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Canine platelet transfusions

Mary Beth Callan, VMD, DACVIM; Elizabeth H. Appleman, VMD, DACVIM and Bruce S. Sachais, MD, PhD

Abstract

Objective – To review potential platelet storage options, guidelines for administration of platelets, and adverse events associated with platelet transfusions.
Data Sources – Data sources included original research publications and scientific reviews.
Human Data Synthesis – Transfusion of platelet concentrates (PCs) plays a key role in the management of patients with severe thrombocytopenia. Currently PCs are stored at 22°C under continuous gentle agitation for up to 5 days. Chilling of platelets is associated with rapid clearance of transfused platelets, and galactosylation of platelets has proven unsuccessful in prolonging platelet survival. Although approved by the American Association of Blood Banks, cryopreservation of human platelets in 6% DMSO largely remains a research technique. Pre-storage leukoreduction of PCs has reduced but not eliminated acute inflammatory transfusion reactions, with platelet inflammatory mediators contributing to such reactions.
Veterinary Data Synthesis – Canine plateletpheresis allows collection of a concentrate with a high platelet yield, typically 3–4.5 $\times$ 10^{11} versus $<1 \times 10^{11}$ for whole blood-derived platelets, improving the ability to provide sufficient platelets to meet the recipient's transfusion needs. Cryopreservation of canine platelets in 6% DMSO offers immediate availability of platelets, with an acceptable posttransfusion in vivo platelet recovery and half-life of 50% and 2 days, respectively. While data on administration of rehydrated lyophilized platelets in bleeding animal models are encouraging, due to a short lifespan (min) posttransfusion, their use will be limited to control of active bleeding, without a sustained increase in platelet count.
Conclusions – Fresh PC remains the product of choice for control of bleeding due to severe thrombocytopenia or thrombopathia. While cryopreservation and lyophilization of canine platelets offer the benefits of immediate availability and long-term storage, the compromise is decreased in vivo recovery and survival of platelets and some degree of impaired function, though such products could still be life saving.

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Introduction

Platelet transfusion therapy poses many challenges in veterinary clinical practice. While packed red blood cells (PRBCs) and fresh frozen plasma constitute the majority of transfusions administered to dogs in hospitals with ready access to blood components, platelet transfusions could be life saving in some situations. Lack of a readily available blood donor, short shelf-life of platelet-rich plasma (PRP) or platelet concentrate (PC), and inability to administer a sufficient number of platelets to meet a dog's transfusion needs are the major difficulties encountered, even in veterinary institutions with well-established blood bank services. The purpose of this paper is to review procedures for platelet donation and preparation of PC, potential platelet storage options as alternatives to fresh PC, guidelines for administration of platelets to thrombocytopenic patients, and potential adverse events associated with platelet transfusions.

From the Department of Clinical Studies, School of Veterinary Medicine (Callan, Appleman), and the Department of Pathology and Laboratory Medicine, School of Medicine (Sachais), University of Pennsylvania, Philadelphia, PA 19104.

Address correspondence and reprint requests to
Dr. Mary Beth Callan, Department of Clinical Studies, School of Veterinary Medicine, University of Pennsylvania, 3900 Delancey St, Philadelphia, PA 19104-6010, USA.
Email: callan@vet.upenn.edu

Indications for Platelet Transfusions

Platelet transfusions are indicated in the management of uncontrolled or life-threatening bleeding due to severe thrombocytopenia or thrombopathia. In clinical practice, immune-mediated thrombocytopenia (IMT) is the most common cause of severe thrombocytopenia in dogs. Blood transfusions may be required in dogs with

IMT experiencing severe mucosal surface bleeding, most commonly into the gastrointestinal tract. In such cases, PRBC transfusions are indicated to provide additional oxygen-carrying support. Platelet transfusions are uncommonly administered to dogs with IMT due to the belief that transfused platelets are rapidly destroyed following administration. However, in dogs with IMT experiencing uncontrolled or life-threatening bleeding (eg, suspected bleeding into the brain, myocardium, or lungs), platelet transfusions may provide short-term hemostasis despite a negligible increase in platelet count posttransfusion. In addition, bleeding associated with hereditary thrombopathias, such as Basset hound thrombopathia, Glanzmann's thrombasthenia in the Great Pyrenees, and δ-storage pool disease in the American Cocker Spaniel, may be severe and require platelet transfusions to achieve hemostasis. Prophylactic platelet transfusions may be considered in dogs with hereditary thrombopathias and a known bleeding tendency before surgery, with compatible PRBCs available in the event of excessive bleeding.

Another potential indication for administration of platelets is massive transfusion, defined as transfusion of a volume of whole blood or blood components that is greater than the patient's estimated blood volume within a 24-hour period or replacement of half the patient's estimated blood volume in 3 hours. Human trauma resuscitation protocols have emphasized the use of crystalloids and PRBCs to improve cardiac output and oxygen delivery, with the use of plasma and platelets reserved for patients with persistent hypotension unresponsive to crystalloid infusion, transfusion of >6 units PRBCs, documented abnormal coagulation laboratory parameters, or obvious microvascular bleeding.[1] However, based on recent retrospective studies showing improved outcomes in massively transfused human trauma patients receiving plasma and platelet transfusions earlier and at a lower ratio to RBC transfusions, there are some investigators advocating the administration of both plasma and platelet transfusions more aggressively in the trauma resuscitation process in an effort to prevent development of coagulopathy and thrombocytopenia.[1,2] Given the retrospective nature of these studies, however, these changes are not being universally adopted. Prospective, randomized clinical trials would be of value in determining the benefits and risks of this more aggressive transfusion policy.

Fresh Platelet Products

Blood products that may be administered to dogs in need of functional platelets include fresh whole blood, PRP, and PC. Fresh whole blood is the only readily available platelet-containing product in many veterinary practices. While there are few clinical indications for fresh whole blood, it may be appropriate in the management of dogs with anemia and bleeding due to thrombocytopenia or thrombopathia as described above. Generally, canine PRP and PC are prepared from fresh whole blood on an as-needed basis by a few veterinary blood banks. During the past decade there has been a trend in human blood banking toward increased use of plateletpheresis, with an estimated 75% of platelet transfusion products prepared from single donors by apheresis (selective removal of platelets from the donor's blood via an automated cell separator with return of RBCs and plasma to the donor) as opposed to platelets derived from a unit of whole blood.[3]

Whole Blood-Derived Platelets

In human blood banks in North America, the standard method for platelet component production from whole blood is the PRP-derived PC method, whereas in Europe the buffy coat-derived PC method is used.[4] In the former method, fresh whole blood is held at 22°C for up to 8 hours before 'soft spin' centrifugation ($2{,}000 \times g$ for 3 min) to produce PRP, which can then be leukoreduced through an integrated filter as the PRP is expressed off the RBC product.[4,5] The leukoreduced PRP then undergoes 'hard spin' centrifugation ($5{,}000 \times g$ for 5 min) to produce PC, with the potential to pool 4–6 PC units just before transfusion; single PCs are stored for up to 5 days, whereas pooled PCs may be stored for up to 4 hours.[4,5] In the buffy coat-derived PC method, butanediol plates within the storage containers rapidly cool and hold whole blood at 22°C for up to 24 hours before processing; the whole blood undergoes 'hard spin' centrifugation to concentrate 90–95% of the platelets along with the white blood cells (WBCs) in the buffy coat.[4] Typically 4–6 buffy coats are pooled, 'soft centrifuged' to remove contaminating WBCs and RBCs, and then passed through a leukoreduction filter.[4] The platelet yields are reported to be similar for both methods. The proposed benefits of the buffy coat-derived PC method include the convenience of storing whole blood for up to 1 day before processing, prestorage pooling of platelets, and greater plasma yield ($\sim$ 30–75 mL/U of whole blood).[4] As part of the quality control of whole blood-derived PC preparation, the American Association of Blood Banks requires that at least 90% of the units tested must contain $\geq 5.5 \times 10^{10}$ platelets and have a plasma pH ≥ 6.2 at the end of the allowable storage time.[5] In addition, pre-storage leukocyte-reduced platelets derived from filtration of PRP must contain $<8.3 \times 10^5$ residual leukocytes per unit to be labeled as leukocyte reduced.[5]

Similar to the human PRP-derived PC method, canine PRP is harvested from fresh whole blood following 'soft spin' centrifugation ($1{,}000 \times g$ for 4 min).[6] The supernatant PRP is then expressed into a satellite bag and may be centrifuged further using a 'hard spin' ($2{,}000 \times g$ for 10 min) to produce PC.[6] The supernatant (platelet-poor plasma) is then expressed into a second satellite bag, leaving 40–70 mL of plasma for resuspension of platelets.[6] In a study evaluating preparation of canine PCs from fresh whole blood using this method, the mean platelet yield was 8×10^{10} per PC unit, with 80% of the units containing $>5.5 \times 10^{10}$ platelets and 24% of the units having a platelet yield $>1 \times 10^{11}$.[6] The mean platelet yield from fresh whole blood to PC was 74%, indicating that approximately 25% of platelets in a unit of whole blood are lost during processing.[6] The leukocyte content of the PC units ranged from 1.0×10^8 to 2.3×10^9 WBCs, and the HCT ranged from 0.1% to 26.2%, with 62% of the units having a HCT > 1%.[6] In a subsequent study of canine PCs by the same group, the HCT of all PC units was reduced to <1% by stopping expression of PRP when the RBC-plasma interface was 1 cm from the top of the bag.[7]

Apheresis Platelets

An alternative approach to preparation of PC from fresh whole blood is plateletpheresis. During the plateletpheresis procedure, blood is removed from the donor, anticoagulated with citrate (typically, acid-citrate-dextrose formula A [ACD-A]) in the extracorporeal circuit, and separated into components by centrifugation, allowing production of a PC, while the other blood components are returned to the donor. The advantages of PC prepared by apheresis in comparison to PRP or PC prepared from a unit of fresh whole blood are greater platelet yield (typically $3–4.5 \times 10^{11}$ versus $<1 \times 10^{11}$) and negligible RBC and WBC contamination.[3] Transfusion of apheresis-derived platelets also decreases recipient exposure to donors, as pooling of products is not required.[3]

Plateletpheresis is generally well tolerated by human donors, with hypovolemia being an uncommon complication. The donor remains in a positive fluid balance throughout the course of the donation because of administration of priming solution (mixture of ACD-A and 0.9% sodium chloride) at the start of the procedure, the return of PRBCs and plasma throughout the procedure, and the use of 0.9% sodium chloride to return donor cells at the completion of the procedure.[8] However, citrate-related effects due to decreased levels of ionized calcium and magnesium occur commonly during plateletpheresis.[9,10] The anticoagulated blood being returned to the donor contains citrate, and the effect of the infused citrate on plasma levels of ionized calcium and magnesium depends on the rate of citrate infusion and its rate of distribution into extracellular fluids, rate of diffusion into cells, rate of renal excretion, and rate of intracellular metabolism (primarily in mitochondria of liver, kidney, and muscle).[11] Symptoms of citrate-induced hypocalcemia reported in humans are typically mild and include paresthesias, chills, headache, lightheadedness, carpopedal spasm, nausea, vomiting, chest tightness, and cramping.[9,12–14] It has been postulated that hypocalcemia causes paresthesias by lowering the threshold for cell membrane depolarization.[15] Because hypomagnesemia exerts a similar effect on the cell membrane, citrate-induced reductions in serum ionized magnesium may also contribute to these clinical signs.[15] During a 90-minute plateletpheresis procedure performed on 7 healthy human donors at a continuous, fixed citrate infusion rate of 1.6 mg/kg/min, ionized calcium and magnesium decreased by 33% and 39%, respectively, below baseline.[9] Serum citrate levels increased continuously during plateletpheresis (final mean serum citrate concentration 1.87 mmol/L), and there was a strong negative correlation between ionized calcium and magnesium and increasing serum citrate levels.[9] In addition, 33% of donors experienced muscle cramps and paresthesias.[9] Oral calcium supplementation (2 g calcium carbonate given 30 min before the procedure) has been evaluated in adult donors undergoing plateletpheresis and was found to exert minimal effects on the development of citrate-related symptoms and a modest effect on ionized calcium concentration, with the end procedure ionized calcium decreased by 32.3% after placebo compared with 29.6% after prophylactic oral calcium supplementation.[13] Calcium prophylaxis is, therefore, not routinely recommended for plateletpheresis in human donors unless there is a prior history of clinically significant citrate-related effects or a high risk of such effects (eg, female donors receiving high citrate infusion rates).[13]

Automated blood cell separators that have been used for canine plateletpheresis include the AS104,[a,16] MCS Plus,[b,c] and COBE Spectra.[d,17] A recent study evaluated the clinical and clinicopathologic effects of plateletpheresis on 14 healthy donor dogs weighing approximately 20 kg using the COBE Spectra (dual needle, leukocyte reduction system).[17] The disposable tubing set had a total volume of 272 mL and was primed with 0.9% sodium chloride and ACD-A, resulting in a calculated mean donor whole blood volume of 131 mL (based on donor HCT of 40%) in the disposable tubing set during the run; this blood volume represents approximately 7% of a 20 kg dog's total blood volume (total blood volume estimated at 90 mL/kg). A high-quality PC was collected from all dogs, with a mean

total platelet yield of 3.3×10^{11} platelets in a mean collect volume of 246 mL, similar to the yield and volume collected from adult human donors. The mean donor platelet count decreased by 55% from 356,000 to 159,000/μL 2 hours after apheresis but returned to baseline level by day 6. The plateletpheresis procedure was generally well tolerated with no evidence of hypotension. In order to obtain the target platelet yield within 90–120 minutes (mean run time, 105 min), the mean citrate infusion rate was 2.5 mg/kg/min, considerably higher than the rate of 1.6 mg/kg/min as described above for human plateletpheresis donors. Serum citrate concentration progressively increased during the plateletpheresis procedure (final mean serum citrate concentration 3.76 mmol/L, double the concentration noted in adult humans undergoing plateletpheresis as indicated above), causing the ionized magnesium concentration to decrease by 45% and ionized calcium to decrease to <1 mmol/L (mean baseline, 1.2 mmol/L) in 10 of 14 dogs. The decrease in ionized calcium concentration was noted despite the dogs receiving 0.9 mg of calcium ion per mL of ACD-A (mean total amount of IV 10% calcium gluconate, 1.7 mL/kg). Clinical signs of hypocalcemia were noted in 3 dogs, all of which exhibited intermittent lip licking and agitation. Generalized tremors and sporadic ventricular ectopy were also noted in the dog with the lowest ionized calcium concentration (0.76 mmol/L at 120 min into the procedure). During a pilot plateletpheresis procedure performed on a dog without calcium supplementation, the dog's ionized calcium concentration decreased from 1.2 mmol/L at baseline to 0.72 mmol/L within 30 minutes, and the dog experienced marked agitation and hypertension.[17] Thereafter, all dogs received calcium supplementation. While plateletpheresis is a feasible option for production of canine PC, citrate-induced hypocalcemia is a potential serious adverse effect. Based on the high serum citrate concentrations reached during plateletpheresis in all 14 dogs and the decrease in iCa to <1 mmol/L in 10 of 14 dogs despite aggressive calcium supplementation, prophylactic calcium administration is warranted to limit clinical signs of hypocalcemia when plateletpheresis is performed in dogs using high citrate infusion rates.[17]

Storage of Fresh Platelets

Fresh platelet products have a short shelf-life. Fresh whole blood may be stored at room temperature (22°C) for up to 8 hours, whereas PRP and PC may be stored at 22°C with continuous gentle agitation for up to 5 days.[4,5] The time limitation on storage of platelets at 22°C is primarily related to concerns about the potential for rapid proliferation of contaminating bacteria at this temperature and a progressive decrease in platelet viability and function over time.[5] During storage, platelets undergo a variety of in vitro changes which are collectively referred to as the 'platelet storage lesion.' This lesion is characterized by a change in platelet shape from discoid to spherical, the generation of lactate from glycolysis with an associated decrease in pH, the release of granule contents, a decrease in various measurements of in vitro platelet function, and reduction in posttransfusion platelet recovery (percentage of transfused platelets that survive in the recipient) and survival (lifespan of transfused platelets).[5] There has been much interest in both human and veterinary transfusion medicine to develop alternative storage options that would increase the shelf-life and availability of platelet products in clinical practice.

Chilled Platelets

Refrigeration of PC could reduce the problem associated with proliferation of contaminating bacteria at room temperature. However, as early as 1969, there was strong evidence that chilled storage of human platelets had a deleterious effect on platelet viability.[18] Storage of human PRP at 4°C for as little as 18 hours with subsequent infusion into the same donor resulted in a markedly reduced platelet survival with a half-life of 1.3 days in comparison to 3.9 days for platelets stored at 22°C for 18 hours.[18] Following this discovery, it became standard procedure for human PRP to be stored at 22°C. The mechanism responsible for rapid clearance from the circulation of refrigerated platelets has been recently elucidated. Chilling of human and mouse platelets clusters their von Willebrand factor (vWF) receptors ($GPIb_{\alpha\beta}$-IX complex) on the platelet surface. The integrin receptor $\alpha_M\beta_2$ (complement receptor type 3 [CR3]) of hepatic macrophages recognizes clustered GPIbα, and the macrophages ingest the platelets.[19] The rapid clearance of refrigerated and rewarmed platelets from the circulation of CR3-expressing but not CR3-deficient mice supports the role of the CR3 receptor in the phagocytosis of chilled platelets.[19] Chilled platelets bind vWF and function normally in vitro, suggesting that the hemostatic and cold-induced clearance functions of the GPIb complex are separable.[19] It has been demonstrated that clustering of GPIbα on cooled platelets results in recognition of exposed β-*N*-acetylglucosamine residues of N-linked glycans by the CR3 receptors.[20] The interaction between GPIbα and hepatic macrophages can be blocked by galactosylation of exposed β-*N*-acetylglucosamine residues.[20] Galactosylation of human and murine platelets does not impair their in vitro function and restores survival of short-term chilled platelets in the mouse.[20]

Studies testing the feasibility of galactosylation of human platelets under standard blood bank conditions and assessing in vitro functions of refrigerated human platelets following long-term storage were encouraging.[21] Platelets were successfully galactosylated following injection of uridine diphosphate-galactose into PCs.[21] Platelets galactosylated and refrigerated for 14 days maintained their ability to aggregate when exposed to agonists in a standard aggregometry assay, showed less pronounced changes in surface expression of GPIbα compared with room temperature-stored platelets, and were poorly phagocytized by differentiated human monocytic THP-1 cells (an in vitro model for refrigerated platelet clearance).[21] Overall, these refrigerated human platelets retained in vitro function better than platelets stored at 22°C.[21] However, results of a phase 1 clinical trial in humans evaluating posttransfusion survival of radiolabeled autologous apheresis platelets stored for 48 hours at 4°C with and without pretreatment with uridine diphosphate-galactose indicated that galactosylation did not prevent the accelerated platelet clearance routinely observed after 4°C storage (mean platelet survival 2.2 and 2.9 d, respectively, compared with mean survival of 6.9 d for fresh platelets).[22]

To date, there are no published reports on in vivo survival of chilled canine platelets. In a study of storage of canine PCs at 22° and 4°C, it was noted that platelets stored at 22°C lost their ability to aggregate in vitro in response to ADP and collagen after 4 days, whereas platelets stored at 4°C did not lose their ability to aggregate before 8 days of storage.[23] Similar loss of platelet function as assessed by the resonance thrombogram was noted over the same time frame.[23] Consequently, it was concluded that canine PC may be stored for 4 days at 22°C or 8–10 days at 4°C.[23] However, in light of the effect of chilling on survival of both murine and human platelets as noted above, refrigerated storage of canine PC cannot be recommended at this time without studies documenting adequate posttransfusion survival. Given potential species variability in survival of chilled platelets (with or without galactosylation or other modification), further investigation of cold storage of canine platelets may be warranted.

Cryopreserved Platelets

During the past several decades, platelet cryopreservation has been extensively investigated as a means to provide long-term storage and immediate availability of platelet products for transfusion.[24–36] The American Association of Blood Banks has approved cryopreservation of human platelets in 6% DMSO as an acceptable storage procedure,[26] but platelet cryopreservation is largely considered a research technique. Current conditions for storage of human PCs for transfusion remain at 22°C under continuous gentle agitation.[5] Based on the numerous studies evaluating cryopreservation of human platelets, it is clear that regardless of the methodology, cryopreserved platelets inevitably demonstrate impaired in vitro function and reduced posttransfusion recovery in comparison to fresh platelets.[24–26,31,33] However, there is clinical evidence that cryopreserved human platelets are hemostatically effective in vivo.[33,34] Given that human platelets cryopreserved in 6% DMSO must be washed before transfusion to remove the DMSO, there has been interest in developing alternative cryopreservation solutions with lower concentrations of DMSO that could eliminate the washing step, which results in further loss and damage of thawed platelets.[25] More recent studies have shown that cryopreservation of human platelets with 2% DMSO plus Thrombosol (a mixture of amiloride, adenosine, and sodium nitroprusside, second-messenger effectors that inhibit platelet activation) resulted in a significant improvement in in vitro function and posttransfusion platelet recovery (40.2% versus 28.8%) in comparison to 6% DMSO alone.[25,26]

Some of the early studies evaluating platelet cryopreservation were performed using dogs as an experimental model. More than 20 years ago, Valeri demonstrated that autologous canine platelets cryopreserved in 6% DMSO and stored at −80°C for up to 1 year had an in vitro recovery of 70% and an in vivo survival 1–2 hours posttransfusion 40% that of fresh platelets.[28] The half-life of the cryopreserved platelets was 2 days, in comparison to 3.5 days for fresh platelets.[28] In addition, autologous cryopreserved platelets administered to lethally irradiated thrombocytopenic dogs were reported to be hemostatically effective, resulting in a reduction in clinical bleeding (GI bleeding, ecchymoses, oozing from venipuncture sites).[28] Valeri et al[28] concluded that canine platelets could be 'satisfactorily preserved' in 6% DMSO in plasma with storage at −80°C for 1 year. A subsequent study evaluating in vitro function of canine platelets cryopreserved in 6% DMSO for 6 months demonstrated marked impairment in platelet aggregation and response to hypotonic shock, leading the investigators to question how well these platelets would have performed in vivo.[7] A more recent study evaluating a commercially available cryopreserved canine PC demonstrated decreased aggregation in response to thrombin and increased premature platelet activation associated with the addition of DMSO or the freeze-thaw process.[35] However, it has been documented that human platelets with poor aggregation responses in vitro may still have good function and viability in vivo.[37]

In light of the improved in vitro platelet function and posttransfusion platelet recovery noted with cryopreservation of human platelets in 2% DMSO plus Thrombosol in comparison to 6% DMSO alone, a study of allogeneic canine platelet transfusions was undertaken to compare these 2 methods for cryopreservation of canine platelets.[36] PCs collected via apheresis from 10 healthy adult mixed breed dogs were each split into 3 units: fresh and cryopreserved in 6% DMSO and 2% DMSO plus Thrombosol. Cryopreserved units were evaluated 1–10 weeks post-freezing, and platelets were not washed before transfusion. Both convulxin- and γ-thrombin-induced platelet aggregation were markedly diminished (<15% increase in light transmittance) in both cryopreserved PCs in comparison to fresh platelets (>60% increase in light transmittance). Mean baseline P-selectin expression was <1% for fresh and cryopreserved platelets but increased in response to thrombin stimulation; thrombin-induced P-selectin expression for both cryopreserved PCs (~ 20%) was significantly less for fresh PC (49%). The mean maximum in vivo platelet recovery for fresh PC was 80%, significantly greater than platelet recovery for 6% DMSO (49%) and 2% DMSO plus Thrombosol PC (44%). The mean half-life of fresh PC (3.8 d) was significantly greater than that of 6% DMSO (1.9 d) and 2% DMSO plus Thrombosol (2.4 d) PC, with no difference between cryopreserved PC. This study did not identify any appreciable benefit of 2% DMSO plus Thrombosol in comparison to 6% DMSO for maintaining in vitro function or prolonging in vivo survival of cryopreserved canine platelets but documented that cryopreserved platelets can be activated, as demonstrated by thrombin-induced P-selectin expression, and survive in the circulation long enough to potentially be of benefit in the management of life-threatening hemorrhage in severely thrombocytopenic or thrombopathic patients. However, further studies are needed to assess in vivo function of cryopreserved platelets.

Canine platelets cryopreserved in 6% DMSO are commercially available through 2 veterinary blood banks, Midwest Animal Blood Services (http://www.midwestabs.com) and Sun States: Blood Banks for Animals (http://www.sunstates.org). Cryopreserved PCs from the former are prepared from apheresis platelets and are reported to contain 1×10^{11} platelets/U while cryopreserved PCs from the latter are derived from whole blood with the platelet yield not specified. Both blood banks recommend storage for up to 6 months at −20°C or below and administration without post-thaw washing to remove DMSO.

Lyophilized Platelets

Due to the time limitation on storage of fresh PC and the cumbersome processes of platelet cryopreservation and washing platelets to remove DMSO before transfusion, there has been considerable interest in the development of lyophilized human platelets. Efforts to freeze-dry or lyophilize platelets for long-term storage and ease of availability for transfusion began more than 50 years ago. The process has been continually refined since that time with the end result being a lyophilized human platelet product (StasiX)[e] that is reported to be nearing phase I clinical trials.[38] After decades of research, it became evident that fixation of platelets by a cross-linking agent rendered platelets able to withstand the stresses of dehydration and rehydration. The platelet lyophilization method perfected primarily by investigators at the University of North Carolina at Chapel Hill and East Carolina University includes washing of platelets (PRP) in a phosphate washing buffer containing 0.1% bovine serum albumin, resuspending, incubating washed platelets for 1 hour in a buffer solution containing paraformaldehyde at concentrations of 1.8% for human platelets and 0.68% for canine platelets, washing platelets with phosphate washing buffer to remove paraformaldehyde, resuspending platelets at a concentration of $\sim 1 \times 10^9$/mL in phosphate washing buffer containing 5% bovine serum albumin as a bulking agent, freezing aliquots at −70°C, and finally drying in a lyophilizer over 1–3 days.[39–41] Dried platelets are stored at −80°C and resuspended in sterile saline just before infusion.[41]

During the past 13 years there have been extensive in vitro studies of rehydrated lyophilized platelets. Transmission electron microscopy showed that rehydrated lyophilized platelets are morphologically similar to fresh washed platelets, with intact and randomly distributed organelles and some pseudopod formation.[39] Flow cytometry using an anti-GPIb monoclonal antibody indicated that fresh and rehydrated lyophilized platelets have similar surface densities of GPIb.[39,42] In addition, ristocetin (an antibiotic that interacts with the platelet GPIb receptor to induce an active conformation for vWF binding)-mediated platelet agglutination was similar for rehydrated lyophilized and fresh platelets, indicating that rehydrated lyophilized platelets retain native vWF-mediated adhesion function.[42] Using the Baumgartner perfusion technique, it was shown that human rehydrated lyophilized platelets adhered to the thrombogenic surface of denuded arterial vessel segments exposed to high shear forces, although to a lesser extent than fresh platelets (39% versus 73%, respectively, for percent vessel coverage) and, as importantly, did not adhere to areas of intact endothelium.[40] Fischer determined that rehydrated lyophilized platelets retain some of the hemostatic stimulus-response function of fresh platelets by demonstrating that thrombin stimulation resulted in enhanced phosphorylation of several

proteins, including the protein kinase C substrate pleckstrin, and, therefore, do more than simply serve as a thrombogenic membrane.[43] In addition, it was noted that rehydrated lyophilized platelets both degranulated and increased surface membrane thrombogenicity in an activation-dependent manner, providing evidence for intracellular signaling and positive feedback.[43]

The role of rehydrated lyophilized platelets in thrombus formation and lysis has been studied in detail.[44] In evaluating the interaction between rehydrated lyophilized platelets and fibrinogen, the surface density of unligated, activated GPIIb/IIIa (as detected with the monoclonal antibody 10E − 5) on rehydrated lyophilized platelets was approximately half that of fresh platelets.[42,44] Similarly, the ability of activated rehydrated lyophilized platelets to bind ^{125}I-fibrinogen was approximately 40% that of fresh platelets.[42,44] Interestingly, evaluation of fibrinogen on the surface of unactivated rehydrated lyophilized platelets indicated that the lyophilization/rehydration process was associated with an approximately 10-fold increase in the number of fibrinogen molecules bound to the surface of the platelets with the fibrinogen most likely originating from alpha granule sources after processing.[44] In evaluating the ability of rehydrated lyophilized platelets to catalyze and amplify the clot formation process in an activation-dependent manner, it was demonstrated that rehydrated lyophilized platelets degranulated in fibrin clots as demonstrated by transmission electron microscopy and functioned as thrombogenic surfaces for the generation of activated coagulation factors and fibrin generation, with near complete conversion of prothrombin to thrombin noted at 7.5 minutes with fresh platelets activated with collagen and at 8 minutes with rehydrated lyophilized platelets stimulated by a mixture of collagen and thrombin.[44] Finally, given concerns about resistance to clot lysis leading to ischemic events if a thrombus is occlusive, it was determined that rehydrated lyophilized platelet clots were lysed in the presence of tissue plasminogen activator with a similar time course as clots without platelets and faster than when fresh platelets were included in the fibrin mass.[44]

There are far fewer reports on the hemostatic efficacy of rehydrated lyophilized platelets in animal models of bleeding. Initial studies were performed in experimentally induced thrombocytopenia in 2 rats that had been treated with platelet antisera to produce platelet counts $<33 \times 10^9$/L and toenail bleeding times >15 minutes (reference range 0.5–3 min).[39] Infusion of rehydrated lyophilized human platelets (total number of platelets infused, 4.5×10^9 and 3.4×10^{10}, or approx 9×10^9 and 6.8×10^{10}/kg for an average 500 g adult male rat) into the tail vein of the thrombocytopenic rats resulted in normalization of the toenail bleeding time (0.5 and 1.5 min).[39] Similarly, in a thrombocytopenic rabbit bleeding time model, rabbits were subjected to γ-irradiation and administered heterologous platelet antisera, typically resulting in platelet counts $<10 \times 10^9$/L and microvascular ear bleeding times >900 seconds (reference value, 77 ± 14 s).[45] The mean bleeding time of the thrombocytopenic rabbits ($n = 11$) was reduced to 252 seconds 1 hour following administration of rehydrated lyophilized human platelets, not statistically different from the mean bleeding time of 203 seconds in the control group infused with an equivalent dose of fresh human platelets.[46,f] In a review paper, there is mention of infusion of rehydrated lyophilized porcine platelets to a pig in a von Willebrand disease (vWD) colony that was apparently successful in controlling exsanguinating bleeding following injury, although the data have not been published.[38] In a swine model of hypovolemic shock from controlled exsanguination combined with impaired hemostasis from hemodilution as a result of blood exchange using a hemoglobin-based oxygen carrier, administration of rehydrated lyophilized porcine platelets to pigs as their HCTs neared zero resulted in correction of the bleeding time and arrested continuing hemorrhage from previous bleeding time tests.[38,g] It should be noted that completed manuscripts of the rabbit and swine studies have not yet been published, despite research abstracts being presented in 1994 and 2001, respectively.[f,g]

Initial studies of administration of fluorescence-labeled rehydrated lyophilized canine platelets to 3 normal dogs and 1 dog with von Willebrand disease treated with cryoprecipitate confirmed the incorporation of rehydrated lyophilized platelets into the hemostatic plug at sites of wounds from bleeding time tests.[39] In addition, circulating fluorescent rehydrated lyophilized canine platelets participated in carotid arterial thrombus formation induced in 3 normal dogs and were adherent to exposed subendothelium.[39] In a canine model of thrombocytopenia and platelet dysfunction induced by cardiopulmonary bypass (CPB), rehydrated lyophilized canine platelets were infused in a single large bolus dose into splenectomized dogs after 2 hours of perfusion on CPB, resulting in a consistent and persistent lowering of the venous bleeding times (time required for cessation of bleeding from puncture of an exposed jugular vein with a 2-Ga needle).[41] In 3 dogs (body weight, 18–23 kg) that received rehydrated lyophilized canine platelets alone (ie, dogs did not receive epsilon aminocaproic acid, platelet-poor plasma, or RBCs), the dogs' platelet counts after 2 hours of CPB ranged from 37 to 49×10^9/L, HCTs 15–17%, and venous bleeding times 6.7–11 minutes (pre-CPB bleeding times, 0.5–1.8 min). Infusion of $1.5–2.9 \times 10^{11}$

platelets to each dog over 2 minutes resulted in platelet counts ranging from 82 to 124 $\times$ 10^9/L at 1–20 minutes post-infusion and venous bleeding times of 1.8–4.3 minutes at 20–30 minutes post-infusion.[41] In comparison, for 2 control dogs undergoing CPB that did not receive platelet transfusions (1 received platelet-poor plasma), post-CPB platelet counts were $\leq$24 $\times$ 10^9/L, HCTs 15% and 17%, and venous bleeding times >12 minutes.[41] It was noted that measurement of the venous bleeding time in the first 5–10 minutes after infusion of rehydrated lyophilized canine platelets did not show the full effect of correction of the bleeding time, with the bleeding time nadir typically noted at 20–30 minutes post-infusion.[41] No signs of acute toxic side effects or untoward consequences of the rehydrated lyophilized platelet infusions were reported.[41] However, given that there is a crosslinking step in the preparation of lyophilized platelets, generation of antiplatelet antibodies is a potential concern and should be evaluated in future studies. Furthermore, because the dogs in the CPB study were splenectomized and splenic clearance of rehydrated lyophilized platelets may occur and affect in vivo efficacy of rehydrated lyophilized platelets, additional data are needed to evaluate rehydrated lyophilized platelets in dogs with normal splenic function.

The in vivo recovery and lifespan of rehydrated lyophilized canine platelets in the circulation were not addressed in the canine CPB study or any other published reports thus far, but studies evaluating the clearance of rehydrated lyophilized platelets in rats and baboons indicate the in vivo circulation time in those species is minutes rather than hours.[47,48] In studies of rats infused with the equivalent of 10% of their initial total number of endogenous platelets with fluorescent-labeled rehydrated lyophilized rat platelets, the lyophilized platelets were cleared from the circulation with an approximately exponential time course (after an initial rapid drop) with a $\tau(1/k)$ value of 9.5 minutes; for comparison, labeled fresh control platelets yielded τ values over 24 hours.[47] When rats were administered gadolinium (10 mg/kg, IV) 24 hours before the infusion of rehydrated lyophilized platelets to induce macrophage apoptosis, the circulation of rehydrated lyophilized platelets was prolonged, with a τ value of 32.3 minutes.[47] Transmission electron microscopy analysis of splenic tissue after infusion of rehydrated lyophilized platelets, in vitro mixing studies of splenic macrophages with rehydrated lyophilized platelets, and demonstration of a prolonged circulation time of rehydrated lyophilized platelets following administration of IV IgG indicated that rehydrated lyophilized platelets were cleared from the circulation through phagocytosis by splenic macrophages, at least in part mediated by platelet surface bound IgG.[47] In a baboon model evaluating survival times of both radiolabeled (^{111}In) and biotinylated autologous rehydrated lyophilized platelets, the in vivo circulation times were <15 minutes (resulting in in vivo platelet recoveries at 1–2 h post-transfusion of 0%), regardless of labeling procedure. Autologous labeled fresh baboon platelets had in vivo recoveries at 1–2 hours posttransfusion of 80% (^{111}In) and 73% (biotin) and a linear lifespan of 7 days.[48]

Lyophilized platelets offer a few potential advantages over fresh PC: a storage time of several years, ease of storage and transport, and the possibility of true sterility (the paraformaldehyde stabilization step and washing reportedly eliminate all test viruses and bacterial inoculations in the starting material).[46] However, the short lifespan (min) of rehydrated lyophilized platelets in the species reported thus far may be a serious limiting factor in their clinical use. The main investigators involved in the development of rehydrated lyophilized human platelets have emphasized that their goal has been to develop a stabilized material that is safe and efficacious for *arresting active hemorrhage* in patients with thrombocytopenia or platelet dysfunction.[46] Although rehydrated lyophilized platelets have not yet been evaluated in animal models of trauma-induced massive transfusion, if shown to be effective, rehydrated lyophilized platelets could be useful in early and aggressive trauma resuscitation preceding a severely thrombocytopenic state. Due to their short lifespan post-transfusion, rehydrated lyophilized platelets are unlikely to replace fresh platelets in the prevention of bleeding (eg, presurgical administration to a dog with a hereditary thrombopathia and known bleeding tendency). In addition, administration of rehydrated lyophilized platelets to a thrombocytopenic patient would not be expected to result in a sustained increase in platelet count, even for 1–2 hours post-transfusion. Administration of rehydrated lyophilized canine platelets[h] to dogs with naturally occurring thrombocytopenia and active bleeding is currently being evaluated in a multicenter study.

In addition to paraformaldehyde-fixed lyophilized platelets described above, trehalose-loaded lyophilized rehydrated platelets prepared from several species (human, porcine, murine, and equine) have undergone in vitro testing.[49–54] Trehalose, a sugar, enters the cytosol of platelets through endocytic pathways and exerts a lyoprotectant effect.[49,50] Trehalose-loaded lyophilized rehydrated human platelets have been shown to have excellent in vitro recovery (>85%), shelf-life of at least 6 months at room temperature, agonist-induced aggregation comparable to fresh platelets, and the ability to regulate intracellular pH.[49–51] The authors are not aware of published reports evaluating transfusion of trehalose-loaded lyophilized rehydrated platelets in animal models.

Platelet Transfusion Guidelines

Platelet transfusions, both prophylactic (to prevent bleeding) and therapeutic (to control bleeding), play a major role in the management of human patients with thrombocytopenia.[55] Guidelines regarding the platelet count at which it is appropriate to administer platelet transfusions (ie, platelet transfusion trigger) to thrombocytopenic patients and the appropriate number of platelets to give per transfusion are continually being reevaluated in human medicine to identify the most effective strategies for providing safe and efficacious platelet support.[55] Randomized prospective platelet transfusion trials have demonstrated that prophylactic administration of platelets at a platelet threshold of 10×10^9/L was as effective as the traditional threshold of 20×10^9/L based on hemorrhagic morbidity and mortality.[56–58] In a study evaluating loss of radiolabeled RBCs in the stool of thrombocytopenic patients transfused prophylactically at platelet counts of 5, 10, and 20×10^9/L, there was no difference in stool blood loss among the 3 groups, suggesting that it may be safe to lower the platelet transfusion threshold to 5×10^9/L.[55]

Given that the lifespan of human platelets is 9–10 days, it has been estimated that 10–15% of all platelets are removed from the circulation daily in normal individuals. The majority (70–80%) are lost by senescence and the remaining platelets are used in the maintenance of vascular integrity with a calculated requirement for 7.1×10^9 platelets/L/d.[55,59] Administration of ^{51}Cr-labeled autologous and allogeneic platelets to thrombocytopenic patients with bone marrow hypoplasia demonstrated an inverse relationship between platelet survival and recipient platelet count when $<100 \times 10^9$/L with a marked reduction in platelet survival (6.0 d for autologous platelets and 3.4 d for allogeneic platelets, compared with 9.6 d for controls) noted in patients having platelet counts $<50 \times 10^9$/L.[59] The reduced platelet lifespan in these steady-state thrombocytopenic patients was attributed to the fixed requirement of 7.1×10^9/platelets/L/d representing an increasing fraction of circulating platelets as the recipient's platelet count decreased.[55,59] However, in thrombocytopenic human patients there are several other clinically important factors that contribute to platelet loss including splenomegaly, neoplasia, veno-occlusive disease, fever, infection, septicemia, HLA immunization, drugs, and surgical procedures.[55,60] Therefore, patients may require more than their calculated platelet dose to meet their platelet requirements.[55]

Common measures of response to platelet transfusions include the corrected count increment and percent platelet recovery, both calculated to take into account the blood volume of the patient and the number of platelets transfused in contrast to the platelet increment (posttransfusion-pretransfusion platelet count/μL).[61] The corrected count increment is defined as ([platelet increment] × body surface area [m^2]) ÷ (number of platelets transfused $\times 10^{-11}$).[61] The percent platelet recovery is defined as ([platelet increment] × 10^{-6} × blood volume [mL]) ÷ (number of platelets transfused $\times 10^{-11}$).[61]

The optimal platelet transfusion dose for thrombocytopenic human patients remains controversial. In a prospective, randomized, double-blind trial evaluating the effects of paired prophylactic transfusions of lower dose PC (mean 3.1×10^{11} platelets) and higher dose PC (mean 5.0×10^{11} platelets) administered to 46 patients undergoing hematopoietic progenitor cell transplantation (total of 158 platelet transfusions), the platelet increment was 17.01×10^9/L for the lower dose PC and 31.06×10^9/L for the higher dose PC.[62] In addition, the mean transfusion-free interval with lower dose PC was 2.16 versus 3.03 days for the higher dose PC, and administration of lower dose PC was associated with a 39–82% increase in the relative risk (per day) of requiring subsequent platelet transfusions.[62] Another prospective study evaluating transfusion of even higher platelet doses (medium dose, $4–6 \times 10^{11}$ platelets; high dose, $6–8 \times 10^{11}$ platelets; very high dose, $>8 \times 10^{11}$ platelets) also demonstrated a higher platelet increment and longer transfusion interval with an increasing dose of platelets (transfusion intervals of 2.6 d for medium dose, 3.3 d for high dose, and 4.1 d for very high dose).[63] Based on results of these studies, maintenance of a higher platelet count for a greater length of time with high-dose platelet therapy could be interpreted as providing improved hemostasis in comparison to low-dose platelet therapy.[55] However, this notion may be challenged by the lack of difference in hemorrhagic morbidity and mortality when comparing prophylactic transfusions at threshold platelet concentrations of 10 and 20×10^9/L, as noted above.[55] The Platelet Dose Trial, a multicenter study supported by the Transfusion Medicine/Hemostasis Clinical Trials Network funded by the National Heart, Lung, and Blood Institute, was initiated in 2006 to evaluate the safety of low-dose platelet transfusions and to determine the most cost-effective approach to prophylactic platelet therapy in patients with thrombocytopenia (platelet counts $\leq 10 \times 10^9$/L) related to stem cell transplants or chemotherapy.[55,64] Three platelet dose groups were evaluated: lower dose (1.1×10^{11} platelets/m^2), medium dose (2.2×10^{11} platelets/m^2), and higher dose (4.4×10^{11} platelets/m^2).[55,64] The study was completed in January 2008, with 1,351 patients enrolled,[64] but results have not yet been published.

With regard to human trauma patients undergoing massive transfusion, the typical recommendation has been a 1:3 ratio of plasma to RBCs, with little consensus on recommendation for platelet transfusions.[1] In 2 retrospective studies of massively transfused human trauma patients, 30-day survival was increased in patients with high plasma:RBC ratio ($\geq$1:2 or $>$2:3) relative to those with low plasma:RBC ratio ($<$1:2 or $<$2:3).[1,2] Similarly, 30-day survival was increased in patients with high platelet:RBC ratio ($\geq$1:2 or $\geq$1:5) relative to those with low platelet:RBC ratio ($<$1:2 or $<$1:5).[1,2] In light of these findings, some human trauma specialists advocate massive transfusion practice guidelines that aim for a 1:1:1 ratio of plasma:platelets:RBCs.[1] However, many human trauma specialists consider these studies insufficient to change practice. Additional data are required to determine the benefits and risks of this more aggressive transfusion strategy, particularly in a civilian (rather than military) population.

In contrast to the wealth of information regarding platelet transfusion therapy for humans, there have been no studies evaluating prophylactic platelet transfusions or optimal platelet doses to control active bleeding due to thrombocytopenia (or thrombopathia) in dogs. At this time, the majority of platelet transfusions administered to dogs are therapeutic rather than prophylactic. The number of platelets administered to a dog is frequently determined by the availability of blood components. One unit of whole blood-derived PRP or PC ($\sim 8 \times 10^{10}$ platelets) per 10 kg body weight has been suggested as an appropriate platelet dose for a thrombocytopenic dog, which would result in a maximum platelet increment of 40×10^9/L.[65] Alternatively, fresh whole blood may be administered at a dose of 10 mL/kg to raise the recipient's platelet count by a maximum of 10×10^9/L.[65] If administering cryopreserved canine platelets, clinicians should consider that the thawed unit will contain fewer platelets than indicated on the label due to losses associated with the freeze-thaw process ($\sim$ 30% loss).[28,35,36] In addition, due to the lower in vivo recovery of cryopreserved canine platelets ($\sim$ 45%) versus fresh canine platelets ($\sim$ 80%),[28,36] the total number of transfused platelets required to reach a desired platelet increment will be substantially greater for cryopreserved than fresh platelets. Taking into account the lower in vitro and in vivo recoveries of cryopreserved PCs, it has been estimated that approximately 2.5 units of cryopreserved PCs must be given to achieve a comparable increase in platelet count as for a single unit of fresh PC in thrombocytopenic human patients.[26] Potential efficacy of platelet transfusions in thrombocytopenic dogs should be assessed by obtaining platelet counts at 1 hour and 24 hours post transfusion and monitoring for cessation of bleeding.

In veterinary medicine, PRBCs tend to be more readily available than PC and may be beneficial in improving hemostasis in anemic thrombocytopenic patients. A correlation has been reported between anemia and a prolonged bleeding time; when the anemia is corrected, the bleeding time is reduced.[66] Proposed mechanisms for the role of RBCs in hemostasis include: dispersion of platelets from the center of the blood vessel toward the vessel wall, increasing contact of platelets with endothelial cells; release of ADP, leading to activation of platelets; scavenging of endothelial cell nitric oxide (which inhibits platelet function); and increased production of thromboxane by platelets at the bleeding site.[66] In a study comparing bleeding times of healthy humans undergoing 2-unit RBC apheresis to induce a 15% reduction in peripheral venous HCT to those undergoing plateletpheresis to induce a 32% decrease in platelet count (no change in HCT), it was noted that there was a 60% increase in bleeding time associated with reduction in HCT, while there was no change in bleeding time associated with the reduction in platelet count.[67] It has been suggested that anemic thrombocytopenic human patients be transfused with PRBCs to increase the HCT to 35% to correct the platelet dysfunction associated with anemia before the transfusion of platelets.[66]

Platelet Transfusion Refractoriness

Platelet transfusion refractoriness, a failure to achieve an expected increment in platelet count in response to a platelet transfusion, has been defined in several ways, including 2 sequential 1-hour posttransfusion platelet corrected count increments of $<5 \times 10^9$/L platelets/m^2 body surface area and 2 sequential 1-hour posttransfusion platelet increments of $<11 \times 10^9$/ L platelets.[60,68] Platelet refractoriness may develop because of immune or nonimmune reasons, the latter of which includes splenomegaly, bleeding, fever, and infection.[60] It has been estimated that 25–70% of multi-transfused thrombocytopenic human patients, particularly those being treated for malignant hematopoietic disorders, develop platelet transfusion refractoriness.[5] Alloimmune platelet refractoriness is most often the result of HLA sensitization, although antibodies to ABO antigens (shared by RBCs and platelets) and platelet-specific antigens can also cause platelet refractoriness.[5]

Dogs have served as a platelet transfusion model in the evaluation of methods of preventing alloimmunization in human patients requiring long-term platelet therapy because of bone marrow hypoplasia.[69,70] Weekly transfusion of PCs from single unrelated canine donors resulted in development of alloimmune platelet refractoriness in 95% (20 of 21) of recipients

after an average of 3 transfusions.[70] When DLA nonidentical littermates were used as platelet donors, the percentage of recipient dogs developing platelet refractoriness was reduced to 31% (4 of 13), and the average number of transfusions required for platelet alloimmunization increased to 7.3 transfusions.[70] Use of DLA identical littermates as platelet donors resulted in a similar rate of platelet refractoriness (31%, or 4 of 12 dogs) as with DLA nonidentical littermates, but the average number of transfusions administered before development of platelet refractoriness increased to 14 transfusions.[70] When dogs were administered platelets from either 6 random single donors given sequentially (ie, when recipient became refractory to platelets from a donor, the next donor was given) or pooled platelets from the same 6 random donors, the percentage of refractory recipients was similar (60% for sequential single unrelated random donors and 77% for pooled unrelated random donors).[70] However, the number of transfusions to refractoriness was greater for the sequential single unrelated random donors (mean, 14 transfusions) than the pooled unrelated random donors (mean, 5.5 transfusions).[70] Results of these studies provided support for the practice in human transfusion medicine of administering either pooled random donor or single random donor apheresis platelets as initial therapy until refractoriness develops with HLA-matched platelets administered after that point to maintain effective platelet support.[70]

Two currently accepted methods for preventing the development of lymphocytotoxic antibodies and platelet refractoriness due to alloimmunization include leukocyte reduction and ultraviolet B irradiation, both of which were initially evaluated in the dog.[68,69,71] Leukocyte reduction and ultraviolet B irradiation remove and inactivate, respectively, the donor's antigen presenting cells which interact with the recipient's T cells to initiate the immunization process.[68,69,71] Weekly administration of ultraviolet B-irradiated single canine donor platelets (incompatible for 1 or more DLA antigens) for 8 weeks resulted in 11 of 12 (92%) recipients remaining nonimmunized in comparison to 14% of controls.[71] In a similar transfusion model, the combination of centrifuge- and filter-leukoreduction was shown to be superior to either leukoreduction procedure alone with prevention of alloimmunization in 87% (13 of 15) of dogs.[69] A multi-institutional, randomized, blinded trial (Trial to Reduce Alloimmunization to Platelets) demonstrated that reduction of leukocytes by filtration and ultraviolet B irradiation were equally effective in preventing alloantibody-mediated platelet refractoriness during chemotherapy for acute myeloid leukemia and that platelets obtained by apheresis from single random donors provided no additional benefit as compared with pooled PCs from random donors.[68] However, leukoreduction and ultraviolet B irradiation did not prevent development of antibodies against platelet-specific antigens, which were detected in approximately 8% of patients.[68]

Platelet transfusion selection for the alloimmunized patient may be challenging. One recommended approach for humans is to: (1) determine HLA phenotype and ABO type; (2) screen patient's serum for lymphocytotoxic antibody; (3) screen patient's serum for antibodies to platelet-specific antigens; (4) select from donor pool the most compatible antigens in the HLA, and, if possible, ABO systems (if a platelet-specific antibody is detected, matching for this antigen is required); and (5) crossmatch the recipient's serum with the selected potential donors' platelets and select the most compatible crossmatch.[72] It should be noted, however, that human platelet crossmatching is performed infrequently and considered of limited value as many PC units that test compatible are actually incompatible in vivo. In veterinary medicine, DLA phenotyping is primarily available only in research settings; canine platelet-specific antigens have not yet been characterized, and canine platelet crossmatching techniques have not been carefully evaluated. Fortunately, few thrombocytopenic dogs require repeated platelet transfusions, but in dogs with hereditary thrombopathias, repeated platelet transfusions may be necessary to control bleeding tendencies throughout the lives of these patients, and platelet refractoriness may pose a challenge in providing effective transfusion support for these patients. Various leukocyte reduction filters[i] have been documented to effectively reduce the total number of WBCs in canine PCs to a mean of $\sim 8 \times 10^4$ and not adversely effect posttransfusion platelet recovery or survival.[69] Such filters are expensive ($\sim$ US$45 each), and their use may not be warranted in most dogs receiving platelet transfusions, but for dogs expected to require multiple platelet transfusions, leukoreduction of platelet products may be considered.

Adverse Events Associated with Platelet Transfusions

In veterinary medicine there are too few reports of canine platelet transfusions to estimate the frequency of potential adverse events. In the Trial to Reduce Alloimmunization to Platelets study evaluating 530 thrombocytopenic human patients that received a total of 8,000 platelet transfusions, 160 (2%) of the platelet transfusions were associated with a *severe* reaction defined as increase in temperature of more than 2°C, shaking, chills, extensive urticaria, dyspnea, cyanosis, or bronchospasm.[68] Severe reactions occurred with the

same frequency in groups receiving pooled PC from random donors and apheresis PC from a single random donor, and leukoreduction by filtration and ultraviolet B irradiation of the platelet products shortly before infusion had no effect on the incidence of severe reactions in comparison to untreated control platelets.[68]

There are numerous potential adverse reactions to platelet transfusions, but 2 topics that have been investigated extensively include the role of leukocyte and platelet proinflammatory mediators in acute inflammatory transfusion reactions and transfusion-associated bacterial sepsis. Febrile nonhemolytic transfusion reactions, defined as a temperature increase of ≥ 1°C associated with transfusion and without any other explanation, are often accompanied by chills or rigors and occur with a reported frequency of up to 38% of platelet transfusions.[5] It has been documented that interleukin-1β and interleukin-6 concentrations progressively increased during storage of platelets and that the concentrations of these cytokines were related to the leukocyte count in the platelet product.[73] Moreover, adverse reactions, including febrile nonhemolytic transfusion reactions, were noted more frequently upon paired administration of plasma supernatant and the cellular component from PC than to the cellular component (containing platelets and leukocytes) alone. There was a strong positive correlation between adverse reactions and concentrations of interleukin-6 and interleukin-1β in the plasma supernatant.[73] Not surprisingly, exchanging supernatant plasma from stored PCs with fresh ABO compatible plasma before transfusion was shown to be more effective for reducing the frequency of acute reactions to platelets than post-storage leukocyte reduction.[74] However, in a subsequent trial comparing the frequency of acute reactions to plasma-exchanged platelets and prestorage leukoreduced platelets, there was no significant difference noted between these 2 methods.[75] Prestorage leukoreduction of PCs reduced but did not eliminate febrile nonhemolytic transfusion reactions.[75] While most of the attention regarding underlying causes of adverse reactions to platelet transfusions has focused on proinflammatory mediators released by leukocytes, there has been a more recent interest in release of the biologically active CD40 ligand (CD40L, CD154), a potent inflammatory mediator normally sequestered inside the resting platelet that can translocate to the platelet membrane and be shed into the plasma in response to activation.[76,77] In PCs prepared for transfusion, high levels of both platelet membrane-bound CD40L and soluble CD40L (sCD40L) have been detected with maximum levels reached 72 hours after platelet collection.[76] Furthermore, higher levels of sCD40L in supernatants from post-storage leukoreduced PCs were associated with febrile but not allergic reactions to platelet transfusions.[77] In addition, PCs implicated in transfusion-related acute lung injury, a posttransfusion acute pulmonary insufficiency linked to infusion of biologic response modifiers, contained significantly higher levels of sCD40L than control PCs.[78] sCD40L promoted neutrophil-mediated cytotoxicity of human pulmonary microvascular endothelial cells in vitro suggesting that sCD40L in stored PCs has the capacity to activate adherent neutrophils causing endothelial damage and potentially transfusion-related acute lung injury in predisposed patients.[78]

Because PCs are stored at room temperature, contaminating bacteria introduced during phlebotomy or, less likely, during blood processing or transient donor bacteremia, may rapidly proliferate with administration of the contaminated unit potentially causing transfusion-associated sepsis.[72,79–81] The most common aerobic and anaerobic bacteria cultured from contaminated PC units are coagulase-negative *Staphylococcus* (including predominantly *S. epidermidis*) and *Propionibacterium acne*, respectively, both skin contaminants.[79–81] The reported incidence of bacterial contamination of PC units varies from one study to another. In a study reporting on active surveillance of PC units during a 15.5-year period by culturing aliquots of units at time of issue, 50 bacterially contaminated units were detected amongst 102,998 screened (1:2,062).[81] Forty-two of the contaminated units were transfused, and 16 septic transfusion reactions were noted including 1 fatality.[81] The severity of the transfusion reactions was associated with bacterial load and virulence with all severe reactions (ie, change in vital signs requiring intervention, septic shock, vital organ dysfunction, or death) associated with loads of $\geq 10^5$ colony forming units per milliliter or more virulent bacterial species (eg, *Pseudomonas aeruginosa*, *Serratia marcescens*), or both.[81] In a prospective quality control study initiated by the American Red Cross, 186 of 1,004,206 (1:5,399) apheresis PC units had confirmed positive culture results.[80] However, 20 septic transfusion reactions were reported, including 3 fatalities, in patients that had received screened-negative PCs (samples obtained at least 24 h after collection and tested using an automated microbial detection system[j]).[80] Contaminating bacteria in PCs may evade detection by quality control and pose a significant residual transfusion risk.[80]

Conclusion

Platelet transfusions may be essential to the management of dogs with severe thrombocytopenia or thrombopathia experiencing uncontrolled or life-threatening bleeding. Plateletpheresis is an alternative to whole

Table 1: Characteristics of platelet concentrates under various potential storage conditions

	Fresh platelets	Chilled platelets	Cryopreserved platelets	Lyophilized platelets
Storage conditions	22°C with continuous gentle agitation	4°C	6% DMSO, −80°C	−80°C
Shelf-life	5 d	N/D	1 y	Several years
Advantages	Optimal post-transfusion platelet recovery (dog, 80%), survival (dog, half-life 3.8 d), and function	Decreased risk of bacterial proliferation during storage	Long-term storage Immediate availability	Long-term storage Immediate availability Sterility (result of paraformaldehyde stabilization)
Disadvantages	Short shelf-life Limited availability Risk of bacterial proliferation during room temperature storage	Rapidly cleared from circulation (in humans and mice, with and without modification)	Reduced post-transfusion platelet recovery (dog, 49%) and half-life (dog, 2 d) Impaired in vitro function, though evidence of hemostatic efficacy in vivo	Short in vivo lifespan (min in rats and baboons) Use limited to control of active hemorrhage

blood donation for preparation of a high-quality canine PC with a substantially greater total platelet yield from a single donor. While fresh PC provides functional platelets with excellent posttransfusion in vivo recovery and survival, administration of cryopreserved platelets, with in vivo platelet recovery and half-life of approximately 60% and 50% those of fresh PC, may be considered if fresh PC is not immediately available (Table 1). Lyophilized canine platelets will likely be commercially available in the near future, although their use will be limited to control of *active* bleeding given the very short life span (<15 min) of RL platelets documented in other species. As with all blood products, administration of canine PCs may be associated with serious adverse events, and, therefore, platelet recipients should be monitored carefully to evaluate both the safety and efficacy of the transfusion.

Footnotes

[a] AS104, Fresenius AG, Hamburg, Germany.
[b] MCS Plus, Haemonetics, Braintree, MA.
[c] Williamson KD, Hale AS. Biochemical abnormalities associated with plateletpheresis. J Vet Emerg Crit Care 2007;17:S12 (abstract).
[d] COBE Spectra, Gambro BCT, Lakewood, CO.
[e] StasiX, Entegrion, Research Triangle Park, NC.
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[h] Animal Blood Resources International, Stockbridge, MI.
[i] Pall Corporation, East Hills, NY.
[j] BacT/ALERT 3D, bioMérieux, Durham, NC.

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