.[3,9] Platelets that are minimally handled, such as those in fresh whole blood or FRESH survive longest in circulation with a half life of 3.5–7 d.[17,47] Canine platelets frozen with dimethyl sulfoxide have a circulating half life of 2 d.[17,18] In early formulations of LYO platelets, the circulating half life appeared to be limited to minutes.[47] Protective activation inhibitors used in the current LYO product have led to a longer circulating half life but only of hours, not days.[3,19,22,24,48] Future studies looking at a narrower range of thrombocytopenic patients would help to control this factor. In addition, future studies comparing efficacy between LYO and FRESH should focus on the initial few hours after transfusion.

Because of the short life span of the LYO product, they may be best used as a hemostatic agent in the face of catastrophic or life-threatening bleeding while awaiting more definite treatment. The fast reconstitution and administration may allow them to be a bridge in an animal with life-threatening hemorrhage during the time it sometimes takes to obtain fresh whole blood or make FRESH. Bode et al[19] showed maximum correction of bleeding time at 20–30 min post administration in the canine cardiopulmonary bypass model, although bleeding was observed to cease very quickly after administration in some patients in this study. In a recent study, 20 pigs were subjected to a grade III liver injury. They were then given either a placebo or LYO. Two hours later, the liver was repaired. There was 80% survival in the group that received the LYO while only 20% survival in the group receiving placebo.[49] As reported in the results, 2 dogs in our study did undergo surgery and received platelet transfusions prior to the procedures. Although no conclusions can be drawn from these cases, use of platelet transfusions for both immediate hemostasis and prior to invasive procedures should be an area for additional study.

Although this study had a small sample size, adverse reactions were low for both LYO and FRESH, and these products appeared safe for clinical use in dogs with thrombocytopenia and hemorrhage. The shelf life and ease of use of the LYO make them attractive options for acute therapy of severe hemorrhage associated with thrombocytopenia or thrombocytopathia; however, further studies evaluating their use in these and other clinical circumstances are indicated. In addition, further studies to evaluate appropriate dosage, in vivo survival, in vivo function, and duration of hemostatic effects of the LYO in thrombocytopenic dogs, as well as those with normal platelet counts, are warranted.

Footnotes

^a Frozen Canine Platelet Concentrate, Midwest Animal Blood Services, Stockbridge, MI.
^b Lyophilized Platelets. Animal Blood Resources International, Stockbridge, MI.
^c Animal Blood Resources International.
^d Fresh Platelet Concentrate. Animal Blood Resources International.
^e Blood Safe-22, Cryopak, Edison, NJ.
^f Haemonetics MCS+, Haemonetics Corp, Braintree, MA.
^g East Carolina University, Greenville, NC (Dr. Arthur Bode).
^h Coulter AcT Diff Analyzer, Beckman Coulter, Brea, CA.
ⁱ Virtus 600 Freeze-Mobile, Virtus Co, Gardiner, NY.
^j The R Project for Statistical Computing version 2.11.0 (2010-04-22), a web-based statistical program (http://www.r-project.org)

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