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Effect of washing units of canine red blood cells on storage lesions

Ashley C. Coll[1] | Matthew K. Ross[2] | Matthew L. Williams[3] | Robert W. Wills[2] | Andrew J. Mackin[1] | John M. Thomason[1]

[1]Department of Clinical Sciences, College of Veterinary Medicine, Mississippi State University, Mississippi State, Mississippi, USA

[2]Department of Comparative Biomedical Sciences, College of Veterinary Medicine, Mississippi State University, Mississippi State, Mississippi, USA

[3]Department of Pathobiology and Population Medicine, College of Veterinary Medicine, Mississippi State University, Mississippi State, Mississippi, USA

Correspondence
John M. Thomason, Department of Clinical Sciences, College of Veterinary Medicine, Mississippi State University, PO Box 6100, Mississippi State, MS 39762-6100, USA.
Email: thomason@cvm.msstate.edu

Abstract

Background: In humans, washing stored blood products before transfusion reduces storage lesions and incidence of transfusion reactions, but the effectiveness of washing canine blood is unknown.

Objectives: The objective was to determine if manually washing units of stored blood would reduce storage lesions without adversely affecting erythrocytes. We hypothesized that washing stored units would reduce concentrations of storage lesions and cause minimal erythrocyte damage.

Animals: Eight healthy research dogs.

Methods: Repeated measure cohort study. Units of whole blood were stored for 28 days and washed 3 times with 0.9% NaCl. Blood samples were collected before and after storage, after each wash, and after being held at a simulated transfusion temperature. Variables measured included CBC variables, blood gas analysis, erythrocyte morphology, mean corpuscular fragility (MCF), and eicosanoid concentrations. A Friedman's test was used to evaluate changes in variables ($P < .05$ was considered significant).

Results: After the first wash, compared to values after storage, there was a significant decrease in potassium (4.3 mmol/L [4.0-4.7] to 1.2 mmol/L [1-1.6]; $P < .0001$, median [range]), lactate (1.45 mmol/L [1.07-1.79] to 0.69 mmol/L [0.39-0.93]; $P = .002$), and partial pressure carbon dioxide (102 mm Hg [80.2-119.2] to 33.7 mm Hg [24.5-44.5]; $P < .0001$), and increase in MCV (69.3 fL [65.7-72.3] to 74 fL [69.6-79.5]; $P = .0003$), and MCF (0.444 fL [0.279-0.527] to 0.491 fL [0.43-0.616]; $P = .0006$).

Conclusions and Clinical Importance: A single wash of stored whole blood significantly reduces most extracellular storage lesions, and additional washing might cause hemolysis.

ABBREVIATIONS: 6-keto-$PGF_{1\alpha}$, 6-ketoprostaglandin F1alpha; AA, arachidonic acid; CPDA, citrate-phosphate-dextrose solution with adenine; Hct, hematocrit; HETE, hydroxyeicosatetraenoic acid; Hgb, hemoglobin; HODE, hydroxyoctadecadienoic acid; iCa, ionized calcium; iMg, ionized magnesium; LOX, lipoxygenase; MCF, mean corpuscular fragility; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; MetHgb, methemoglobin; pCO_2, partial pressure carbon dioxide; PGD_2, prostaglandin D_2; PGE_2, prostaglandin E_2; $PGF_{2\alpha}$, prostaglandin F2alpha; pO_2, partial pressure oxygen; pRBC, packed red blood cell; RBC, red blood cell; RDW, red blood cell distribution width; SIRS, systemic inflammatory response syndrome; sO_2, oxygen saturation; Tbili, total bilirubin; TCO_2, total carbon dioxide; TRALI, transfusion associate lung injury; TXB_2, thromboxane B_2; WBC, white blood cells.

KEYWORDS
eicosanoid, hemoglobin, manual centrifugation, transfusion

1 | INTRODUCTION

Stored blood products such as whole blood and packed red blood cells (pRBC) are commonly used in veterinary medicine. Both storage duration and environment can have a substantial impact on red blood cell (RBC) fragility, leading to both in vitro (pre-transfusion) and in vivo (post-transfusion) hemolysis. The changes that occur within units of stored blood, known as storage lesions, can create biochemical, biomechanical, and immunologic events that lead to in vitro and in vivo hemolysis.[1] Prolonged storage can cause changes in RBC shape and decrease the circulating survival time of RBCs after transfusion. In humans, the accumulation of vasoactive and pro-inflammatory molecules, including cytokines and arachidonic acid (AA)-derived eicosanoids, and increased RBC fragility[2-4] increase the risk of transfusion reactions.[5]

Several methods have been used to reduce the accumulation of storage lesions, improve erythrocyte health, prolong RBC survival in the recipient, and reduce the risk and severity of transfusion reactions. Leukoreduction and platelet depletion before storage decrease many inflammatory mediators and storage lesions in humans and dogs.[2,6-13] However, leukoreduction and platelet depletion cause an immediate increase in concentrations of TXB_2 and do not eliminate the accumulation of 6-ketoprostaglandin F1alpha (6-keto-$PGF_{2\alpha}$) in units of canine whole blood.[8] Irradiation of stored canine pRBCs to inactivate lymphocytes has minimal impact on storage lesion development.[14] Advancements in cell preservative solutions have improved erythrocyte health and prolonged storage durations but have not eliminated storage lesions.[15]

Washing RBCs before transfusion can remove up to 95% of the plasma/supernatant within the unit, including proteins, electrolytes, cytokines, microparticles, antibodies, cellular debris, and other storage lesions.[10,16-18] In humans, the use of washed blood products before transfusion reduces transfusion reactions, risk of transfusion-associated lung injury, and hyperkalemia in transfusions to infants.[19-22] Washing of blood products can reduce the accumulation of storage lesions and risk of transfusion reactions, but can increase RBC fragility and hemolysis.[10,16,17,23]

There are multiple washing techniques including an automated closed system and centrifugation techniques. These techniques use saline or other isotonic/preservative fluids to eliminate storage lesions while maintaining RBC health.[16] Automated closed systems can be expensive and are not readily available in veterinary hospitals. Therefore, if washing of blood products is going to be utilized in veterinary clinics, manual techniques would probably be the most commonly used method. In veterinary medicine, the most effective manual washing technique, which eliminates storage lesions and perseveres RBC health, is unknown.

The objective of this study was to determine if a manual method of washing can effectively reduce storage lesions that have accumulated in units of stored canine whole blood without causing adverse effects to erythrocytes. Our hypotheses were that washing units of canine whole blood after storage (a) would decrease measurable storage extracellular lesions before being held at a simulated transfusion temperature, with no subsequent increase in lesions while held at a simulated transfusion temperature, (b) would cause minimal erythrocyte damage, evaluated by hemolysis, morphology, and function, and (c) could be performed in a sterile fashion.

2 | METHODS AND MATERIALS

2.1 | Animals

Eight healthy adult research dogs, 5 intact females and 3 castrated males, were used in this study. The mean age of the dogs was 5.1 years (range, 3.5-6 years) and the mean weight was 27.2 kg (range, 24.6-36.1 kg). The dogs were deemed healthy based on no abnormalities on physical examination, CBC (including manual platelet count), serum biochemistry, urinalysis, and heartworm and tick-borne disease testing. The dogs were not exposed to any medications or vaccines for at least 2 weeks before the initiation of the study. A sample size calculation was performed using results from previous studies[10,20,24] performed in humans. The calculation was performed based on concentrations of lactate, potassium, and glucose in blood products before and after washing. Based on these results, 8 dogs were calculated to be needed to have 80% power to find a 25% reduction in storage lesions with an alpha of .05. Animal use was approved by the Mississippi State University College of Veterinary Medicine Institutional Animal Care and Use Committee and was in compliance with the requirements of the American Association of Accreditation of Laboratory Animal Care.

2.2 | Donation and washing technique

Each dog underwent standard blood donation. The donors were sedated with acepromazine (0.02 mg/kg IV) (Henry Schein Inc; Dublin, Ohio) and butorphanol (0.1 mg/kg IV) (Zoetis, Kalamazoo, Michigan) and positioned in either right or left lateral recumbency. The hair overlaying the right jugular vein was clipped and the skin was aseptically cleaned. A 16-gauge needle was inserted into the jugular vein and, under negative pressure, approximately 450 mL of blood was collected into a standard blood banking bag containing citrate phosphate dextrose adenine solution (Teruflex blood bag system, Terumo, Tokyo, Japan). After adequate mixing of blood with storage bag preservative, blood was allowed to fill the collection tubing again and a blood sample was obtained before storage (*pre-storage*). There were no adverse

events detected in the dogs during or after blood collection. All units were stored vertically at 4°C in a dedicated refrigerator. After 28 days of storage, the units were removed from refrigeration and gently rocked for 1 minute. A sterile bag access spike (Clave IV Bag Access Device; ICU Medical, San Clemente, California) was inserted into the unit and a blood sample (8 mL) was collected (*post-storage*).

Each unit was manually washed using a protocol previously used in human medicine.[23] After collection of the post-storage sample, each unit was centrifuged for 10 minutes at 5°C and 4000*g*. To remove the plasma/supernatant after centrifugation, external pressure was applied to the unit, and the supernatant was extracted through a syringe attached to the bag access spike. The unit was resuspended with 250 mL of cold (5°C), sterile 0.9% NaCl (Baxter, Copperfield, Illinois), gently rocked for 1 minute, and a blood sample was collected (*wash 1*). This centrifugation process was repeated until a total of 3 washes were performed. Samples were collected at the completion of each wash step (*wash 2* and *wash 3*). After the third wash, the units were centrifuged, the wash saline was removed and 100 mL of 0.9% NaCl was added to the units. To simulate a transfusion of pRBCs, the units remained at room temperature (25°C) for 5 hours. After being held at a simulated transfusion temperature, the units were gently rocked for 1 minute and a final sample was collected (*post-transfusion temperature*).

2.3 | Blood gas analysis

Blood gas analysis (Stat Profile Prime Plus Critical Care Analyzer, NOVA biomedical, Waltham, Massachusetts) was performed within 30 minutes of sample collection, except for 1 post-transfusion temperature sample that was analyzed 7 hours after collection because of instrument malfunction. If the blood gas analyzer was unavailable, measurement of blood lactate (Lactate Plus, NOVA Biomedical, Waltham, Massachusetts) and blood glucose (AlphTrak-2 glucometer, Zoetis, Kalamazoo, Michigan) was performed separately. The blood gas analyzer included: pH, partial pressure carbon dioxide (pCO_2), partial pressure oxygen (pO_2), oxygen saturation (sO_2), Na, K, Cl, iCa, iMg, glucose, lactate, methemoglobin (MetHgb), total bilirubin (Tbili), fetal hemoglobin (HgbF), and total carbon dioxide (TCO_2).

2.4 | Erythrocyte osmotic fragility

Erythrocyte osmotic fragility was completed within 6 hours of sample collection using a dilution technique.[25,26] For each blood sample, serial dilutions of a 1% NaCl solution (5 mL) were added to 16 tubes to create final concentrations of 0.0% to 0.85% NaCl (increments of 0.05% NaCl). From each sample, 20 μL of blood was added to each tube, gently mixed, and remained undisturbed at room temperature for 30 minutes. The average hematocrit (Hct) of blood added to each tube ranged from 34.6% to 39.8% in the pre-storage, post-storage, wash 1, wash 2, and wash 3 samples, and 53.5% in post-transfusion temperature samples. Samples were centrifuged at 2000*g* for 10 minutes, and the optical density of the supernatant was measured in triplicate at a wavelength of 540 nm by use of a plate reader (SpectraMax M5 multimode microplate reader, Molecular Devices LLC, Sunnyvale, California). The percent hemolysis was calculated by dividing the optical density of the blood samples at each NaCl concentration by the optical density of the blood sample at 0.0% NaCl. The mean corpuscular fragility (MCF) was calculated to determine which NaCl concentration was associated with 50% hemolysis.

2.5 | CBC and erythrocyte morphology

A CBC was performed within 4 hours of collection using an automated hematology analyzer (Cell-dyn 3700, Abbott Laboratories, Taguig City, Philippines). The analyzer provided the following variables: RBC count, white blood cells count, platelet count, Hct, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red blood cell distribution width (RDW), and hemoglobin (Hgb).

Erythrocyte morphology was evaluated by a blinded board-certified veterinary clinical pathologist (MLW). For each blood sample, a blood film was prepared with a standard Wright's stain and 10 high-power fields (hpf; 1000× oil magnification) were evaluated. Microscopic fields were scanned for abnormal RBCs (eg, echinocytes, keratocytes, acanthocytes, schistocytes, and target cells) and the frequency of abnormal cells was scored using a subjective scale as rare (1 to 10 abnormal cells/hpf), few (11 to 50 abnormal cells/hpf), moderate (51 to 100 abnormal cells/hpf), and many (> 100 abnormal cells/hpf).

2.6 | Aerobic and anaerobic bacterial culture

Using blood-agar plates, aerobic and anaerobic cultures were performed on the *post-storage* and *post-transfusion temperature* samples. Samples were considered aseptic if no bacterial growth was detected after 48 hours of observation. If bacterial growth was detected, further identification was performed to determine the species of the isolated bacterial colony.

2.7 | Eicosanoid analysis

After collection, blood samples were centrifuged at 1800*g* for 8 minutes at 25°C, and the supernatant was frozen in liquid nitrogen and stored at −80°C for later analysis. Using a previously established liquid chromatography mass spectrometry technique,[8,27] with modifications to the sample workup procedure described below, the concentrations of AA, prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), prostaglandin E_2 (PGE_2), prostaglandin D_2 (PGD_2), thromboxane B_2 (TXB_2, a stable thromboxane A_2 metabolite), 6-keto-$PGF_{1\alpha}$ (a stable prostacyclin metabolite), hydroxyeicosatetraenoic acid (HETE), and hydroxyoctadecadienoic acid (HODE) were measured.

For analysis, deuterated internal standards were added to the thawed plasma samples in ice-cold 1 : 1 (v/v) methanol/acetonitrile and mixed. After chilling at −20°C for 30 min, the samples were centrifuged at 16100*g* for 10 min at 4°C and the precipitated proteins

removed. The eicosanoids in the concentrated samples were extracted by using a C18 SepPak column (HyperSep Retain PEP 60 mg, 1 mL, Thermo Fisher Scientific, Waltham, Massachusetts). The samples were dried under nitrogen gas, resolubilized, and injected onto a Waters C18 column (1.7 μm particle size, 100 × 2.1 mm^2 internal diameter) (Acquity UPLC BEH C18 column, Waters Corporation, Milford, Massachusetts). The analytes were eluted with a gradient program and introduced into a mass spectrometer (TSQ Quantum Access Max, Thermo Fisher Scientific Inc). The concentration of each eicosanoid was determined by measuring the area under each chromatographic peak and comparing this result to the area under the chromatographic peak for the internal standard. The eicosanoid concentrations were normalized to the volume of plasma used for analysis and expressed as pmol/mL plasma. The estimated limits of detection were between 0.1 and 10 nM.

2.8 | Statistical analysis

Descriptive and inferential statistics were performed using commercially available statistic software (SAS for Windows v. 9.4, SAS Institute, Inc Cary, North Carolina). Impact of stored blood washing on outcomes of blood gas values, CBC values, and eicosanoid concentrations was initially assessed using a generalized linear mixed model; however, models did not meet the assumptions of homoscedasticity and normality of the residuals. Consequently, a method similar to the nonparametric Friedman's test was used. Outcomes were first ranked by dog, then analyzed by linear models. Dog identity and sample were included as fixed effects in each model. The least square means of the following sample times in the washing protocol were compared: pre-storage vs post-storage, pre-storage vs wash 1; pre-storage vs wash 2; pre-storage vs wash 3; post-storage vs wash 1; post-storage vs wash 2; post-storage vs wash 3; post-storage vs. post-transfusion temperature; wash 1 vs wash 2; wash 1 vs wash 3; wash 2 vs wash 3; wash 3 vs post-transfusion temperature. A simulation-based method was used to adjust *P*-values for multiple comparisons among sample times. An alpha level of .05 was used to determine statistical significance.

3 | RESULTS

3.1 | Blood gas analysis

Because of malfunctions with the analyzer, results for post-storage, wash 1, wash 2, wash 3, and post-transfusion temperature samples

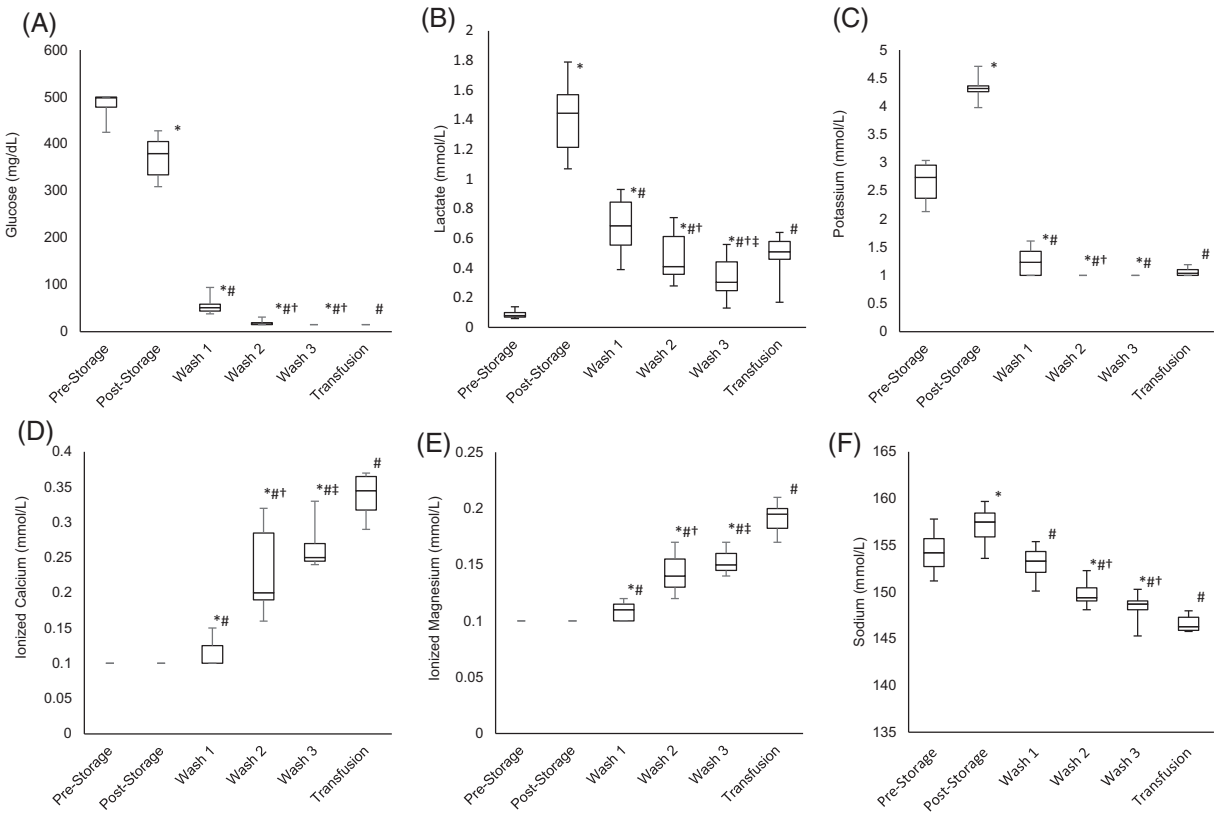

FIGURE 1 Blood gas analysis results (glucose (A), lactate (B), potassium (C), ionized calcium (D), ionized magnesium (E), sodium (F)) in 8 units of canine whole blood at the time of collection (pre-storage), after 28 days of storage at 4°C (post-storage), after 3 washes with 250 mL 0.9% NaCl (wash 1, wash 2, and wash 3), and after being held at a simulated transfusion temperature for 5 hours (transfusion). The box and whiskers plot demonstrate the median (line), interquartile range (box), and total range (whiskers). *Indicates a significant ($P < .05$) difference from pre-storage; #Indicates a significant ($P < .05$) difference from post-storage; †Indicates a significant ($P < .05$) difference from the previous wash; ‡Indicates a significant ($P < .05$) difference after units being held at a simulated transfusion temperature

were not completely evaluated in 1 unit of blood and data for these samples consisted of only blood glucose and lactate measurements.

The results for glucose, lactate, potassium, sodium, iCa, and iMg are represented in Figure 1 and the remaining blood gas results are listed in Table 1. There was a significant ($P < .0001$) increase in lactate concentration after storage, but the lactate concentration decreased after each wash (wash 1, $P = .0016$; wash 2, $P = .0002$, wash 3, $P = .0006$), but increased significantly ($P = .0003$) in the post-transfusion temperature samples. There was a significant decrease in glucose concentration ($P < .0001$) during storage and after each wash (wash 1, $P < .0001$; wash 2, $P < .0001$; wash 3, $P < .0003$). There was no change ($P = .99$) in glucose concentration in the post-transfusion temperature samples.

The potassium concentration increased ($P = .01$) during storage, but decreased significantly after wash 1 ($P < .0001$) and wash 2 ($P = .0006$). There was no change ($P = .69$) in potassium concentration in the post-transfusion temperature samples. The sodium concentration increased ($P = .02$) during storage, but decreased significantly (wash 1, $P < .0001$; wash 2, $P < .0001$; wash 3, $P < .0001$) after each wash. There was no difference ($P = .11$) in sodium concentration between the pre-storage and wash 1 samples. The chloride concentration increased after each wash (wash 1, $P = .0002$; wash 2, $P < .0001$; wash 3, $P = .0002$). There was no change in the ionized calcium (iCa) ($P = 1.000$) and ionized magnesium (iMg) ($P = 1.000$) during storage, but there was a progressive increase in both variables after each wash and in the post-transfusion temperature samples.

3.2 | CBC results and erythrocyte morphology

The CBC results are summarized in Table 2. There were no statistically significant changes in RBC variables between pre- and post-storage samples. There was a significant increase in RBC count ($P = .002$), Hct

TABLE 1 Blood gas analysis results (mean ± SD) from 8 units of canine whole blood at donation (pre-storage), storage (post-storage), 3 washes with 0.9% NaCl (wash 1, wash 2, and wash 3), and after being held at a simulated transfusion temperature (transfusion)

	Pre-storage	Post-storage	Wash 1	Wash 2	Wash 3	Transfusion
pH	6.91 ± 0.21	6.63 ± 0.04[a]	6.59 ± 0.08[a]	6.44 ± 0.31[a,b,c]	6.20 ± 0.43[a,b]	5.88 ± 0.31[b]
pCO_2 (mm Hg)	53.1 ± 12	100.7 ± 16.1[a]	35.8 ± 6.7[a,b]	14.5 ± 6.8[a,b,c]	4.7 ± 3.4[a,b,c]	3.2 ± 4.1[b]
pO_2 (mm Hg)	89 ± 10	95.7 ± 36	107.4 ± 45.6	105.8 ± 34.8[a,b]	106.3 ± 28.1[a,b]	112.4 ± 42.3[b]
sO_2 (%)	91.3 ± 5.5	84.1 ± 7.5[a]	87.9 ± 6.3	88 ± 5.9	89.3 ± 5.3[b]	89.7 ± 5.3
TCO_2 (mmol/L)	12.4 ± 2.2	13.8 ± 1.7	3.7 ± 1.5[a,b]	2.5 ± 0[a,b,c]	2.5 ± 0[a,b]	2.5 ± 0[b]
HgbF (g/dL)	1.2 ± 0.5	1.1 ± 0.7	1.0 ± 0.6	0.7 ± 0.4	1.2 ± 0.5	0.7 ± 0.7
MetHb (%)	0.6 ± 0.2	1 ± 0.4	1.9 ± 0.4[a,b]	1.8 ± 0.4[a,b]	2 ± 0.4[a,b,d]	1.5 ± 0.3[b]
Tbili (mg/dL)	1.8 ± 0.4	2.5 ± 1.8	3.1 ± 2.1[a,b]	3.1 ± 1.8[a,b]	3.2 ± 2[a,b,d]	1 ± 0.6

[a]Indicates a significant ($P < .05$) difference from pre-storage.
[b]Indicates a significant ($P < .05$) difference from post-storage.
[c]Indicates a significant ($P < .05$) difference from the previous wash.
[d]Indicates a significant ($P < .05$) difference after units being held at a simulated transfusion temperature.

TABLE 2 CBC variables and MCF results (mean ± SD) in 8 units of canine whole blood at the time of donation (pre-storage), after 28 days of storage (post-storage), after 3 washes 0.9% NaCl (wash 1, wash 2, and wash 3), and being held at a simulated transfusion temperature (transfusion)

	Pre-storage	Post-storage	Wash 1	Wash 2	Wash 3	Transfusion
WBC ($10^3/\mu L$)	4.43 ± 1.3	4.69 ± 1.4	3.86 ± 1.4[a,b]	3.4 ± 1.5[a,b,c]	3.22 ± 1.5[a,b,d]	5.22 ± 1.6
RBC ($10^6/\mu L$)	5.06 ± 0.8	5.47 ± 0.8	5.36 ± 0.8	5.17 ± 0.8	5.04 ± 0.8[d]	7.01 ± 0.9
HGB (g/dL)	12.51 ± 2.0	13.28 ± 2.0	13.25 ± 2.0[a]	12.76 ± 2.0	12.58 ± 2.0[d]	17.54 ± 2.1
HCT (%)	34.61 ± 6.6	37.76 ± 6.6	39.83 ± 6.6[a]	38.43 ± 6.6[a]	37.46 ± 6.6[d]	53.4 ± 7.1[b]
MCV (fL)	68.5 ± 4.5	69.08 ± 4.4	74.45 ± 4.2[a,b]	74.54 ± 4.2[a,b]	74.58 ± 4.2[a,b]	76.41 ± 4.1[b]
MCH (pg)	24.78 ± 0.9	24.28 ± 0.7	24.79 ± 0.7	24.78 ± 0.7	25.03 ± 0.8[b]	24.99 ± 0.8[b]
MCHC (g/dL)	36.18 ± 1.9	35.15 ± 1.9[a]	33.35 ± 1.9[a,b]	33.3 ± 1.9[a,b]	33.61 ± 1.9[a,b]	32.74 ± 1.9[b]
RDW	14.64 ± 1.1	15.28 ± 1.1	15.85 ± 1.1[a]	16.03 ± 1.1[a,b]	15.95 ± 1.1[a,b,d]	17.1 ± 1.2[b]
PLT ($10^3/\mu L$)	149 ± 38.8	91.25 ± 38.8[a]	73.63 ± 39.3[a]	81.63 ± 39.2[a]	84.75 ± 39.1[a,d]	146 ± 39.6[b]
MCF (%)	0.487 ± 0.1	0.430 ± 0.1	0.504 ± 0.1[b]	0.490 ± 0.1[b]	0.575 ± 0.1[b]	0.569 ± 0.1[b]

[a]Indicates a significant ($P < .05$) difference from pre-storage.
[b]Indicates a significant ($P < .05$) difference from post-storage.
[c]Indicates a significant ($P < .05$) difference from the previous wash.
[d]Indicates a significant ($P < .05$) difference after units being held at a simulated transfusion temperature.

($P = .0006$), Hgb concentration ($P = .0023$), and RDW ($P = .04$) between wash 3 and the post-transfusion temperature samples. The Hct also increased, compared to pre-storage, after wash 1 ($P = .0011$) and wash 2 ($P = .03$).

The MCV, MCHC, and RDW results are presented in Figure 2. Compared to pre- and post-storage samples, there was a significant increase in MCV after each wash, but there no differences between each wash. The MCV was significantly ($P < .0001$) greater in the post-transfusion temperature samples compared to post-storage samples. Compared to pre- and post-storage samples, there was a significant decrease in MCHC after each wash, but not between individual washes. Compared to post-storage samples, there was a significant ($P < .0001$) decrease in the MCHC in the post-transfusion temperature samples. Compared to pre-storage values, the RDW increased after each wash (wash 1, $P = .0009$; wash 2, $P < .0001$; wash 3, $P = .0002$), but not between each wash. When compared to post-storage values, there was no change in RDW after wash 1 ($P = .13$), but there was an increase after wash 2 ($P = .01$), wash 3 ($P = .01$), and the post-transfusion temperature samples ($P < .0001$).

Compared to pre- and post-storage samples, there was a significant decrease in WBC count after all 3 washes. There was a progressive decrease ($P = .03$) in WBC count between the first 2 washes, but there was no difference ($P = .93$) between washes 2 and 3. There was a significant ($P < .0001$) increase between wash 3 and the post-tranfusion temperature samples.

Compared to pre-storage samples, there was a significant ($P < .0001$) decrease in platelets after storage and each wash (wash 1, $P < .0001$; wash 2, $P < .0001$; wash 3, $P = .0003$). However, when compared to post-storage samples, there was no difference in platelet count after each wash, but there was a significant ($P = .0006$) increase between wash 3 and post-transfusion temperature samples.

The erythrocyte morphology results are presented in Table 3.

3.3 | Erythrocyte osmotic fragility

The MCF results are summarized in Table 2. Compared to the post-storage sample, there was a significant increase in MCF after wash 1 ($P = .0006$), wash 2 ($P = .0048$), and wash 3 ($P < .0001$).

3.4 | Eicosanoid analysis

The eicosanoid results are summarized in Table 4. Arachidonic acid concentrations significantly ($P = .0006$) increased during storage, but compared to pre- and post-storage samples, AA concentrations progressively decreased after each wash. Additionally, compared to post-storage samples, the AA concentration significantly ($P < .0001$) decreased in the post-transfusion temperature samples. Compared to pre-storage, the TXB_2 concentrations decreased significantly ($P = .04$) after the first wash, but no other subsequent washes. Compared to post-storage samples, the TXB_2 concentrations increased ($P = .03$) in the post-transfusion temperature samples. There were no changes in HODE concentrations during storage. Compared to post-storage concentrations, there was a decrease in HODE concentration after the third wash and in the post-transfusion temperature samples ($P = .01$ and $P = .0002$, respectively). HETE concentrations increased significantly ($P = .0003$) during storage and did not decrease to below pre-storage values until wash 3 ($P = .02$).

3.5 | Aerobic and anaerobic bacterial cultures

For the post-storage samples, 3 units had a faint growth of a *Bacillus sp.* on enrichment broth. There was no aerobic bacterial growth identified on the post-transfusion temperature samples. Anaerobic bacterial growth was not detected in any sample.

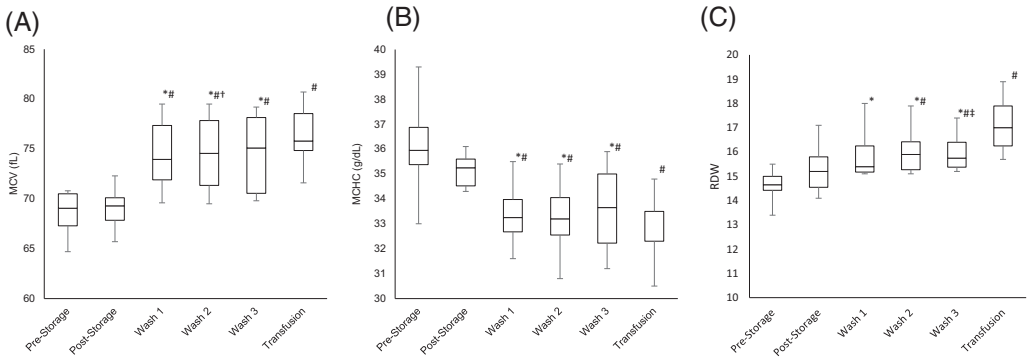

FIGURE 2 CBC results (MCV (A), MCHC (B), RDW (C)) in 8 units of canine whole blood at the time of collection (pre-storage), after 28 days of storage at 4°C (post-storage), after 3 washes with 250 mL 0.9% NaCl (wash 1, wash 2, and wash 3), and after being held at a simulated transfusion temperature for 5 hours (transfusion). The box and whiskers plot demonstrate the median (line), interquartile range (box), and total range (whiskers). *Indicates a significant ($P < .05$) difference from pre-storage; #Indicates a significant ($P < .05$) difference from post-storage; †Indicates a significant ($P < .05$) difference from the previous wash; ‡Indicates a significant ($P < .05$) difference after units being held at a simulated transfusion temperature

TABLE 3 Abnormal erythrocyte morphology from 8 units of canine whole blood at the time of donation (pre-storage), after 28 days of storage (post-storage), after 3 washes 0.9% NaCl (wash 1, wash 2, and wash 3), and after being held at a simulated transfusion temperature (transfusion)

Morphology	Pre-storage	Post-storage	Wash 1	Wash 2	Wash 3	Transfusion
Echinocytes	7 (rare to moderate)	7 (moderate to many)	7 (moderate to many)	1 (moderate)	ND	ND
Codocytes	1 (rare)	3 (rare)	ND	ND	ND	ND
Keratocytes	5 (rare to mild)	1 (rare)	ND	ND	ND	ND
Eccentrocyte	1 (rare)	ND	ND	ND	ND	ND
Dacrocyte	ND	ND	2 (mild to moderate)	2 (rare to mild)	2 (rare to mild)	2 (rare to mild)
Ovalocyte	ND	ND	1 (mild)	3 (rare to mild)	1 (rare)	2 (rare to mild)
Poikilocytosis	1 (mild)	1 (mild)	ND	ND	ND	ND
Other descriptions						
Smudge cells	ND	ND	8 (mild to many)	7 (many)	7 (many)	7 (many)
Rouleaux	ND	ND	3	5	7	3
Lack of central pallor	ND	ND	5	8	8	7
Cells not spread out	ND	ND	3	3	3	4

Note: Results reported are the number of samples for which the RBC and WBC morphology was present. Frequency of abnormalities was scored by use of a subjective scale as rare (1–10 abnormal cells/hpf), mild (11-50 abnormal cells/hpf), moderate (51-100 abnormal cells/hpf), and many (>100 abnormal cells/hpf).
Abbreviation: ND, not detected.

TABLE 4 Eicosanoid concentrations (mean ± SD) in 8 units of canine whole blood at the time of donation (pre-storage), after 28 days of storage (post-storage), after 3 washes 0.9% NaCl (wash 1, wash 2, and wash 3), and after being held at a simulated transfusion temperature (transfusion)

	Pre-storage	Post-storage	Wash 1	Wash 2	Wash 3	Transfusion
AA (pmol/mL)	56.0 ± 42.2	96.8 ± 41.5[a]	18.3 ± 40.0[a,b]	3.7 ± 40.1[a,b,c]	.9 ± 40.2[a,b,c,d]	0.3 ± 40.2[b]
$PGF_{2\alpha}$ (pmol/mL)	2.4 ± 2.8	2.1 ± 2.8	2.0 ± 2.7	2.3 ± 2.7	1.7 ± 2.7	1.7 ± 2.6
TXB_2 (pmol/mL)	7.2 ± 6.4	6.7 ± 5.8	4.6 ± 4.2[a]	7.1 ± 5.9	9.0 ± 8.6	73.5 ± 110.8[b]
PGE_2 (pmol/mL)	0.4 ± 0.3	0.2 ± 0.3	0 ± 0.3	0 ± 0.3[a]	0.1 ± 0.3	0 ± 0.3
PGD_2 (pmol/mL)	0.082 ± 0.1	0.059 ± 0.1	0.004 ± 0.1	0.004 ± 0.1	0.069 ± 0.1	0.009 ± 0.1
6-keto-$PGF_{1\alpha}$ (pmol/mL)	0.004 ± 0	0.005 ± 0	0.005 ± 0	0.000 ± 0	0.001 ± 0	0.008 ± 0
12-HETE (pmol/mL)	34.5 ± 63.7	129.4 ± 65.5[a]	106.9 ± 68.9	42.1 ± 68.7[b]	12.0 ± 68.8[a,b,c]	5.1 ± 69.0[b]
HODE (pmol/mL)	8.5 ± 10.4	11.1 ± 10.4	8.0 ± 10.2	12.7 ± 10.1	4.8 ± 5.4[b]	1.3 ± 5.0[b]

[a]Indicates a significant ($P < .05$) difference from pre-storage.
[b]Indicates a significant ($P < .05$) difference from post-storage.
[c]Indicates a significant ($P < .05$) difference from the previous wash.
[d]Indicates a significant ($P < .05$) difference after units being held at a simulated transfusion temperature.

4 | DISCUSSION

The manual washing technique used in our study reduced several storage lesions that had developed in units of stored canine whole blood. However, 3 sequential washes adversely affected erythrocyte health by causing an increase in erythrocyte fragility, RDW, MCV, Hgb concentration, and changes in erythrocyte morphology. Fortunately, most of the measured plasma storage lesions decreased to below pre-storage concentrations after a single wash, suggesting that only 1 wash would be needed to effectively remove most of the storage lesions.

Similar to previous studies, our study demonstrated an increase in lactate concentrations and pCO_2 during storage.[14,28-30] During storage, erythrocyte metabolic function is more dependent on anaerobic metabolism, which requires the conversion of intra-cellular pyruvate to lactic acid.[28,31] During storage, the erythrocyte intracellular concentration of 2,3-diphosphoglycerage (2,3-DPG) decreases, which reduces RBC oxygen carrying capacity and promotes anaerobic metabolism.[32,33] In both canine and human blood products, lactate begins to increase within 7 days of storage.[14,28] The neutralization of this excess lactate results in an excess production of CO_2. In the presence of carbonic anhydrase and water, the excessive CO_2 will be

converted to carbonic acid,[28] contributing to the decrease in the pH of stored units. After the first wash, our study demonstrated a significant decrease in both lactate and pCO_2, but the pH inside the unit continued to decrease, even after 2 additional washes. A potential explanation for the progressive decrease in pH is continued anaerobic metabolism. A second potential explanation for the progressive decrease in pH is the loss of the buffer, TCO_2, during the washing process. A third potential explanation for the continual decrease in unit pH is the wash solution used in our study. The solution used in our washing technique, 0.9% NaCl, has a pH of 5.0, and it is unknown if the use of a pH-neutral wash solution would have prevented the pH from continuing to decrease.

Similar to previous studies, our study demonstrated an increase in potassium concentrations during storage.[14,17,20,28,30,31] The increase in potassium could be due to ATP depletion within the unit, and to the inhibitory effects that low temperatures have on the enzymatic function of Na-K-ATPase.[34] There is an increase in RBC sodium uptake and a loss of intracellular potassium from red cells during storage and shipment.[34] The transfusion of blood products with increased concentrations of potassium has not been associated with clinically significant adverse events.[28] However, serum potassium concentration could potentially increase during massive transfusions,[35] especially in patients that are small and have concurrent hyperkalemia.

In both human and veterinary medicine, storage of blood products can increase erythrocyte fragility, causing an increase in in vitro hemolysis and free Hgb concentrations.[15,29,36-38] An increase in red cell fragility and hemolysis can result in the release of cell-free Hgb, which can contribute to multiple organ failure,[39] lung injury,[40-44] bacterial infections,[19] systemic and pulmonary hypertension,[40] kidney injury, and endothelial dysfunction.[21] Hemoglobin effectively scavenges nitric oxide, causing endothelial dysfunction, vasoconstriction, platelet aggregation, increased vascular permeability, and leukocyte adhesion.[45] In the present study, total Hgb increased after the first, but not subsequent washes. A similar finding was demonstrated with a cell salvage device that also used 0.9% NaCl to wash canine erythrocytes.[46] Careful handling of these units, including using the least number of washes, would reduce risk of in vitro hemolysis. Our study also showed an increase in total Hgb between the final wash and the post-transfusion temperature samples. Potential explanations for this increase in Hgb could be the continued decline in RBC health while held at room temperature,[34] and reduced dilutional effects of the wash solution. The volume of 0.9% NaCl infused (100 mL) into the unit before being held at room temperature was less than the wash samples (250 mL), potentially increasing the Hgb concentration purely because of the reduced volume of diluting solution.

By washing old vs fresh blood before transfusion in a canine pneumonia model, we found that washing older blood reduced multiple organ injury, lung injury, plasma iron, and cell-free Hgb concentrations and improved survival times.[41] However, washing of fresh blood caused RBC membrane damage and increased cell-free Hgb and iron, and worsened infection and outcomes.[41] A second study found that washing of blood stored for 14-35 days caused a separation of Kaplan Meyer curves, but the differences in survival in washed vs non-washed blood products were not statistically different.[42]

Studies in both human and veterinary medicine have demonstrated that the progressive depletion of ATP during storage can cause increased senescent signaling[47,48] and degenerative changes to erythrocyte shape, including echinocytes and spherocytes.[38,49,50] Our study showed that echinocytes were detected in 7 dogs in the pre-storage, post-storage, and wash 1 groups, but that only 1 dog demonstrated echinocytes after the second wash. Loss of central pallor was another red cell change seen with storage. The loss of central pallor, increased RDW and MCV, and decreased MCHC with washing supports previous reports of human and canine RBC swelling after manual or automated RBC washing.[46,51] The cellular swelling during washing is widely considered to be secondary to build up of osmotically active substances (lactate, chloride, and 2,3-DPG) intracellularly.[49,52] These osmotically active substances develop in RBCs during storage and, after washing, the extracellular osmolality decreases significantly depending on the washing media used,[53,54] resulting in an intracellular shift of fluid. Additionally, a storage-related dysfunction of Na-K-ATPase could increase intracellular sodium concentration lead to an osmotic pull of water into the RBC.[34,38,46] In humans, washing with solutions containing dextrose, mannitol, or albumin that exhibit osmotic or colloidal oncotic effects improves red cell morphology as evidenced by normalized MCV and increased MCHC, as well as decreased erythrocyte water content and improved cell health.[23,24,51,54]

Ionized calcium and iMg concentrations increased after each wash, suggesting that any residual and unbound citrate-phosphate-dextrose solution with adenine (CPDA) was removed during washing. The use of washed blood products could provide a clinical benefit for patients with a concurrent coagulopathy, receiving massive transfusions, or at risk of developing citrate intoxication. Although our study did not measure citrate concentration in the unit, increases in both calcium and magnesium after the first wash suggest removal of most citrate. With each wash step, it is possible that intracellular calcium and magnesium leaked out of the RBCs, which could indicate cellular fragility during the washing process. Another possible cause for the increased iCa is platelet activation; however, citrated storage media has been shown to decrease both platelet and leukocyte activation and, after prolonged storage, this mechanism is not expected to significantly contribute to the changes in calcium concentrations measured in this study.[55,56] Additionally, albumin was removed along with the plasma after the first washing step, and it is possible that the iCa moved into the washing media to maintain calcium homeostasis. Finally, the iCa concentration in the units could have been affected by the progressive decrease in pH. As the units become more acidic, hydrogen ions could compete with calcium for binding sites on any residual proteins that were not been removed through the washing process. This competition with hydrogen ions would then cause an increase iCa.

During the washing process, there appeared to be minimal immediate evidence of bacterial contamination. After the 3 washes and the

post-transfusion temperature samples, there was no aerobic or anaerobic bacterial growth on cultures. However, on the post-storage samples, 3 units had a faint growth of *Bacillus* sp. on enrichment broth. Although bacterially contaminated blood products could adversely affect RBCs and potentially increase the risk of storage lesions, it is unclear how much the *Bacillus* sp. growth affected our results. A comparison between units with a light growth of *Bacillus* and units without a light growth revealed no obvious difference in results. Additionally, the same *Bacillus* sp. was not detected on cultures or enrichment broth in the post-transfusion temperature samples. It is possible that the washing process eliminated the *Bacillus* sp., or that the positive growth on enrichment broth was a contaminant. To reduce the risk of bacterial contamination, all washing was performed with sterile supplies on a sterile surface, and the individual performing the washing procedure wore sterile gloves.

Automated washing systems will safely and efficiently wash units of blood, but are not usually available in most veterinary hospitals. If a blood bag centrifuge is available, the manual washing protocol described in this study is straight-forward and can be easily completed within 30 minutes. Although this washing protocol is feasible for the clinical setting, the benefits of washing blood products before transfusion are unknown.

Elevated concentrations of eicosanoids have been suggested as a possible cause of transfusion associate lung injury (TRALI) in humans,[57] and a reduction in eicosanoids has been proposed as a mechanism to reduce transfusion reactions.[3,58] In humans, the use of leukoreduction filters before storage can reduce the accumulation of eicosanoids in stored units[5]; however, in dogs, leukoreduction causes an immediate increase in TXB_2 and $PGF_{2\alpha}$, and does not decrease 6-keto-$PGF_{1\alpha}$.[8] Our study demonstrated that manual washing decreased TXB_2 and PGE_2 concentrations, after 1 and 2 washes, respectively, when compared to concentrations before storage. These results are similar to studies in units of human blood, which showed that washing caused a 95% reduction of eicosanoids that accumulated during storage, including TXB_2 and PGE_2.[3] Compared to post-storage concentrations, there was an increase in TXB_2 concentrations in the post-transfusion temperature samples. However, a significant difference in TXB_2 concentrations between the post-transfusion temperature sample and other samples was not detected, despite the mean TXB_2 value being substantially greater than the samples collected at other time points. Of the 7 post-transfusion temperature samples analyzed, 5 samples had a TXB_2 value of less than 21 pmol/mL, for an average of 10.9 pmol/mL. However, 2 samples had a substantially higher TXB_2 values, 278.7 and 181.5 pmol/mL, which caused the mean and SD to be greater in the combined post-transfusion temperature samples compared to the previous samples.

HETEs and HODEs are lipoxygenase (LOX)-induced oxidative byproducts of AA that have vasoactive effects. 12-HETE directly contributes to platelet activation and aggregation and influences the expression of the platelet adhesion molecule P-selectin.[59] HODEs are proinflammatory eicosanoids that contribute to leukocyte migration; specifically, human and bovine neutrophil chemotaxis[60] and cytotoxic NK cell migration.[61] Increases in both HETEs and HODEs have been associated with vascular dysfunction, hypertension, and microalbuminuria in greyhounds.[62] Our study is the first to demonstrate the presence of 12-HETE and HODEs in stored canine whole blood; however, the roles that 12-HETE and HODEs play in transfusion reactions are unknown. In mice, the accumulation of HETEs and HODEs during storage is associated with a poor 24-hour RBC recovery in mice after transfusion.[63] Furthermore, AA and HETEs have been implicated as the "second hit" in TRALI in people.[57] Other potential clinical consequences of increased exposure to eicosanoids include immunomodulation,[64] enhancement of the systemic inflammatory response syndrome (SIRS),[65-68] and leukocyte and platelet activation.[59,69,70] Further investigation into the effects of eicosanoids and their physiologic effects and clinical significance in transfusion recipients is needed.

The present study had several limitations. Firstly, there were numerous technical problems with the blood gas analyzer that caused absent or partial data for several analytes. Secondly, 1 blood unit ruptured during the final centrifugation, which prevented the collection of the post-transfusion temperature samples. Thirdly, our study only evaluated 1 type of wash media, 0.9% NaCl, and the use of different wash solutions could have caused less cellular damage and potentially reduced the amount of hemolysis.[24,71] The 0.9% NaCl was selected because it has been used previously in studies evaluating washing units of human blood, and it is readily available in most veterinary clinics.[10,23,24] Similarly, using different washing protocols could have removed most storage lesions and reduced erythrocyte damage.[10,23,24] Finally, our study only evaluated some storage lesions, and other storage lesions (extracellular and intracellular) and indicators of erythrocyte health (cell-free Hgb) were not evaluated. The objective of this study was to determine if a manual washing technique could remove most extracellular storage lesions without damaging the erythrocytes. Based on our results, this washing technique could be used for future studies to evaluate the effects of washing on the removal of other storage lesions.

The present study demonstrates the manual washing of units of canine whole blood significantly reduced most storage lesions within the units, with many lesions at concentrations below that present before storage. Based on the results of this study, a single wash eliminated the storage lesions examined in this study. Further investigation into alternative washing solutions and protocols, and the in vivo benefits of transfusing washed blood products, might enable greater reductions in plasma storage lesions and improve erythrocyte health.

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CONFLICT OF INTEREST DECLARATION

Authors declare no conflict of interest.

OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

This study was approved by the Mississippi State University College of Veterinary Medicine IACUC, protocol number 19-215.

HUMAN ETHICS APPROVAL DECLARATION

Authors declare human ethics approval was not needed for this study.

ORCID

John M. Thomason https://orcid.org/0000-0001-5678-8923

REFERENCES

1. Patterson J, Rousseau A, Kessler RJ, Giger U. In vitro lysis and acute transfusion reactions with hemolysis caused by inappropriate storage of canine red blood cell products. *J Vet Intern Med*. 2011;25(4):927-933. doi:10.1111/j.1939-1676.2011.0737.x
2. Shanwell A, Kristiansson M, Remberger M, Ringden O. Generation of cytokines in red cell concentrates during storage is prevented by prestorage white cell reduction. *Transfusion*. 1997;37(7):678-684. doi:10.1046/j.1537-2995.1997.37797369441.x
3. Jacobi K, Walther A, Lörler H, et al. Plasma levels of eicosanoids after transfusion of intraoperatively salvaged blood. *Vox Sang*. 2000;78(1):31-36. doi:10.1159/000031146
4. Fu X, Felcyn JR, Odem-Davis K, Zimring JC. Bioactive lipids accumulate in stored red blood cells despite leukoreduction: a targeted metabolomics study. *Transfusion*. 2016;56(10):2560-2570. doi:10.1111/trf.13748
5. Jacobi KE, Wanke C, Jacobi A, Weisbach V, Hemmerling TM. Determination of eicosanoid and cytokine production in salvaged blood, stored red blood cell concentrates, and whole blood. *J Clin Anesth*. 2000;12(2):94-99. doi:10.1016/S0952-8180(00)00122-7
6. Richter JR, Sutton JM, Hexley P, Johannigman TA, Lentsch AB, Pritts TA. Leukoreduction of packed red blood cells attenuates proinflammatory properties of storage-derived microvesicles. *J Surg Res*. 2018;223:128-135. doi:10.1016/j.jss.2017.09.052
7. Nagura Y, Tsuno NH, Tanaka M, Matsuhashi M, Takahashi K. The effect of pre-storage whole-blood leukocyte reduction on cytokines/chemokines levels in autologous CPDA-1 whole blood. *Transfus Apher Sci*. 2013;49(2):223-230. doi:10.1016/j.transci.2013.01.006
8. Muro SM, Lee JH, Stokes JV, et al. Effects of leukoreduction and storage on erythrocyte phosphatidylserine expression and eicosanoid concentrations in units of canine packed red blood cells. *J Vet Intern Med*. 2017;31(2):410-418. doi:10.1111/jvim.14664
9. McMichael MA, Smith SA, Galligan A, Swanson KS, Fan TM. Effect of leukoreduction on transfusion-induced inflammation in dogs. *J Vet Intern Med*. 2010;24(5):1131-1137. doi:10.1111/j.1939-1676.2010.0561.x
10. Loh YS, Tan S, Kwok M, Stark MJ, Marks DC. Reduction of biological response modifiers in the supernatant of washed paediatric red blood cells. *Vox Sang*. 2016;111(4):365-373. doi:10.1111/vox.12442
11. Lozano BL, Blois SL, Wood RD, et al. A pilot study evaluating the effects of prestorage leukoreduction on markers of inflammation in critically ill dogs receiving a blood transfusion. *J Vet Emerg Crit Care*. 2019;29(4):385-390. doi:10.1111/vec.12857
12. Callan MB, Patel RT, Rux AH, et al. Transfusion of 28-day-old leucoreduced or non-leucoreduced stored red blood cells induces an inflammatory response in healthy dogs. *Vox Sang*. 2013;105(4):319-327. doi:10.1111/vox.12058
13. Yang H, Kim W, Bae J, et al. Effects of irradiation and leukoreduction on down-regulation of CXCL-8 and storage lesion in stored canine whole blood. *J Vet Sci*. 2019;20(1):72-78. doi:10.4142/jvs.2019.20.1.72
14. Press SA, Cooper ES, Stull JW. Electrolyte, acid-base, and hemoglobin oxygen affinity alterations following irradiation and storage of canine packed red blood cells. *Vet Clin Pathol*. 2017;46(4):580-588. doi:10.1111/vcp.12539
15. Yoshida T, Prudent M, D'Alessandro A. Red blood cell storage lesion: causes and potential clinical consequences. *Blood Transfus*. 2019;17(1):27-52. doi:10.2450/2019.0217-18
16. Schmidt A, Refaai M, Kirkley S, Blumberg N. Proven and potential clinical benefits of washing red blood cells before transfusion: current perspectives. *Int J Clin Transfus Med*. 2016;4:79-88. doi:10.2147/IJCTM.S101401
17. Grabmer C, Holmberg J, Popovsky M, et al. Up to 21-day banked red blood cells collected by apheresis and stored for 14 days after automated wash at different times of storage. *Vox Sang*. 2006;90(1):40-44. doi:10.1111/j.1423-0410.2005.00719.x
18. Cholette JM, Henrichs KF, Alfieris GM, et al. Washing red blood cells and platelets transfused in cardiac surgery reduces postoperative inflammation and number of transfusions. *Pediatr Crit Care Med*. 2012;13(3):290-299. doi:10.1097/PCC.0b013e31822f173c
19. Cholette JM, Pietropaoli AP, Henrichs KF, et al. Longer RBC storage duration is associated with increased postoperative infections in pediatric cardiac surgery. *Pediatr Crit Care Med*. 2015;16(3):227-235. doi:10.1097/PCC.0000000000000320
20. Bansal I, Calhoun BW, Joseph C, Pothiawala M, Baron BW. A comparative study of reducing the extracellular potassium concentration in red blood cells by washing and by reduction of additive solution. *Transfusion*. 2007;47(2):248-250. doi:10.1111/j.1537-2995.2007.01095.x
21. Woźniak MJ, Qureshi S, Sullo N, et al. A comparison of red cell rejuvenation versus mechanical washing for the prevention of transfusion-associated organ injury in swine. *Anesthesiology*. 2018;128(2):375-385. doi:10.1097/ALN.0000000000001973
22. Klein HG. The red cell storage lesion(s): of dogs and men. *Blood Transfus*. 2017;15(2):107-111. doi:10.2450/2017.0306-16
23. Weisbach V, Ringwald J, Zimmermann R, et al. The in vitro quality of washed, prestorage leucocyte-depleted red blood cell concentrates. *Vox Sang*. 2004;87(1):19-26. doi:10.1111/j.1423-0410.2004.00526.x
24. Proffitt S, Curnow E, Brown C, Bashir S, Cardigan R. Comparison of automated and manual methods for washing red blood cells. *Transfusion*. 2018;58(9):2208-2216. doi:10.1111/trf.14781
25. McDevitt RI, Ruaux CG, Baltzer WI. Influence of transfusion technique on survival of autologous red blood cells in the dog. *J Vet Emerg Crit Care*. 2011;21(3):209-216. doi:10.1111/j.1476-4431.2011.00634.x
26. Cooley-Lock KM, Williams JP, Williams ML, et al. Assessment of erythrocyte damage and in-line pressure changes associated with simulated transfusion of canine blood through microaggregate filters. *Am J Vet Res*. 2019;80(9):852-861. doi:10.2460/ajvr.80.9.852
27. Xie S, Borazjani A, Hatfield MJ, Edwards CC, Potter PM, Ross MK. Inactivation of lipid Glyceryl Ester metabolism in human THP1 monocytes/macrophages by activated Organophosphorus insecticides: role of carboxylesterases 1 and 2. *Chem Res Toxicol*. 2010;23(12):1890-1904. doi:10.1021/tx1002194
28. Rodrigues RR, Kayano CY, dos Santos VP, Moroz LR, Fantoni DT, Ambrósio AM. Evaluation of hematologic, biochemical, and blood gas variables in stored canine packed red blood cells, and the impact of storage time on blood recipients. *Vet Clin Pathol*. 2020;49(2):198-206. doi:10.1111/vcp.12865
29. Blaine KP, Cortés-Puch I, Sun J, et al. Impact of different standard red blood cell storage temperatures on human and canine RBC hemolysis and chromium survival. *Transfusion*. 2019;59(1):347-358. doi:10.1111/trf.14997
30. Smith T, Riley W, Fitzgerald D. In vitro comparison of two different methods of cell washing. *Perfusion*. 2013;28(1):34-37. doi:10.1177/0267659112458960

31. Rosenstein PG, Tennent-Brown BS, Huges D. Clinical use of plasma lactate concentration. Part1: physiology, pathophysiology, and measurement. *J Vet Emerg Crit Care*. 2018;18(2):85-105.
32. Price GS, Armstrong J, McLeod DA, et al. Evaluation of citrate-phosphate-dextrose-adenine as a storage medium for packed canine erythrocytes. *J Vet Intern Med*. 1988;2(3):126-132. doi:10.1111/j.1939-1676.1988.tb02808.x
33. Olson J, Talekar M, Sachdev M, et al. Potassium changes associated with blood transfusion in pediatric patients. *Am J Clin Pathol*. 2013;139(6):800-805. doi:10.1309/AJCP2MP8OIIXNKXF
34. Makhro A, Huisjes R, Verhagen LP, et al. Red cell properties after different modes of blood transportation. *Front Physiol*. 2016;7:288. doi:10.3389/fphys.2016.00288
35. Jutkowitz LA, Rozanski EA, Moreau JA, Rush JE. Massive transfusion in dogs: 15 cases (1997-2001). *J Am Vet Med Assoc*. 2002;220(11):1664-1669. doi:10.2460/javma.2002.220.1664
36. Lacerda LA, Hlavac NRC, Terra SR, Back FP, Jane Wardrop K, González FHD. Effects of four additive solutions on canine leukoreduced red cell concentrate quality during storage. *Vet Clin Pathol*. 2014;43(3):362-370. doi:10.1111/vcp.12163
37. Offner PJ. Age of blood does it make a difference? *Crit Care*. 2004;8:S24-S26.
38. Obrador R, Musulin S, Hansen B. Red blood cell storage lesion. *J Vet Emerg Crit Care*. 2015;25(2):187-199. doi:10.1111/vec.12252
39. Zallen G, Offner PJ, Moore EE, et al. Age of transfused blood is an independent risk factor for postinjury multiple organ failure. *Am J Surg*. 1999;178(6):570-572. doi:10.1016/S0002-9610(99)00239-1
40. Solomon SB, Wang D, Sun J, et al. Mortality increases after massive exchange transfusion with older stored blood in canines with experimental pneumonia. *Blood*. 2013;121(9):1663-1672. doi:10.1182/blood-2012-10-462945
41. Cortés-Puch I, Wang D, Sun J, et al. Washing older blood units before transfusion reduces plasma iron and improves outcomes in experimental canine pneumonia. *Blood*. 2014;123(9):1403-1411. doi:10.1182/blood-2013-11-539353
42. Cortés-Puch I, Remy KE, Solomon SB, et al. In a canine pneumonia model of exchange transfusion, altering the age but not the volume of older red blood cells markedly alters outcome. *Transfusion*. 2015;55(11):2564-2575. doi:10.1111/trf.13275
43. Wang D, Cortés-Puch I, Sun J, et al. Transfusion of older stored blood worsens outcomes in canines depending on the presence and severity of pneumonia. *Transfusion*. 2014;54(7):1712-1724. doi:10.1111/trf.12607
44. Suffredini DA, Xu W, Sun J, et al. Parenteral irons versus transfused red blood cells for treatment of anemia during canine experimental bacterial pneumonia. *Transfusion*. 2017;57(10):2338-2347. doi:10.1111/trf.14214
45. Tousoulis D, Kampoli AM, Tentolouris C, et al. The role of nitric oxide on endothelial function. *Curr Vasc Pharmacol*. 2012 Jan;10(1):4-18. doi:10.2174/157016112798829760
46. Hofbauer N, Windberger U, Schwendenwein I, Tichy A, Eberspächer E. Evaluation of canine red blood cell quality after processing with an automated cell salvage device. *J Vet Emerg Crit Care*. 2016;26(3):373-383. doi:10.1111/vec.12472
47. Raat NJ, Ince C. Oxygenating the microcirculation: the perspective from blood transfusion and blood storage. *Vox Sang*. 2007;93:12-18. doi:10.1111/j.1423-0410.2007.00909.x
48. Oliver CS, Andrews GA, Smith JE, et al. Erythrocyte structure and function. In: Weiss DJ, Wardrop KJ, eds. *Schalm's Veterinary Hematology*. 6th ed. Hoboken, NJ: Wiley-Blackwell; 2010:123-129.
49. Harvey JW. Erythrocyte biochemistry. In: Weiss DJ, Wardrop KJ, eds. *Schalm's Veterinary Hematology*. 6th ed. Hoboken, NJ: Wiley-Blackwell; 2010:131-135.
50. Kozlova E, Chernysh A, Moroz V, Sergunova V, Gudkova O, Kuzovlev A. Nanodefects of membranes cause destruction of packed red blood cells during long-term storage. *Exp Cell Res*. 2015;337(2):192-201. doi:10.1016/j.yexcr.2015.07.009
51. Zhao Y, Luo G, Luo H, Ye X, Rong X, Huang K. Modify washing solutions in the process of deglycerolization in ACP 215 and storage at 4°C in 0.9% sodium chloride in 24h. *Transfus Apher Sci*. 2010;43(2):163-165. doi:10.1016/j.transci.2010.07.009
52. Beutler E. Liquid preservation of red cells. In: Rossi E, Simon T, Moss G, et al., eds. *Principles of Transfusion Medicine*. 2nd ed. Baltimore, MD: Williams & Wilkins; 1996:51-60.
53. Blumberg N, Heal JM. Immunomodulation by blood transfusion: an evolving scientific and clinical challenge. *Am J Med*. 1996;101(3):299-308. doi:10.1016/S0002-9343(96)00124-6
54. Reinhart WH, Piety NZ, Deuel JW, et al. Washing stored red blood cells in an albumin solution improves their morphologic and hemorheologic properties. *Transfusion*. 2015;55(8):1872-1881. doi:10.1111/trf.13052
55. Gubensek J, Strobl K, Harm S, et al. Influence of citrate concentration on the activation of blood cells in an in vitro dialysis setup. *PLoS ONE*. 2018;13(6):2-13. doi:10.1371/journal.pone.0199204
56. Fürstenberger G, Marks F, Krieg P. Arachidonate 8(S)-lipoxygenase. *Prostaglandins Other Lipid Mediat*. 2002;68-69:235-243. doi:10.1016/S0090-6980(02)00033-3
57. Silliman CC, Moore EE, Kelher MR, Khan SY, Gellar L, Elzi DJ. Identification of lipids that accumulate during the routine storage of prestorage leukoreduced red blood cells and cause acute lung injury. *Transfusion*. 2011;51:2549-2554.
58. Lannan KL, Sahler J, Spinelli SL, Phipps RP, Blumberg N. Transfusion immunomodulation — the case for leukoreduced and (perhaps) washed transfusions. *Blood Cells Mol Dis*. 2013;50(1):61-68. doi:10.1016/j.bcmd.2012.08.009
59. Powell WS, Rokach J. Biosynthesis, biological effects, and receptors of hydroxyeicosatetraenoic acids (HETEs) and oxoeicosatetraenoic acids (oxo-ETEs) derived from arachidonic acid. *Biochim Biophys Acta—Mol Cell Biol Lipids*. 2015;1851(4):340-355. doi:10.1016/j.bbalip.2014.10.008
60. Henricks PAJ, Engels F, van der Vliet H, Nijkamp FP. 9-and 13-hydroxy-linoleic acid posses chemotactic activity for bovine and human polymorphonuclear leukocytes. *Prostaglandins*. 1991;41(1):21-27. doi:10.1016/0090-6980(91)90101-K
61. Rolin J, Al-Jaderi Z, Maghazachi AA. Oxidized lipids and lysophosphatidylcholine induce the chemotaxis and intracellular calcium influx in natural killer cells. *Immunobiology*. 2013;218(6):875-883. doi:10.1016/j.imbio.2012.10.009
62. Martinez-Sogues L, Blois SL, Manzanilla EG, Abrams-Ogg AO, Cosentino P. Exploration of risk factors for non-survival and for transfusion-associated complications in cats receiving red cell transfusions: 450 cases (2009 to 2017). *J Small Anim Pract*. 2020;61(3):177-184. doi:10.1111/jsap.13108
63. Zimring JC, Smith N, Stowell SR, et al. Strain-specific red blood cell storage, metabolism, and eicosanoid generation in a mouse model. *Transfusion*. 2014;54(1):137-148. doi:10.1111/trf.12264
64. Dennis F, Landers DF, Gary EH, et al. Blood transfusion-induced immunomodulation. *Anesth Analg*. 1996;82:187-204.
65. Suchner U, Senftleben U. Effekte von mehrfach ungesittigten Fettsauren auf den Immunstatus: Bedeutung als Strukturund Mediatorbausteine. *Infusionsther Transfusionsmed*. 1994;21:59-70.
66. Suchner U, Senftleben U. Immune modulation by polyunsaturated fatty acids during nutritional therapy: interactions with synthesis and effects of eicosanoids. *Infusionsther Transfusionsmed*. 1994;21:167-182.
67. Matera G, Cook JA, Hennigar RA, et al. Beneficial effects of a 5-lipoxygenase inhibitor in endotoxic shock in the rat. *J Pharmacol Exp Ther*. 1988;247:363-371.
68. Cook JA, Wise WC, Halushka PV. Protective effect of a selective leukotriene antagonist in endotoxemia in the rat. *J Pharmacol Exp Ther*. 1985;235:470-474.
69. Okada M, Kodama T, Tominaga A, Kon K, TS, Utsumi S. Cytotoxicity of activated platelets to autologous red blood cells. *Br J Haematol*. 1992;82:142-150.

70. Sieunarine K, Langton S, Lawrence-Brown MMD, Goodman MA, Prendergast FJ, Hellings M. Elastase levels in salvaged blood and the effect of cell washing. *Aust N Z J Surg*. 1990 Aug;60(8):613-616. doi: 10.1111/j.1445-2197.1990.tb07441.x
71. Sasaki J, Tirotta C, Lim H, et al. Comparison of stored red blood cell washing techniques for priming extracorporeal circuits. *Perfusion*. 2018;33(2):130-135. doi:10.1177/0267659117730134

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