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Retrospective evaluation of the effect of red blood cell product age on occurrence of acute transfusion-related complications in dogs: 210 cases (2010–2012)

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Abstract

Objective – To determine whether red blood cell (RBC) product age influences the occurrence of acute transfusion-related complications and mortality in dogs. The hypothesis was that acute transfusion-related complications and mortality would increase with age of product.
Design – Retrospective study (2010–2012).
Setting – University teaching hospital.
Animals – Two hundred and ten clinical canine patients.
Interventions – None.
Measurements and Main Results – Medical records were reviewed for dogs receiving RBC-containing products. Patient signalment; reason for transfusion; product type, dose, age, and source; pretransfusion compatibility; rate, route, and method of administration; administration of multiple transfusions; underlying disease; occurrence of transfusion-related complications (eg, fever, hemolysis, gastrointestinal distress, cardiovascular, neurologic, and respiratory complications); various hematologic parameters; and survival were recorded. Data were analyzed for association between potential risk factors and occurrence of transfusion-related complications as well as between transfusion-related complications and survival. Of 333 transfusion events in 210 patients, 84 transfusion-related complications occurred. Fever was most common (41/333), followed by hemolysis (21/333). For every additional day of product age, the odds of hemolysis increased significantly (odds ratio, 1.11; 95% confidence interval, 1.06–1.16; $P < 0.0001$). Transfusion-related complications when considered as a whole were associated with higher dose of product, longer duration of administration per transfusion event, and immune-mediated disease, but not with source of product or general category of anemia. Administration rate was significantly slower in patients with febrile transfusion-related complications ($P < 0.0001$). Product age was not associated with increased mortality.
Conclusions – Age of stored RBC products is associated with increased risk of transfusion-related hemolysis, but not with fever. Prospective clinical studies evaluating the influence of storage duration on development of in vitro versus in vivo hemolysis are warranted.

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Keywords: anemia, canine, febrile nonhemolytic transfusion reaction

Abbreviations

AHTR	acute hemolytic transfusion reaction
AS-5	additive solution 5
DEA	dog erythrocyte antigen
IMHA	immune-mediated hemolytic anemia
ITP	immune-mediated thrombocytopenia
pRBC	packed red blood cell
U-BB	University Blood Bank
VTH	Veterinary Teaching Hospital

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Introduction

Transfusion of any type of blood product has the potential to cause harm to the recipient, and standard blood collection, storage, and administration protocols are recommended in order to minimize the risk of adverse events. Despite such precautions, adverse transfusion reactions and transfusion-related complications may still occur. Acute reactions are typically noted during or within hours following a transfusion.[1,2] Delayed reactions may not manifest until days after the transfusion, as dogs may develop antibodies to foreign antigens anywhere from 4 to 14 days after a transfusion.[3]

The use of stored blood products for component therapy is desirable because the products are available for immediate use and allow pretransfusion compatibility testing of donor blood to the recipient.[4–6] However, administration of stored red blood cell (RBC) products can decrease transfusion efficacy since 2,3-diphosphoglycerate levels decrease as the RBCs age, which increases hemoglobin-oxygen affinity and thus decreases off-loading of oxygen at peripheral tissues.[7] In older RBC units, deformability, functional capacity, and survival of RBCs is also reduced.[8–10] Additional drawbacks of stored RBC products include the risk of bacterial proliferation in the unit during storage secondary to contamination at the time of collection, as well as increased concentrations of potassium and ammonia with increasing product age.[11,12] Storage of RBCs can result in in vitro and in vivo hemolysis, as well as an inflammatory response in the recipient. Transfusions of older, leukoreduced, stored RBCs to mice can cause extravascular hemolysis due to destruction of senescent RBCs, and can provoke release of inflammatory cytokines.[13] A recent veterinary study demonstrated that nonleukoreduced, stored canine RBCs had a higher concentration of IL-8, which in addition to IL-1 and TNF-α, may contribute to a pyrogenic response in the donor by priming leukocytes.[14]

In people, the age of the transfused blood product is positively correlated with the risk of transfusion-related complications,[15,16] and transfusion of RBCs >14 days old is associated with an increased risk for multiple organ failure.[17–19] One similar veterinary study, although underpowered, did not find an association between product age and the development of transfusion-associated complications.[20] A larger veterinary study determined that there was no association between the duration of the packed RBC (pRBC) storage time and febrile nonhemolytic transfusion reactions or acute hemolytic transfusion reactions (AHTR).[21] However, this study discovered that increased pRBC storage time was a negative risk factor for survival in dogs with anemia induced by hemolysis.[21] Available information from the veterinary literature reports that transfusion reactions in dogs occur in 0–28% of transfusions[20–24]; 2 of these reports considered age of the RBC product.[20,21]

The purpose of this retrospective study was to determine whether the age of RBC product is associated with the occurrence of acute transfusion-related complications or mortality in dogs receiving transfusions. It was hypothesized that both the occurrence of transfusion-related complications and mortality rate would increase with increasing age of the RBC product.

Materials and Methods

Case selection

All dogs that received a whole blood or pRBC transfusion at the University of Georgia Veterinary Teaching Hospital (VTH) between January 2010 and December 2011, and May 2012 and December 2012 were identified by searching the VTH blood transfusion case log. The months of January through April 2012 were missing from the logbook; thus, due to an inability to identify the product age, no transfusions performed during this time were included in the investigation. Only dogs with a complete medical record, including signalment, clinical history, initial physical examination, reason for transfusion, a pre- and posttransfusion spun packed cell volume (PCV), and a detailed record of the transfusion event were included in the study.

Data collection

Medical records for the dogs identified in the case log were retrospectively reviewed. From each medical record, patient data were collected, including the patient signalment, body weight in kilograms, patient blood type and crossmatch results (if performed), general cause of anemia (blood loss, hemolysis, lack of production), final diagnosis, history of prior transfusions, concurrent immunosuppressive medications (if applicable), and survival to hospital discharge and to 30 days posttransfusion. Pre- and posttransfusion PCV measurements were the last before transfusion and the first after transfusion, respectively, both within the 24 hours surrounding the transfusion event. Other clinicopathologic data were gathered (pre- and posttransfusion CBC, results of blood gas analysis, serum biochemistry profile, and urinalysis) if obtained within a 24-hour window pre- or posttransfusion event. The net change (Δ = postvalue − prevalue) in these clinicopathologic measurements was also calculated.

Transfusion data that were collected included the recipient blood type (dog erythrocyte antigen [DEA] 1.1 positive or negative), source of the blood product (in-house blood bank vs commercial blood bank), type of blood product (pRBC, fresh whole blood, stored

whole blood), unit size of blood product administered (mL), dose of blood product (mL/kg), duration of transfusion administration (hours), rate of administration (mL/kg/h), age of the blood product (days), administration of diphenhydramine, expiration date of the blood product, donor identification, and donor blood type. A transfusion event was defined as administration of an RBC product from a single donor. Transfusion of non-RBC blood-derived products (fresh frozen plasma, stored plasma, cryoprecipitate, platelet-rich plasma, albumin) was not included in the statistical analysis.

Data indicating 3 specific, acute transfusion related complications (allergy/anaphylaxis, fever, and hemolysis) were collected from the record. Allergy/anaphylaxis reactions were defined as development of urticaria, erythema, pruritus, facial swelling, or vasodilatory shock during or within 4 hours posttransfusion administration. Fever was defined as an increase in rectal temperature ≥1.1°C (2°F) from baseline in previously normothermic patients, during or within the 4 hours following transfusion. Subjects that were hypothermic at the beginning of the transfusion were allowed to have an increase in body temperature >1.1°C (2°F) as long as the body temperature did not increase above the reference interval in the absence of external warming. For example, if a patient was recovering from anesthesia during a transfusion, and the body temperature increased from 35°C (95°F) to 37.8°C (100°F) in the presence of a forced-air warming system, a reaction was not noted if all other clinical parameters were within normal limits. Transfusion-related hemolysis was defined as development of hemolyzed serum or pigmenturia in a patient in which these were previously undocumented (eg, in a patient with extravascular immune-mediated hemolytic anemia [IMHA] that developed intravascular hemolysis associated with transfusion), or worsened compared to baseline. Worsening hemolysis or pigmenturia was identified by clear documentation of these changes in the medical record, such as when data on serial samples of hematocrit tubes or urine were saved, compared, and recorded, during or within 12 hours of the transfusion. If a patient had intravascular IMHA and hemolysis did not clearly worsen or change during or after transfusion, it was not considered a complication. For all transfusion-related hemolysis, identifying whether hemolysis was immunologic (such as AHTR) versus nonimmunologic, or in vitro versus in vivo in origin could not be determined from review of the medical records.

Other clinical signs that were temporally associated with transfusion were also recorded. These signs were considered transfusion-related complications if they were temporally related and could not be definitively attributed to any other cause. Changes in cardiovascular parameters, respiratory status, neurological status, or blood pressure, in addition to the presence of gastrointestinal distress, were noted during the transfusion and for a defined time period following the transfusion, as outlined below. Changes in cardiovascular parameters included new or worsening sinus tachycardia or arrhythmias, ventricular premature complexes, ventricular tachycardia, or supraventricular tachycardia during the administration period of the transfusion event. Changes in respiratory status were defined as an increase in the patient's respiratory rate or effort from baseline, or by a decline in SpO_2 from baseline, during or within 4 hours following transfusion. Neurologic abnormalities included new onset of seizures (generalized) or progressive mental dullness (with the patient characterized as mentally dull, obtunded, stuporous, or comatose) during the transfusion administration event. Blood pressure changes included the development of hypotension (defined as a systolic blood pressure measured using a Doppler ultrasonic flow probe of <80 mm Hg) during the transfusion, as long as the hypotension was not attributable to underlying disease or known hemorrhage. Gastrointestinal distress was defined as regurgitation, vomiting, or diarrhea that was new or worsened during the transfusion or within the following 4 hours.

Death as a transfusion-related complication was defined as unexpected cardiopulmonary arrest or euthanasia as a result of clinical deterioration that occurred during or within 1 hour after the transfusion. An effort was made to differentiate death that resulted from underlying disease, prognosis, or financial constraints. Patients in cardiac arrest at the time the transfusion was initiated or those that were euthanized (due to financial constraints or perceived prognosis of the underlying disease) were not included as transfusion-related mortality.

Blood product storage and collection protocol

All blood products were sourced either locally from the University of Georgia blood bank (U-BB) or from a commercial blood bank.[a] The U-BB uses donor dogs owned by faculty, staff, and students of the University of Georgia. These healthy donors receive yearly physical examinations and clinicopathologic and infectious disease testing as recommended by the American College of Veterinary Internal Medicine consensus statement on blood donor screening for infectious disease.[25] Blood collection for the U-BB is performed in an aseptic manner through jugular venipuncture into a closed collection system using a citrate-phosphate-dextrose-adenine anticoagulant.[b] Some of the U-BB pRBC products contained the nutritive additive solution 5 (AS-5),[c] a crystalloid solution containing sodium chloride, dextrose, adenine, and mannitol, which has been approved to extend human RBC storage for up to 42 days.[2,26,27] The blood collection bags that were available at the time of

the blood donation determined which pRBC units contained AS-5. The U-BB supplemented in-house donations with purchased products from a commercial blood bank during times of extreme need. Packed RBC units originating from the commercial blood bank were all collected in citrate-phosphate-dextrose-adenine with AS-5, with a product shelf life of 42 days from collection date.

Regardless of the origin of the blood product, RBC products were stored in a dedicated and locked blood product refrigerator[d] with continuous internal temperature monitoring. Storage of the blood products was performed according to the standards set by the American Association of Blood Banks.[26] This refrigerator was only opened for retrieval of blood products, or when a designated staff member was restocking or rotating blood products.

Fresh whole blood products originating from U-BB donors were used immediately after collection. Stored blood products (pRBC, stored whole blood) originating from the in-house blood bank had a shelf life of either 35 or 42 days, depending on whether they contained AS-5. Since it was not possible to determine retrospectively whether U-BB products contained AS-5 or not, the age of expiration for all in-house stored RBC products, unless otherwise specified on the unit, was averaged to 38 days (range 35–42 d). The age of the product was calculated by counting the days left by subtracting the date of administration from expiration date.

Blood product administration

During the time periods of the study, blood type was determined using blood typing cards.[e] Patients were administered type-specific blood products. If a blood type was not performed prior to administration of the transfusion due to presence of autoagglutination or in cases of extreme emergency, the patient received a DEA 1.1 negative blood product. Except in extreme emergencies, the hospital protocol recommends that any patient that receives a blood transfusion ≥4 days[28-30] after an initial transfusion should be crossmatched. For patients that have never received blood products, crossmatching is at the discretion of the clinician. Crossmatch protocol included both major and minor crossmatches, performed via tube agglutination method at 24°C, 4°C, and 37°C. When applicable, only major and minor crossmatch-compatible units were transfused to a recipient.

A blood product administration form is completed for every transfusion event completed at the VTH. The form contains standard information regarding patient signalment, medical record number, date of transfusion, donor and patient blood type, donor identification information, crossmatch information if available, the reason for transfusion, and a chart for recording vital parameters such as rectal temperature, heart rate, respiratory rate, mucous membrane color, capillary refill time, and mentation. Vital parameter assessment was performed every 10 minutes for the first half hour of the transfusion, every 15 minutes for the second half hour of the transfusion, and then every 30 minutes until the transfusion was completed. Additional comments, blood pressure measurements, and other diagnostic tests performed at the discretion of the ICU technician or clinician were also noted. Standard notification parameters for blood transfusions are as follows: unexpected increase in rectal temperature >1.1°C (2°F), increased heart rate, increased respiratory rate or effort, urticaria, itching, vomiting, hypotension, signs of anaphylaxis, shaking or restlessness, dark colored (pigmented) or red urine, icterus, or decreased urine output (<1 mL/kg/h). If a transfusion was administered by the anesthesia staff to a patient under general anesthesia, the ICU transfusion sheet was not used, in favor of the anesthesia monitoring forms on which esophageal temperature, heart rate, respiratory rate, blood pressure, and any other pertinent diagnostic results or comments were recorded every 5 minutes.

Blood product administration in the ICU setting was primarily performed using a standard straight type blood transfusion line[f] with a built-in 170–260 μm filter and a volumetric peristaltic infusion pump.[g] Administration sets were not reused when consecutive transfusions were administered. Transfusions that were administered as a rapid bolus were administered either by gravity flow or using a 3-way stopcock and a 60-mL syringe attached to the end of the transfusion administration set for rapid administration. If administered to a patient under general anesthesia, gravity flow was the primary method of infusion. In both ICU and anesthesia patients, low-volume transfusions (≤60 mL of blood) were administered using a syringe pump[h] and an 18 μm filter.[i] Blood transfusion dose (mL/kg) and delivery rate (mL/h) were at the discretion of the primary clinician. If the rate of administration was not designated, the ICU protocol dictates administration over 4 hours. A maximum flow rate was calculated based on the specified duration of administration, and the transfusion was started at time zero at approximately 25% of the maximum administration rate. The delivery rate (mL/h) was then increased by 25% every 10 minutes for the first half hour, until the maximum rate was attained.

Statistical analysis

Data were analyzed for association between potential risk factors and occurrence of transfusion-related complications as well as for association between the occurrence of transfusion-related complications and survival. Transfusion-related complications were evaluated for an association with the presence of immunosuppressive therapy, transfusion dose and duration, and type and

source of product. Of the gathered clinicopathologic data (CBCs, serum biochemistry panels, urinalyses), changes in specific hematologic parameters were analyzed, including PCV; toxic-appearing WBCs seen on a microscopic review of a blood smear by a certified medical technologist or board-certified clinical pathologist (distinguished by such characteristics as cytoplasmic vacuolation, cytoplasmic basophilia, and Dohle bodies); and icterus noted in the serum. These parameters were selected as potential indicators of inflammatory response or hemolysis occurring in the transfusion recipient. For dogs receiving multiple transfusions, survival data were attributed to the oldest bag of blood that was administered during the most recent group of transfusion events.

Statistical analyses were performed using commercial statistical software.[j] Simple logistic regression was performed to test for relationships between various risk factors (eg, age of transfused product, multiple transfusions administered to the same animal) and the probability of a reaction. Student's *t*-tests were used to compare dose, pretransfusion PCV, posttransfusion PCV, pre- to posttransfusion change of PCV, and rate of administration, between patients with and without reactions. The folded form *F*-statistic was used to test whether variances were equal between groups. If unequal, then Satterwaithe's approximation for degrees of freedom for Student's *t*-test was used. Analysis of variance was used to compare age of the blood product between the survival categories of survived, euthanized, or died. Linear and multiple linear regressions were used to test for a relationship between change in PCV pre- to posttransfusion, product dose in milliliters per kilogram, and age of the blood product in days. Chi-square analysis was utilized to test for an association between hemolysis as a transfusion-related complication, and the presence of toxic WBCs, serum total bilirubin concentration, use of diphenhydramine prior to transfusion, concurrent immunosuppressive therapy, general reasons for transfusion (blood loss, hemolysis, or lack of production), specific reason for transfusion (definitive diagnosis), other transfusion-related complications, and product delivery method. Chi-square analysis was also utilized to test for an association between the presence of fever or hemolysis and survival to discharge as well as survival to 30 days posttransfusion. All hypothesis tests were 2-sided and the significance level was $\alpha = 0.05$.

Results

Patient data

There were 378 canine RBC transfusion events in 244 patients logged during the study period. Of these, 45 transfusion events occurring in 34 patients were excluded due to incomplete medical records, leaving 333 transfusion events in 210 patients for inclusion in this study.

Of the 210 patients, there were 103 males (84 castrated, 19 intact) and 107 females (94 spayed, 13 intact). The median age at the time of the transfusion event was 8 years (range, 5 mo to 16.5 y). The median body weight was 23.1 kg (range, 2.52–65 kg). There were 56 breeds represented, in which mixed breed dogs and Labrador Retrievers each represented 10.9% of the study population. The other common breeds included Golden Retriever (7.1%), Cocker Spaniel (4.7%), Shih Tzu (4.3%), Boxer (3.8%), German Shepherd Dog (3.8%), Miniature Schnauzer (3.3%), Pit Bull type dog (3.3%), Doberman Pinscher (2.8%), Boston Terrier (2.4%), Pomeranian (2.4%), Weimaraner (2.4%), Beagle (1.9%), Chihuahua (1.9%), Dachshund (1.9%), and Standard Poodle (1.9%). All other breeds each represented <1.5% of the remaining population.

Transfusion event data

Transfusion-related data are provided in Table 1. Of 333 transfusion events, 321 were pRBC units, 193 were administered to first-time blood product recipients, and 140 were administered anywhere from 30 minutes to 3.17 years (median 24 h) after a previous transfusion. Thirty-nine transfusion events in 18 patients occurred >4 days after a previous RBC transfusion, of which 10 transfusion events in 5 patients were administered without a crossmatch.

Of the 333 transfusions administered, 84 transfusions (25%) resulted in 112 transfusion-related complications, with some transfusion events having >1 complication (Table 2). The 2 most common types of transfusion-related complications were fever, which occurred in 41/333 events (12.3%) and transfusion-related hemolysis, which occurred in 21/333 events (6.3%). Eight of the transfusion-associated hemolysis events were associated with extravascular IMHA cases that had no prior documentation of hemolyzed serum or pigmenturia until during or immediately after the transfusion event. No patients with intravascular IMHA could be concluded as having hemolysis that worsened in association with transfusion. Additional transfusion-related complications included 11 transfusion events in 11 patients associated with gastrointestinal upset. Vomiting or regurgitation was noted in 9 dogs during transfusion and in 2 patients within 2 hours following completion of the transfusion. Of these 11 cases, 8 showed signs of another transfusion-related complication such as hyperthermia, collapse, or tachycardia. Sixty of the 210 dogs received a total of 89 FFP transfusion events at some time within the same hospitalization period; 3 of these FFP transfusion events were associated with fever, urticaria,

Table 1: General transfusion information by number of patients and transfusion events

	No. of patients	No. of events
Total number of patients	210	
Total number of transfusion events		333
Number of fresh whole blood transfusions	10	11
Number of stored whole blood transfusions	1	1
Number of pRBC transfusions	207	321
Number of FFP transfusions during same hospitalization	60	89
Blood typing prior to transfusion		
DEA 1.1 negative blood type	79	
DEA 1.1 positive blood type	72	
Not performed due to autoagglutination	28	
Not performed due to reasons unknown	31	
Crossmatch prior to transfusion		41
Administered units per donor blood type		
DEA 1.1 negative unit		236
DEA 1.1 positive unit		97
Origin of administered units		
University blood bank		230
Commercial blood bank		103
Administered to a first-time recipient		193
Recipient had prior exposure to blood products		140
Hospital service overseeing transfusion		
Intensive care unit		289
Anesthesia		42
Intensive care unit and anesthesia		2
Administration method used for transfusion delivery		-
Peristaltic pump		244
Gravity drip or bolus		53
Syringe pump		32

DEA, dog erythrocyte antigen; FFP, fresh frozen plasma; pRBC, packed red blood cell.

Table 2: Packed RBC transfusion-related complications recorded in 84 transfusion events

Transfusion-related complications	Number of occurrences ($n = 112$)	Percentage of complications of the total 333 transfusions	Percentage of 84 transfusion events with complications
Allergy/ anaphylaxis	0	0	0
Fever	41	12.3	48.8
Hemolysis	21	6.3	25.0
Gastrointestinal signs (vomiting, diarrhea)	11	3.3	13.1
Tachypnea	13	3.9	15.5
Tachycardia/ arrhythmias	12	3.6	14.3
Neurologic signs	5	1.5	6.0
Hypotension	3	0.9	3.6
Death/cardiac arrest	6	1.8	7.1

or both. The reaction data for the FFP transfusion events were not further included in the data analysis.

Blood type was determined prior to transfusion for 151/210 patients. Of the 59 patients for which blood type was not determined, 28 had clinicopathologic evidence of autoagglutination and 31 were not blood typed for unstated reasons. There was no association between blood typing prior to a transfusion and the occurrence of any transfusion-related complication ($P = 0.83$), including the occurrence of transfusion-related hemolysis ($P = 0.72$). Of the 292 events administered without crossmatch, 75 (26%) had a transfusion-related complication compared to 9/41 (21%) of the events that were crossmatched prior to transfusion ($P = 0.61$). Among first-time blood product recipients, 27.2% (52/191) of dogs experienced signs of a transfusion-related complication compared to 22.5% (32/142) of dogs with history of a prior transfusion ($P = 0.13$). Of the 10 transfusion events given >4 days after a previous transfusion and without a crossmatch, 1 was associated with a fever during the transfusion. There was no statistical difference between delivery method (peristaltic pump, gravity flow, bolus, syringe pump) and the occurrence of transfusion-related hemolysis ($P = 0.46$).

Reasons for transfusion administration and associated subcategories are listed in Table 3. Four events were listed in >1 general category of anemia. The most common specific reasons for transfusions were RBC lysis due to IMHA (106 events, 31.8%), cavitary hemorrhage secondary to neoplasia (61 events; 18.3%), and surgical blood loss (53 events; 15.9%). There were no significant associations between the general category of anemia (blood loss, hemolysis, or ineffective erythropoiesis) and occurrence of transfusion-related complications.

There were 132/333 (40%) transfusion events in which 1 or more immunosuppressive drugs were being concurrently administered as therapy for underlying disease, primarily IMHA. There were 129 (39%) transfusion events in dogs that were receiving corticosteroids, 84 (25%) in dogs being treated with mycophenolate mofetil, 20 (6%) in dogs being treated with cyclosporine, 6 (2%) in dogs receiving azathioprine, and 5 (2%) in dogs receiving other imunosuppressive agents at the time of the transfusion. One hundred and three (31%) transfusion events occurred in dogs that were concurrently receiving >1 immunosuppressant. Of the transfusion events in which dogs were receiving immunosuppressive therapy, 32.5% (43/132) had a transfusion-related complication (fever being the most common, 27/43). In contrast, 20% of patients not receiving immunosuppressive therapy experienced a transfusion-related

Table 3: General and specific causes of anemia

General and specific causes of anemia	Number of patients*	Number of transfusion events	*P*-value†
Red blood cell loss	**138**	**197**	0.0857
Hemorrhage secondary to neoplasia	45	61	
Surgical loss	42	53	
Gastrointestinal hemorrhage	15	33	
Coagulopathy	21	31	
Traumatic loss	14	17	
Immune-mediated thrombocytopenia	6	15	
Gastric dilatation-volvulus	4	5	
Undefined hemorrhage	5	6	
Postoperative anemia (not attributable to surgical loss)	2	3	
Aortic aneurysm rupture	1	2	
Ruptured (benign) splenic mass	1	1	
Diffuse subcutaneous hemorrhage of undefined origin	1	1	
Red blood cell lysis	**55**	**107**	0.1045
Immune-mediated	54	106	
Infectious (babesiosis)	1	1	
Ineffective erythropoiesis	**22**	**35**	0.6299
Bone marrow disease (neoplastic)	12	16	
Lack of production (chronic disease)	7	13	
Bone marrow disease (nonneoplastic)	3	5	

*Some patients are classified in >1 category of anemia. Bolded lines reflect general category for the cause of anemia.

†*P*-value denotes causes of anemia (per transfusion event) versus the occurrence of a transfusion reaction.

complication ($P = 0.01$). A diagnosis of IMHA was the most common reason for immunosuppression. There was an association between the occurrence of all transfusion-related complications and a diagnosis of IMHA ($P = 0.018$). Fever occurred in 23/106 transfusion events (22%) in patients with IMHA ($P = 0.0004$), and intravascular hemolysis ($P = 0.089$) that had not been identified prior to transfusion developed in 8/106 (8%) of these events. By comparison, in dogs receiving similar immunosuppressive medications as therapy for immune-mediated thrombocytopenia (ITP), 3/15 (20%) transfusion events led to fever, and 1/15 events (7%) was associated with new hemolysis.

There were 30 transfusion events in which diphenhydramine was administered as a premedication. Diphenhydramine was administered in 17 of these events because of clinician preference, in 11 events due to a previous transfusion-related complication, and in 2 events because the dog was already receiving diphenhydramine, 1 for mast cell neoplasia and 1 for anaphylaxis due to bee stings. There was no significant association between diphenhydramine administration and the occurrence of transfusion-related complications ($P = 0.07$).

The median dose of pRBC was 9.40 mL/kg (range, 3.09–46.15 mL/kg; mean, 10.6 mL/kg). The dose of pRBC was higher in transfusion events that provoked transfusion-related complications (11.5 mL/kg) than in those that did not (10.3 mL/kg; $P = 0.04$), and in transfusions that resulted in hemolysis (13.3 mL/kg) than in those that did not (10.4 mL/kg; $P = 0.009$). The median duration of pRBC product administration was 2.7 hours (range, 0.25–6 h). Administration duration was significantly longer in animals with any transfusion-related complication (3.1 h) than in animals without signs of complications (2.6 h; $P = 0.001$). The median administration rate of pRBCs was 3.8 mL/kg/h (range, 1.1–40.7 mL/kg/h; mean, 6.2 mL/kg/h). The administration rate of the transfusion was significantly slower in transfusion events associated with fever (6.3 mL/kg/h; $P < 0.001$). The median dose of the 11 whole blood transfusions was 17.51 mL/kg (range, 9.03–34.59 mL/kg; mean, 17.10 mL/kg), and the median administration rate was 6.1 mL/kg/h (range, 3.69–18.86 mL/kg/h; mean, 8.25 mL/kg/h). There were no transfusion-related complications noted with any fresh whole blood transfusion events.

PCV increased to a greater degree following transfusion events with any transfusion-associated complication (10.3 percentage points from baseline; eg, increased from 10% to 20.3%) than it increased in those without complication (increased 7.4 percentage points; $P = 0.003$). There was not a significant association between change in PCV and age of product, with or without consideration of the total dose of blood. There were not sufficient pre- and posttransfusion clinicopathologic data available for Δ values to be analyzed.

The median age of the RBC product in the 333 blood transfusions studied was 28 days (range, 0–46 d; mean, 18 d). When comparing the development of transfusion-related complications to the age of the blood product, the odds of developing any transfusion-related complication significantly increased for every additional day by which the product aged (odds ratio, 1.05; 95% confidence interval, 1.03 – 1.07; $P < 0.001$). Hemolysis was the only transfusion-related complication associated with the product age, with the odds of hemolysis significantly increasing for every additional day by which the product aged (odds ratio, 1.11; 95% confidence interval, 1.06–1.16; $P < 0.0001$; Table 4).

There were 149 patients, encompassing 236 transfusion events, which survived to hospital discharge;

Table 4: Packed RBC transfusion related-complication proportions and odds by age classes of RBC product

Age of RBC product (in days)	Number of units	No. of events developing transfusion-related complication	Odds of transfusion-related complication (percent)	No. of events with transfusion-related hemolysis	Odds of transfusion-related hemolysis (percent)
Fresh	13	1	1:12 (0.08)	0	0:13 (0)
1–7	60	13	13:47 (0.28)	3	3:57 (0.05)
8–14	68	11	11:57 (0.19)	0	0:68 (0)
15–22	63	9	9:55 (0.17)	0	0:63 (0)
22–28	51	14	14:37 (0.38)	3	3:48 (0.06)
29–35	39	17	17:22 (0.77)	4	4:35 (0.11)
>35	39	19	19:20 (0.95)	11	11:28 (0.39)

43 patients (74 transfusion events) that were euthanized during their hospital stay; and 18 patients (23 transfusion events) that experienced in-hospital cardiac arrest. Seven of 18 natural in-hospital deaths occurred during or immediately after transfusion. One of these had consumptive coagulopathy secondary to heatstroke, and was in cardiac arrest at the time the transfusion was initiated; this dog was not included in further mortality analysis. In 4 patients that died naturally, death was anticipated regardless of the transfusion administration based on severity of the underlying condition, including 1 dog with caval syndrome recovering from manual heartworm extraction, 2 dogs with septic peritonitis (1 secondary to ruptured jejunal lymphoma, 1 with septic bile peritonitis), and 1 dog with multiple myeloma and pancytopenia. The final 2 patients were not expected to die. One was a previously stable patient with IMHA and the other was a dog that was recovering 24 hours following surgical excision of a deep axillary abscess. The patient with IMHA had stable physical examination parameters at the initiation of the transfusion; however, it suddenly developed respiratory distress and respiratory arrest 2 hours into the transfusion event. The dog was euthanized without a necropsy at the owner's request. The dog recovering from axillary abscess excision was anemic secondary to suspected nonsteroidal anti-inflammatory-induced gastrointestinal hemorrhage and received 2 pRBC transfusions 24 hours after surgery. Despite having no evidence of complications during the transfusions, the patient experienced cardiopulmonary arrest 45 minutes after the second transfusion was completed, and there was no return to spontaneous circulation despite resuscitative efforts. A necropsy on this dog did not reveal a direct cause of death, but did reveal severe bacterial cellulitis in the affected axilla, multifocal gastrointestinal ulceration, and acute hemoglobinuric nephrosis.

There was not a significant effect of product age ($P = 0.471$), the presence of transfusion-related complications ($P = 0.109$), fever ($P = 0.076$), or hemolysis ($P = 0.301$) on survival to discharge. Additionally, there was not a significant effect of the source of RBC product (U-BB vs commercial blood bank) on the odds of fever ($P = 0.545$), hemolysis ($P = 0.809$), or other transfusion-related complication ($P = 0.781$). Multiple transfusions administered to a single patient did not affect the odds of transfusion-related complication ($P = 0.131$).

Discussion

Consistent with reports in human[31] and veterinary medicine,[20,22] fever was the most commonly documented transfusion-related complication in this study, and occurrence of fever was not associated with increasing age of the RBC product. Hemolysis with fever is associated with activation of complement and production of cytokines.[3,32,33] Nonhemolytic fever, which occurs more commonly, is associated with recipient production of leukocyte-derived cytokines or anti-leukocyte antibodies.[1,34–36] Prior studies have shown that concentrations of leukocyte-derived pro-inflammatory cytokines increase in stored human blood products.[34,37,38] Concentration of the pro-inflammatory cytokine IL-8 increases with the age of stored, nonleukoreduced canine RBCs.[14] Likewise, blood stored with AS-5, as were some units used in this study, has been documented to have increased concentrations of pro-inflammatory cytokines, which can be attenuated by leukoreduction.[39] With the association between increased concentrations of inflammatory cytokines with increasing age of products, it is interesting that the occurrence of fever did not increase with product age in this study. Instead, fever occurred at all time points, raising the question as to whether leukoreduction would mitigate this febrile response. IL-8 does not have direct pyrogenic effects; rather, it is believed to prime leukocytes for pyrogenic responses to other cytokines.[14] Prospective veterinary studies comparing occurrence of febrile nonhemolytic transfusion reactions between leukoreduced and nonleukoreduced blood are warranted.

The development of fever in this population was significantly associated with slower product administration rate; however, it is unclear which came first. It is common

practice to slow or stop administration of a transfusion for a short time in patients with minor reactions, such as fever.[1] Alternately, a slower administration rate may have offered more opportunities for documentation of a transfusion reaction due to a prolonged period of focused monitoring.

The odds of transfusion-related hemolysis increased with each day the RBC products aged. Hemolysis can occur in vitro due to physical damage to RBCs during collection, processing, and storage of product, or during administration from shearing injury to the RBCs. It is also possible that poor venipuncture technique may have led to ex vivo hemolysis in recipient blood samples when blood was sampled for posttransfusion PCV evaluation. Hemolysis can occur in vivo due to storage-associated increases in RBC fragility, or from an in vivo immune-mediated process such as AHTR. In this study, dogs that received crossmatch-compatible blood products developed hemolysis at the same rate as dogs whose blood had not been crossmatched to donor product prior to transfusion. Lack of effect of crossmatch in this population could reflect the large number of first-time transfusion recipients and those receiving subsequent transfusions within 4 days of the initial transfusion. Also, administration of crossmatch-compatible product does not affect the likelihood of nonimmunologic transfusion reactions or complications. It is unclear what caused the transfusion-related hemolysis documented in this study because there was no information in the records regarding the presence or degree of hemolysis in any of the RBC products prior to or during administration. At the study institution, transfusion protocol recommends saving samples of patient and donor blood in the case of reaction; however, the attending clinician ultimately decides what to do in the event of an adverse reaction.

It is interesting that approximately 1/3 of the transfusion-associated hemolysis occurred in dogs with IMHA that were previously documented to have only extravascular hemolysis. While IMHA is a dynamic disease process, in the authors' experience, it would be unusual for a patient with evidence of only extravascular IMHA to develop signs of intravascular IMHA during the course of treatment. This frequent occurrence of transfusion-associated hemolysis in dogs with IMHA could reflect that IMHA was the single most common reason for patients to require a transfusion. It could also reflect true immune-mediated destruction, with or without the confounding factor of RBC fragility due to storage changes that may lead to shortened RBC lifespan.

Free hemoglobin concentration increases over time in stored RBC units, and can be used as a measure of the quality of RBC product.[40–42] The Food and Drug Administration allows for human RBC products to be stored for a maximum of 42 days with an additive nutrient solution such as AS-5.[13,43] However, the Food and Drug Administration also mandates quality control such that at the end of a storage period, ≥75% of the RBCs be recoverable 24 hours after transfusion, with <1% hemolysis in the bag prior to transfusion.[13,44] There are no such mandates for veterinary blood products at this time, although the generation of specific guidelines for allowable hemolysis may be of benefit.[45] With a median age of 28 days for this study's pRBC units, many of the transfused units were older, and may have contained higher free hemoglobin concentrations than younger units. Given the retrospective nature of this study, it is impossible to know the exact origin of transfusion-related hemolysis in these cases. Preadministration identification of gross hemolysis in a stored blood product or measurement of large amounts of free hemoglobin in the product should preclude product use. For future studies, free hemoglobin concentration measurement in blood products prior to transfusion, and in patients prior to, during, and after transfusion, are recommended to better determine the nature of transfusion-related hemolysis.

Free circulating hemoglobin can be detrimental to patients regardless of the source. Circulating free hemoglobin can lead to vasoconstriction due to nitric oxide scavenging.[46] Clinical consequences of nitric oxide depletion include increased platelet aggregation and thrombosis,[47] altered endothelial permeability,[48] and smooth muscle contraction.[49] Hemolysis may also cause complement cascade activation and induce systemic inflammatory response syndrome due to cytokine release from leukocytes.[49,50] Additional documented deleterious effects of circulating cell-free hemoglobin include vascular damage, hypertension, and kidney injury.[51]

Transfusion of older RBCs is also associated with extravascular clearance of senescent RBCs by the reticuloendothelial system, leading to increases in circulating nontransferrin-bound iron that deposits in tissues and causes inflammation.[52,53] In human volunteers, transfusions of 42-day-old autologous RBCs were associated with increased serum total bilirubin and serum iron concentrations, consistent with extravascular hemolysis.[53] Elevated concentrations of nontransferrin-bound iron can impair host defenses against Gram-negative bacteria,[52] and thus may contribute to complications such as infection.[53]

Administration techniques, such as use of a rotary infusion pump, may also contribute to hemolysis in blood products.[54] A decrease in short-term in vivo RBC survival has been demonstrated in canine RBCs administered by volumetric pump (through a 170–260 μm filter) or by syringe pump (through an 18 μm filter), when compared to administration by gravity flow drip through a 170–260 μm filter.[55] In the current study, most transfusions were administered by a volumetric peristaltic

pump and there was no statistical association detected between delivery method and the occurrence of hemolysis. While boluses in ICU were most often delivered using the syringe technique and those in anesthesia using gravity drip, there was no definitive way to determine retrospectively how a bolus was administered in most cases.

There was a small but statistically significantly greater increase in PCV in patients with any type of transfusion-related complication than in those without. Acute hemolytic transfusion reactions can present within 24 hours after transfusion,[56,57] and collection times for post-transfusion PCV sampling were not standardized in this retrospective study. It is possible that additional AHTR were missed due to timing of the sample collection. Additionally, since the dose of blood and the change in PCV was higher in patients with transfusion-related complications than in those without, the higher volume of blood administered may reflect disease severity. A recent retrospective study in critically ill dogs suggested that a higher dose of RBCs may be a risk factor for mortality.[20] These findings, along with the published benefits of a conservative transfusion target demonstrated in people, highlight the need for prospective veterinary studies regarding possible benefits of more conservative transfusion strategies.[58,59]

There was no association between the 3 general categories of anemia and the occurrence of transfusion-related complications. The most common general category of anemia requiring transfusion was blood loss, which is similar to findings of other veterinary studies regarding transfusion.[21–23] Similar to other studies,[20,21] immune-mediated disease was the most common definitive diagnosis for patients requiring transfusion, with the majority having IMHA (106 transfusion events in 54 patients), and some having hemorrhage associated with ITP (15 events in 6 patients). Dogs with immune-mediated disease were more likely to have signs of any type of transfusion reaction. There was an association between provision of immunosuppressive therapy for IMHA and occurrence of all transfusion-related complications, as well as transfusion-related fever specifically. However, there was no association between transfusion-related hemolysis and immunosuppressive therapy for IMHA, which is consistent with previous findings.[24] Fever could be attributable to IMHA itself, since these patients have pro-inflammatory disease.[60] However, fevers in these patients appeared to be isolated events, associated only with transfusion administration and not specifically with underlying disease. The low number of patients with ITP precluded separate statistical analysis of this group.

Administration of DEA 1.1 negative blood to untyped patients was not associated with greater occurrence of transfusion-related complications. Lack of such association may be due to the relatively small population of untyped patients in this study, and the large number of first-time blood product recipients. Since autoagglutination precludes blood typing via card agglutination method, this institution has since changed to an immunochromatographic blood typing test,[k] which enables more reliable determination of blood type in the face of autoagglutination.[61]

The results of this study suggest that administration of older RBC products contributed to development of transfusion-related hemolysis. Regardless of the cause of hemolysis, it is possible that reduction in RBC product storage duration may reduce the incidence of transfusion-related hemolysis. Reducing storage duration of RBC products could have implications on blood banking procedures. Veterinary practices that rely on stored RBC products may need to more tightly control inventory, may experience more frequent product expiration or shortages, or may require the addition of more blood donors. Commercial blood banks could also see a change in patterns of product demand. Any of these changes could translate to an increased cost for blood products.

Use of prestorage leukoreduction of veterinary blood products has been recommended to reduce the frequency of transfusion reactions.[62,63] The cost versus clinical efficacy of leukoreduction has been debated, as the theoretical benefits of leukoreduction do not necessarily translate into reduction in clinical transfusion reactions. Multiple in vitro studies have shown that leukoreduction may decrease the inflammatory effect of RBC transfusions, diminish the frequency of febrile nonhemolytic reactions in people, and may decrease the numbers of bacterial contaminants.[64-66] Leukoreduction can reduce the concentration of WBCs and platelets within the stored product,[66,67] which could reduce potential for febrile transfusion reactions. In some studies, leukoreduction has decreased the incidence of febrile nonhemolytic reactions associated with RBC transfusions,[68] and in vitro storage-associated hemolysis in pRBC units by almost 50%.[69] However, Callan et al[70] showed that transfusion of either leukoreduced or non-leukoreduced older autologous transfusions to healthy dogs caused an inflammatory response. The current study found that fever lacked impact on patient outcome and was not associated with product age. As such, it is unclear whether leukoreduction would be clinically beneficial. Additional studies in this area are warranted.

There are several limitations to this study, many due to its retrospective nature. The study population consisted of a large, heterogeneous group of canine patients, and thus stratification by illness severity using the acute patient physiologic and laboratory evaluation (APPLE)

score or survival prediction index (SPI2) would have mitigated some external factors that may have affected the analyses.[71,72] Unfortunately, only approximately half of the dogs studied had all the data necessary to calculate either score. There were 4 months of transfusion events missing from the logbook, which is the only place where product age was recorded. The approximate age of blood products was calculated based on the dates of expiration and administration, rather than by the date of actual blood collection. Some dogs received multiple transfusions in a short period of time, which made it difficult to ascribe clinical signs to 1 particular RBC product, and thus the development of transfusion-related complications was attributed to the oldest transfusion, which confounds the emphasis placed on product age. Additionally, there was no record for which pRBC units contained an additive solution such as AS-5, although all of the units from the commercial blood bank contained AS-5 and most units from U-BB contained it.

Consistent monitoring of transfusion events and documentation of transfusion-related complications were another limitation for this study. Transfusion monitoring was done by different people with different levels of clinical experience. There are standardized transfusion monitoring protocol sheets in the ICU; however, different observers may have interpreted situations differently. While only 1 author retrospectively analyzed the files for transfusion-related complication data, interpretation of records is still subjective in nature and is reliant on accurate medical record keeping. This study only identified acute transfusion related complications, and did not record any delayed complications. As mentioned previously, preadministration analysis of donor units for hemolysis was not performed, making it difficult to determine whether in vitro versus in vivo hemolysis (immunologic versus nonimmunologic), or both, contributed to the events included here as transfusion-related hemolysis. Due to the retrospective nature of this study, not all of the complications could be definitively linked to transfusions or differentiated from clinical signs of underlying disease, resulting in subjectivity of interpretation. Finally, transfusion-related complications and clinical signs associated with transfusion administration do not necessarily denote causality.

Conclusion

Fever was the most commonly identified transfusion-related complication and was not associated with the age of transfused product. Blood product age was associated with an increased odds of transfusion-related complications and, more specifically, with development of transfusion-related hemolysis. Whether hemolysis was immunologic or nonimmunologic in origin could not be determined. Product age did not affect acute mortality rate. In an attempt to reduce the frequency of transfusion-related hemolysis, prospective veterinary clinical trials should be designed to further describe the effects of RBC storage time on occurrence of hemolysis in vivo and in vitro, and to differentiate in vivo hemolysis due to immune destruction from storage-induced change. Finally, higher doses of blood product were associated with increased frequency of transfusion-related complications, which warrants further studies regarding the potential benefit of a conservative transfusion strategy in dogs.

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Footnotes

[a] Animal Blood Resources International (ABRI), Dixon, CA.
[b] TERUFLEX Blood Bags TerumoBCT, Inc, Lakewood, CO.
[c] Optisol, TerumoBCT, Inc.
[d] Fischer Scientific Isotemp Plus, Fisher Scientific Company LLC, Pittsburgh, PA.
[e] RapidVet-H Canine 1.1, DMS Laboratories, Flemington, NJ.
[f] Baxter INTERLINK System, Straight Type Blood Set (10 drops/mL), Baxter Healthcare Corporation, Deerfield, IL.
[g] Baxter Travenol FLO-GARD ™ 6200 Volumetric Infusion Pump, Baxter Healthcare Corporation.
[h] Baxter Model AS50 Infusion Pump, Baxter Healthcare Corporation.
[i] HEMO-NATE Filter, Utah Medical Products, Midvale, UT.
[j] SAS Statistical Software, Version 9.2, SAS Institute, Cary, NC.
[k] AlvediaVET QUICK Test DEA 1.1, Alice Veterinary Diagnostic, Limonest, France.

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