Effect of Duration of Packed Red Blood Cell Storage on Morbidity and Mortality in Dogs After Transfusion: 3,095 cases (2001–2010)

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Background: Accumulating evidence suggests that transfusion of packed red blood cells (PRBCs) stored for >14 days is associated with increased rates of sepsis, multiple organ dysfunction, and mortality in human patients.

Objective: To determine if duration of PRBC storage has an effect on morbidity and mortality in dogs after transfusion.

Animals: Dogs admitted to the Matthew J Ryan Veterinary Hospital of the University of Pennsylvania.

Methods: A retrospective case review of dogs identified through blood bank logbooks that received PRBC transfusions (minimum, 5 mL/kg) between 2001 and 2010. Dogs were categorized according to major cause of anemia (eg, hemorrhage, hemolysis, ineffective erythropoiesis) for analysis.

Results: A total of 3,095 dogs received 5,412 PRBC units. Longer duration of PRBC storage was associated with development of new or progressive coagulation failure ($P = .001$) and thromboembolic disease ($P = .005$). There was no association between duration of PRBC storage and survival for all dogs overall. However, a logistic regression model indicated that for dogs with hemolysis, 90% of which had immune-mediated hemolytic anemia, longer duration of PRBC storage was a negative risk factor for survival. For every 7 day increase in storage, there was a 0.79 lesser odds of 30 day survival (95% CI, 0.64–0.97; $P = .024$).

Conclusions and Clinical Importance: Duration of PRBC storage does not appear to be a major contributing factor to mortality in the overall canine population. However, longer duration of PRBC storage may negatively impact outcome in dogs with immune-mediated hemolytic anemia, thus warranting further investigation with prospective studies.

Key words: Anemia; Canine; Hemolysis; Immune-mediated hemolytic anemia.

Red blood cell (RBC) transfusions generally are considered to be life-saving for patients with severe anemia. Many hospitals rely on stored packed RBCs (PRBCs) as a readily available blood component for transfusion. The shelf-life of refrigerator-stored PRBCs varies depending on the anticoagulant and preservative solution used, but typically is 35 and 42 days for canine and human PRBCs, respectively.[1,2] Given that blood is a precious and limited resource, oldest units generally are dispensed first to reduce wastage. However, large observational studies of human patients, especially those with trauma,[3] after cardiac surgery,[4] or in a pediatric intensive care unit,[5] found associations between transfusions of older PRBCs (generally defined as >14 days of storage) and increased sepsis, multiple organ dysfunction syndrome (MODS), mortality or some combination of these. Such studies raise important concerns regarding whether older PRBC units should be used in certain critically ill patient populations.

Abbreviations:

AHTR	acute hemolytic transfusion reaction
aPTT	activated partial thromboplastin time
DIC	disseminated intravascular coagulation
FNHTR	febrile nonhemolytic transfusion reaction
IE	ineffective erythropoiesis
IMHA	immune-mediated hemolytic anemia
MODS	multiple organ dysfunction syndrome
NTBI	nontransferrin-bound iron
PABB	Penn Animal Blood Bank
PCV	packed cell volume
PRBC(s)	packed red blood cell(s)
PTE	pulmonary thromboembolism
PT	prothrombin time
SIRS	systemic inflammatory response syndrome
TED	thromboembolic disease
TP	total plasma protein concentration

The "RBC storage lesion" refers to cumulative biochemical and biomechanical changes that occur in RBCs during storage in vitro that decrease their function and survival in vivo. The Food and Drug Administration mandates that the maximal allowable shelf-life of stored human RBCs requires maintenance of cellular integrity (ie, free hemoglobin <1% of total hemoglobin in the RBC unit) and adequate 24-hour RBC recovery post-transfusion (ie, on average, ≥75% of transfused RBCs must remain in the circulation). Although the specific mechanism or mechanisms by which transfusion of older, stored RBCs may increase morbidity and mortality is not yet known, potential mediators include the accumulation of microparticles in RBC units during storage,[6,7] release of free hemoglobin,[8] and increased plasma concentration of nontransferrin bound iron (NTBI) after rapid clearance of

storage-damaged RBCs by the reticuloendothelial system.[a],[9,10]

Administration of older (21–28 days) stored autologous RBCs has been shown to induce an inflammatory response in healthy dogs.[10,11] In an experimental model of massive exchange transfusion of RBC units stored for 7 or 42 days in dogs with experimentally-induced sepsis, older RBC transfusions were associated with increased mortality (100% versus 33% for dogs receiving 42- and 7-day-old stored RBCs, respectively).[8] None of the units stored for 42 days fulfilled the FDA standards for RBC storage time, because the 24-hour RBC recovery post-transfusion ranged from 60 to 63%.[8] Potential adverse effects associated with transfusion of older stored RBCs to critically ill dogs have not yet been reported.

The purpose of this study was to determine, by retrospective analysis of data accrued over a 10-year period, whether or not the duration of storage of RBCs had an effect on morbidity and mortality in dogs receiving PRBC transfusions.

Materials and Methods

Penn Animal Blood Bank (PABB) logbooks were reviewed to identify dogs that received PRBC transfusions between January 2001 and December 2010. The anticoagulant-preservative and RBC additive solutions used for all units were citrate-phosphate-dextrose and adenine-dextrose-saline-mannitol,[b] respectively; the PRBC units were not leukoreduced. The Penn Animal Blood Bank's guideline for maximum duration of PRBC storage was 28 days, but storage time was extended during times of increased demand and limited supply to 33 days, within a range previously deemed to be acceptable based on RBC post-transfusion recovery.[1] Information recorded from the logbooks included date of collection of blood from the donor, date of administration of PRBCs, number and volume of PRBC transfusions, and volume of concurrent blood products (eg, fresh frozen plasma) administered. Medical records of all dogs were reviewed; if the record was not available or incomplete, or the patient received <5 mL/kg of PRBCs, the dog was excluded from the study. All dogs were blood typed, received DEA 1.1-negative RBCs, or both. Information recorded included signalment, body weight, history of previous RBC transfusion, major cause of anemia (eg, hemorrhage, hemolysis, or ineffective erythropoiesis [IE]), major underlying disease process, and concurrent medical conditions.

The following parameters before and closest to the first PRBC transfusion were recorded: packed cell volume (PCV); total plasma protein concentration (TP); absolute reticulocyte count; platelet count; total white blood cell count; neutrophil count; serum creatinine, bilirubin, albumin, and glucose concentrations; blood lactate concentration; venous pH; body temperature; and, presence or absence of a blood crossmatch. In addition, medical records were evaluated for evidence of any of the following pre-existing conditions (defined as): MODS[12] (failure of 2 or more organs, including hemodynamic failure [systolic blood pressure <90 mmHg, mean blood pressure <60 mmHg, or vasopressors required], respiratory failure [oxygen supplementation or mechanical ventilation required], renal failure [serum creatinine concentration >3.4 mg/dL or urine output <0.25 mL/kg/h after fluid resuscitation], neurologic failure [abnormal mentation not attributable to drug treatment, seizures, or other objective evidence of central nervous system dysfunction], hepatic failure [serum total bilirubin >5.0 mg/dL without anemia, posthepatic obstruction, or pancreatitis; increased blood ammonia concentration], pancreatic

failure [vomiting, abdominal pain, and ultrasonographic changes consistent with pancreatitis or pancreatic neoplasia], coagulation failure [prothrombin time {PT} or activated partial thromboplastin time {aPTT} >25% above upper reference interval or platelet count <40,000/μL]), sepsis[13] (histological or microbiological confirmation of infection and ≥3 of the following: hypo- or hyperthermia [<100 or >103°F]; tachycardia [>140 beats per minute]; tachypnea [>40 breaths per minute]; and leukopenia [<6,000/μL], leukocytosis [>16,000/μL], or >3% band neutrophils), systemic inflammatory response syndrome (SIRS, as for sepsis, minus evidence of infection, but evidence of an inflammatory focus), thromboembolic disease (TED, visualization of a clot by ultrasound examination or CT angiography or at necropsy), disseminated intravascular coagulation (DIC, ≥2 of the following: PT or aPTT >25% above the upper reference interval, platelet count <100,000/μL, and increased D-dimer concentration), or nosocomial infection (pneumonia [new or progressive ventral pulmonary infiltrate on thoracic radiographs with or without suppurative inflammation on tracheal wash cytology with positive bacterial culture], urinary tract infection [pyuria, bacteriuria, and positive bacterial culture {10^5 colony-forming units per mL}], or surgical site infections).

Additional information recorded for the period during and post-transfusion included PCV and TP (measured within 4 hours after the first PRBC transfusion), body temperature, evidence of a febrile nonhemolytic transfusion reaction (FNHTR; increase in body temperature by ≥2°F during or within 4 hours after transfusion without evidence of hemolysis) or acute hemolytic transfusion reaction (AHTR; presence of fever, hemoglobinemia, hemoglobinuria, and lack of increase in PCV), and development of new or progressive organ failure, SIRS, TED, DIC, or nosocomial infection. It was also noted if the patient had surgery before, during, or after transfusion or some combination of these. Patient outcome (discharged, died, or euthanized), and survival at 30 days after the patient's first and oldest (if the dog received >1 PRBC unit) PRBC transfusion also were recorded.

Statistical Analysis

Descriptive statistics were calculated. Because of non-normality of the data, continuous data were expressed as median values and ranges. Categorical data were expressed as frequencies. Fisher's exact test was used to evaluate the association between categorical variables. A Mann–Whitney test was used to compare continuous variables among categorical variables with 2 categories, and a Kruskal–Wallis test was used to compare continuous variables among categorical variables with ≥3 categories. All tests were 2-tailed, and $P < .05$ was considered statistically significant.

Logistic regression analysis was used to determine the association of duration of PRBC storage with 30-day survival while controlling for any potentially confounding factors (ie, factors extracted from the medical record as described above). Two-way interactions among the main effects were investigated first and a variable was retained as an effect modifier if the interaction term had a $P < .05$. Univariate analysis then was performed to determine what additional variables would be further considered in the model based on a $P < .20$. Factors were retained in the model based on $P < .05$ or if found to be a confounder (changing model coefficients by >15%). All analyses were completed using STATA 11.[c]

Results

During the 10-year period, 3,205 dogs received PRBC transfusions, and 3,095 dogs were included with a total of 3,261 hospital visits. Reasons for exclusion

included missing or incomplete medical records or a total PRBC transfusion volume <5 mL/kg. One-hundred-and-fifteen dogs received transfusions during >1 hospitalization. In total, 5,412 PRBC units were administered.

Patient Baseline Characteristics

The median age and body weight of the dogs were 7.9 years (range, 0.1–19.8) and 24.2 kg (range, 0.4–86), respectively. The median pretransfusion PCV was 18% (range, 4–58). The most common reason for RBC transfusion was hemorrhage (n = 2,103, 68%), with neoplasia (n = 766), trauma (n = 285), and surgical blood loss (n = 249) being the most frequent underlying causes of hemorrhage. Hemolysis was the major cause of anemia in 500 dogs (16%), with immune-mediated hemolytic anemia (IMHA) accounting for 90% of these cases. There were 492 dogs (16%) with IE, most commonly caused by immune-mediated disease (n = 138), neoplasia (n = 126), and metabolic disease (n = 86).

For the overall population of dogs requiring RBC transfusions, pre-existing morbidities included MODS (13.8%), with coagulation failure (n = 891) and hemodynamic failure (n = 387) being the most common organ failures, followed by DIC (n = 349), SIRS (n = 165), sepsis (n = 96), and TED (n = 32).

PRBC Transfusion Characteristics

There was a wide distribution of PRBC unit storage times, ranging from 0 to 40 days with a median of 23 days for the oldest PRBC unit administered (Fig 1 and Table 1). Units stored >33 days inadvertently were not removed from the stock in the blood bank refrigerator during extended holidays. Approximately 20% of dogs received only PRBC units stored ≤14 days (Table 1). The total volume of PRBCs administered during a single hospitalization ranged from 5 to 187.5 mL/kg, with a median of 15.2 mL/kg.
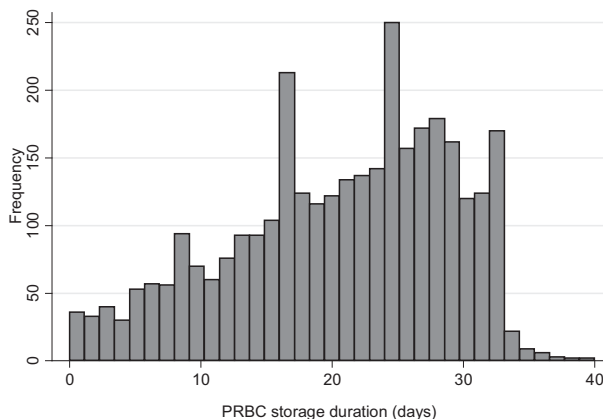

Fig 1. Distribution of packed red blood cell (PRBC) unit storage times. Figure shows the number of units transfused versus their length of storage.

Adverse Events

The incidence of FNHTRs was 3% (98 of 3,261 hospital visits). There was no difference in the frequency of FNHTRs among the 3 main transfusion groups, and there was no association with previous RBC transfusion or the presence or absence of a blood crossmatch. The median duration of RBC storage for dogs not experiencing a FNHTR was 22 days (range, 0–40) versus 23.5 days (range, 2–37) for those experiencing a FNHTR ($P = .003$). AHTRs were noted in 7 of 3,261 transfusion events (0.21%), all in patients with hemorrhage. None of the 7 dogs had received a previous RBC transfusion, and a blood crossmatch had not been performed for any of these dogs. Duration of PRBC storage was not associated with development of an AHTR. The dogs experiencing AHTRs received a median total PRBC volume of 34 mL/kg (range, 10.7–128.7), more than twice the median volume for all dogs with hemorrhage (15.1 mL/kg; $P = .009$).

Regarding post-transfusion complications, new or progressive MODS was noted in 2.54% of 3,261 total hospital visits, TED in 1.53%, and DIC in 1.35%. There was an association between longer duration of PRBC storage and development of coagulation failure and TED after administration of oldest rather than first PRBC transfusion (Table 2). Furthermore, a larger total volume of PRBCs administered was strongly associated with development of new or progressive MODS, individual organ failures, sepsis, DIC, and TED (Table 2).

Patient Outcome

The percentage of dogs discharged from the hospital was significantly higher when the major cause of anemia was hemolysis rather than hemorrhage (Table 3). Dogs with anemia caused by hemorrhage had a higher percentage of in-hospital deaths (not including euthanasia) than dogs with hemolysis (3.7% versus 1.6%; $P = .009$). There was no difference in percentage of dogs being euthanized among the 3 major causes of anemia. Survival at 30 days after the first PRBC transfusion was longest for dogs with hemolysis, followed by hemorrhage and IE (Table 3). Results were nearly identical for 30-day survival after the oldest, as opposed to the first, PRBC transfusion administered because all dogs, except for 1 with IE, which survived to 30 days after its first RBC transfusion, also survived to 30 days after their oldest RBC transfusion (data not shown).

When comparing dogs that received only PRBC units stored ≤14 days (n = 675) to those that received PRBC units stored for >14 days (n = 2,586), there was no difference in outcome (discharged, died, or euthanized) or 30-day survival for all dogs overall or for any subset (hemorrhage, hemolysis, or IE). However, for dogs with hemolysis, a longer duration of PRBC storage (ie, considering oldest rather than first PRBC unit administered) was associated with not surviving to discharge from the hospital and decreased 30-day

Table 1. Duration of packed red blood cell (PRBC) storage and volume of PRBCs administered.

	All Dogs	Dogs with Hemorrhage	Dogs with Hemolysis	Dogs with Ineffective Erythropoiesis
Duration of PRBC storage for first transfusion (days)	22 (0–40)	22 (0–40)	22 (0–35)	21 (0–36)
Volume of PRBCs administered during first transfusion (mL/kg)	11.3 (4.2–55.5)	11.1 (5–43.8)	11.7 (5–35)	11.2 (4.2–55.5)
Longest duration of PRBC storage for any transfusion (days)	23 (0–40)	23 (0–40)	24 (1–35)	22 (0–36)
Volume of oldest PRBCs administered (mL/kg)	11.3 (5–61.1)	11.2 (5–50)	11.8 (5.1–35)	11.4 (5–61.1)
Total volume of PRBCs administered during single hospitalization (mL/kg)	15.2 (5–187.5)	15 (5–187.5)[a]	18.4 (5.2–163.1)[a]	13.7 (5.3–141.4)[a]
Number of dogs receiving only PRBC units stored ≤14 days	675 (20.7%)	447 (20.8%)	92 (17.6%)	136 (23.2%)

None of the data are normally distributed, thus are presented as median with range, except for number of dogs receiving only PRBC units stored ≤14 days.

[a]$P < .001$ hemorrhage versus hemolysis, hemorrhage versus IE, and hemolysis versus IE.

Table 2. Post-transfusion complications noted during 3,261 hospital visits and association with total volume of PRBCs administered and duration of PRBC storage.

Post-Transfusion Complications	Total Volume of PRBCs Administered (mL/kg)	P Value	Duration of PRBC Storage (days) – First Transfusion	P Value	Duration of PRBC Storage (days) – Oldest Transfusion	P Value
MODS						
Absent (n = 3,178)	15.1 (5.2–187.5)	<.001	22 (0–40)	.214	23 (0–40)	.053
Present (n = 83)	23.7 (5–160)		23 (2–34)		25 (2–35)	
Hemodynamic failure						
Absent (n = 3,129)	15.1 (5.2–187.5)		22 (0–40)		23 (0–40)	
Present (n = 132)	20.2 (5–160)	<.001	21.5 (2–34)	.952	23 (2–35)	.193
Respiratory failure						
Absent (n = 3,056)	15 (5.2–170)	<.001	22 (0–40)	.820	23 (0–40)	.147
Present (n = 205)	21 (5–187.5)		22 (1–35)		23 (2–35)	
Neurological failure						
Absent (n = 3,222)	15.2 (5–187.5)	.004	22 (0–40)	.294	23 (0–40)	.109
Present (n = 39)	21.6 (8.3–80.7)		23 (5–34)		25 (5–34)	
Coagulation failure						
Absent (n = 3,211)	15.1 (5–187.5)	<.001	22 (0–40)	.060	23 (0–40)	.001
Present (n = 50)	23.9 (8.1–74.5)		24.5 (2–33)		28 (2–34)	
Sepsis						
Absent (n = 3,245)	15.2 (5–187.5)	<.001	22 (0–40)	.373	23 (0–40)	.708
Present (n = 16)	32.5 (10–112)		19 (3–32)		21.5 (3–32)	
DIC						
Absent (n = 3,217)	15.2 (5–187.5)	<.001	22 (0–40)	.239	23 (0–40)	.061
Present (n = 44)	24 (7.8–74.5)		24 (4–33)		25.2 (4–33)	
Increased D-dimer concentration						
Absent (n = 3,216)	15.2 (5–187.5)	<.001	22 (0–40)	.310	23 (0–40)	.132
Present (n = 45)	24.6 (7.8–74.5)		23 (4–35)		25 (4–35)	
TED						
Absent (n = 3,211)	15.2 (5–187.5)	<.001	22 (0–40)	.70	23 (0–40)	.005
Present (n = 50)	23.8 (7–112)		25 (1–34)		27 (9–34)	

None of the data are normally distributed, thus are presented as median with range. n is the number of hospital visits during which a post-transfusion complication was observed. "Absent" refers to dogs for which a post-transfusion complication was not observed, whereas "present" refers to dogs experiencing a post-transfusion complication.

survival after the first (and oldest – data not shown, as noted above) PRBC transfusion (Table 3). In a logistic regression model controlling for all other variables, longer duration of PRBC storage was a negative risk factor for survival in dogs with hemolysis, but not for dogs with hemorrhage or IE. For every 7 day increase in storage, there was a 0.79 lesser odds of 30-day survival ($P = .024$; Table 4).

Although a larger total volume of PRBCs administered was associated with post-transfusion complications as noted above (Table 2), there was no association with 30-day survival overall or for each subset (hemorrhage, hemolysis, or IE). For all dogs, the median total volume of PRBCs administered to those not surviving to 30 days was 15.3 mL/kg (range, 5–187.5) compared to 15.1 mL/kg (range, 5.2–170) for

Table 3. Patient outcome and association with duration of PRBC storage.

Outcome	Number of Hospital Visits	Duration of PRBC Storage (days) – First Transfusion	P Value	Duration of PRBC Storage (days) – Oldest Transfusion	P Value
Discharged from hospital					
Overall (n = 3,261)					
Yes	2,113 (64.8%)	22 (0–40)	.474	22 (0–40)	.053
No	1,148 (35.2%)	22 (0–37)		23 (0–39)	
Hemorrhage (n = 2,152)					
Yes	1,367 (63.5%)	22 (0–40)	.998	23 (0–40)	.347
No	785 (36.5%)	22 (0–37)		23 (0–39)	
Hemolysis (n = 523)					
Yes	361 (69%)[a]	21 (0–35)	.227	23 (1–35)	.015
No	162 (31%)	24 (1–35)		26 (2–35)	
Ineffective erythropoiesis (n = 586)					
Yes	385 (65.7%)	20 (0–36)	.510	21 (0–36)	.475
No	201 (34.3%)	22 (2–36)		22 (2–36)	
30-day survival after first PRBC transfusion					
Overall (n = 3,174)					
Yes	1,646 (51.9%)	22 (0–40)	.927	23 (0–40)	.544
No	1,528 (48.1%)	22 (0–37)		23 (0–39)	
Hemorrhage (n = 2,094)					
Yes	1,090 (52.1%)[b]	22 (0–40)	.405	23 (0–40)	.712
No	1,004 (47.9%)	22 (0–37)		23 (0–39)	
Hemolysis (n = 509)					
Yes	301 (59.1%)[b]	21 (0–35)	.067	23 (1–35)	.003
No	208 (40.9%)	24 (1–35)		26 (1–35)	
Ineffective erythropoiesis (n = 571)					
Yes	255 (44.7%)[b]	20 (0–36)	.985	21 (0–36)	.890
No	316 (55.3%)	21 (0–36)		22 (0–36)	

Values for duration of PRBC storage are median and range. 30-day survival data were not available for all patients. "Yes" indicates either survival to discharge or 30-day survival post first transfusion, "No" indicates patients did not survive.

[a]P < .02 for hemolysis versus hemorrhage.

[b]P < .005 for hemolysis versus IE, hemorrhage versus IE, and hemorrhage versus hemolysis.

Table 4. Multivariate logistic regression model for 30-day survival among dogs with hemolysis.

Variables	Odds Ratio	Lower 95% CI	Upper 95% CI	P Value
Every 3 year increase in dog age	0.71	0.57	0.88	<.001
Every 3 mmol/L increase in pretransfusion lactate	0.53	0.41	0.68	<.001
Every 7 day increase in duration of PRBC unit storage	0.79	0.64	0.97	.024

dogs surviving to 30 days after their first PRBC transfusion. There was a statistically but not clinically relevant difference in the total volume of PRBCs administered to all dogs being discharged (median, 15 mL/kg; range, 5.2–170) compared to those not discharged (median, 16 mL/kg; range, 5–187.5; P = .008).

Discussion

A recent editorial in *Transfusion*, the official journal of the American Association of Blood Banks, stated that the "question of whether or not older blood is less safe is the most critical issue facing transfusion medicine."[14] In this study, we describe an independent association between longer duration of PRBC storage and decreased survival of dogs with hemolysis. In addition, longer PRBC storage time was associated with development of new or progressive coagulation failure and TED post-transfusion. However, when considering all dogs, regardless of cause of anemia, there was no association between the PRBC storage time and survival.

In contrast to the overall population, and dogs with hemorrhage or IE, duration of PRBC storage was found to be a negative risk factor for 30-day survival in dogs with hemolysis, 90% of which were diagnosed with IMHA. The case fatality rate in dogs with IMHA has been reported to be approximately 25–40%,[15] with development of TED being a leading contributing factor to mortality.[16] Although likely multifactorial, cross-talk between systemic inflammation and the coagulation system may cause a prothrombotic state.[17] A pro-inflammatory cytokine response has been observed in dogs with IMHA,[16,18] with increased serum concentrations of monocyte chemoattractant protein-1[16,18] and interleukin-18[16] independently associated with mortality. Administration of 28-day-old, but not 7-day-old, stored autologous RBCs to healthy dogs induced a pro-inflammatory cytokine response, exemplified by increased serum monocyte

chemoattractant protein-1 concentration, increased neutrophil counts and decreased platelet counts.[10] A synergistic effect of older PRBC transfusion-associated inflammation with the inflammatory response in dogs with IMHA may contribute to mortality.

Increasing patient age and pretransfusion plasma lactate concentration also were found to be negative risk factors for survival of dogs with hemolysis. A recent prospective observational cohort study of 126 dogs in an intensive care unit also identified these same 2 factors as being significantly higher in nonsurvivors compared to survivors.[19] As increased plasma lactate concentration reflects decreased oxygen delivery to tissues, it is not surprising that increasing lactate concentration was strongly associated with increased risk of mortality.

Although several observational studies of critically ill human patients have documented an association between older PRBCs and post-transfusion complications, including MODS and sepsis,[4,5] we identified an association of longer duration of PRBC storage only with new or progressive coagulation failure and TED. For this study, we used strict definitions of coagulation failure and TED. Therefore, dogs with more subtle coagulation abnormalities and thrombotic events were not included, potentially underestimating the impact of duration of PRBC storage on coagulation and development of TED. Transfusion of older PRBCs may activate the endothelium by release of free hemoglobin or potential harmful mediators that accumulate during storage (eg, microparticles, isoprostanes),[6] resulting in platelet consumption, activation of coagulation factors, and thrombotic events. The median pretransfusion platelet count for the dogs of this study was 127,000/μL (range, 0–464,000/μL; reference interval, 177,000–398,000/μL), indicating that many dogs were already thrombocytopenic because of their underlying disease. Although there is a statistical association between duration of PRBC storage and coagulation failure and TED, we cannot conclude that a cause and effect relationship exists, because new or progressive coagulation failure and TED may have been caused by progression of the patient's underlying disease rather a transfusion-associated complication. However, given the devastating consequences of hemorrhagic and thrombotic tendencies, the potential impact of duration of RBC storage on development of these morbidities warrants further evaluation.

The incidence of FNHTR and AHTR in this study was 3% and 0.2%, respectively, similar to previous reports.[20,21] A notation in the medical record of hemolyzed plasma alone was insufficient to be included in our study as an AHTR, because it was impossible to retrospectively differentiate hemoglobinemia from an iatrogenically hemolyzed plasma sample; therefore, the incidence of AHTRs may have been underestimated. A pretransfusion blood cross-match was not performed for any of the 7 dogs with AHTR because they were not known to have been previously transfused. All 7 dogs were transfused because of hemorrhage and received a larger volume

(median, 34 mL/kg) and older PRBCs (27–33 day storage time for 6 dogs) than all dogs overall. Potential causes of the AHTR include the presence of RBC alloantibodies (acquired from a previous transfusion unknown to the current owner or naturally-occurring, but clinically relevant naturally-occurring hemolysins have not yet been recognized in dogs), improperly stored PRBCs,[22] lysis of RBCs during rapid administration through small gauge catheters, and bacterial contamination of the PRBC unit. In the 16 dogs that developed sepsis as a post-transfusion complication, none experienced an acute reaction to the RBC transfusions that would suggest bacterial contamination of the PRBC units nor was there a notation that the PRBC units appeared discolored. We cannot, however, retrospectively eliminate the possibility of bacterial contamination.

Although there was a statistically significant association between longer PRBC storage and development of a FNHTR, the clinical relevance of this observation is questionable given that the median durations of PRBC storage were 23.5 and 22 days, respectively. In another study,[d] there was no association of duration of RBC storage with febrile transfusion reactions.

Our study included dogs with a wide range of underlying disease processes of varying severity, and many dogs received blood components other than PRBCs. Clearly, when evaluating morbidity and mortality in this population, there are many factors to consider other than duration of PRBC storage. The total volume of PRBCs administered was strongly associated with the development of new or progressive MODS, sepsis, DIC, and TED, although there was no association with 30-day survival. One likely explanation is that dogs requiring large volumes of PRBCs are more critically ill and, therefore, predisposed to developing MODS and other complications independent of their transfusions. Increases in circulating NTBI follow administration of a large volume of stored damaged RBCs because the released iron overwhelms the acquisition capacity of transferrin.[a,9,10] Serum from humans containing increased NTBI post-transfusion resulted in enhanced bacterial growth in vitro.[23] Thus, NTBI may play a role in the development of sepsis after transfusion. In addition, increased plasma concentrations of NTBI in humans have been shown to correlate with markers of endothelial cell activation,[24] which could contribute to development of MODS, DIC, and TED. Other potential mediators of the harmful effects of stored RBCs include free hemoglobin, isoprostanes, and microparticles that accumulate during storage.[6] Although duration of RBC storage (median, 23 days) was not associated with increased mortality overall for the dogs of this study, dogs that received a larger total volume of PRBCs would be more likely impacted by these potential harmful mediators. Interestingly, in human trauma patients, administration of larger volumes of RBC transfusions, regardless of duration of storage, was associated with increased odds of mortality, but the transfusion of blood

stored beyond 2 weeks appeared to potentiate this association.[25]

The median duration of storage for the oldest PRBC unit transfused in our study was 23 days, with a range of 0–40 days. If defining fresh and old PRBCs as being stored for ≤14 days and >14 days, respectively, the majority of PRBC units administered to the dogs were old. As is the practice in many blood banks to avoid wastage, PABB has followed a "first in, first out" policy, with the oldest PRBC units administered before they reach their expiration date. Based on our findings that there was no association between duration of PRBC storage and overall mortality, there is insufficient data at this point to recommend shortening the refrigerator shelf-life of canine PRBCs. However, in light of the association between longer duration of PRBC storage and increased mortality in dogs with hemolysis, as well as development of coagulation failure and TED in the overall population of transfused dogs, storage of canine PRBCs for 5–6 weeks may not be optimal.

The main limitation of this study is its retrospective nature and inherent difficulties associated with determination whether a patient experienced a post-transfusion complication, thereby potentially underestimating the incidence of adverse effects of PRBC transfusions. Although the large number of patients represented is a strength of this study, the patient population was heterogeneous, making it difficult to differentiate progressive underlying disease from post-transfusion complications. In addition, approximately 80% of dogs in this study received blood stored >14 days, potentially preventing detection of differences in morbidity and mortality between patients receiving fresh and old PRBCs.

In conclusion, results of this study suggest that duration of RBC storage is not a major contributing factor to mortality in the overall population of dogs receiving PRBC transfusions. However, longer storage may negatively impact outcome in dogs with IMHA and contribute to increased morbidity, particularly coagulation failure and TED, thus warranting further investigation with prospective studies.

Footnotes

[a] Wurlod V, McMichael M, Smith S, O'Brien M. Effects of packed red blood cells storage time on iron metabolism in healthy transfused dogs. J Vet Emerg Crit Care 2013;23:S11
[b] Adsol, Fenwal, Lake Zurich, IL
[c] STATA11, College Station, TX
[d] Maglaras C, Koenig A, Bedard D, et al. Effect of red blood cell product age on incidence of canine transfusion reactions. J Vet Emerg Crit Care 2013;23:S9

Acknowledgment

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