BLOOD STORAGE LESIONS AND LEUKOREDUCTION

Dr Sarah Purcell BVSc (hons) DACVECC
Murdoch University Veterinary Hospital, Murdoch, Perth, Australia

Processing and storage of blood products for use in anaemic animals is commonplace in referral veterinary practice. Blood transfusions improve tissue oxygen delivery and can be life-saving, however they are not innocuous, and many complications can occur.

Morbidity and Mortality
Blood transfusion is an independent risk factor for increased morbidity and mortality in critically-ill people. A large scale study called Anaemia and Blood Transfusion in Critically Ill Patients, the ABC study, evaluated 3534 critically-ill people from 146 ICUs.\(^1\) Transfusion was associated with longer ICU stays, with a mean ICU length of stay of 7.2 days compared with 2.6 days for non-transfused patients, and higher mortality in transfused patients, 29% vs. 14.9%. A similar study called Anaemia and Blood Transfusion in the critically ill, the CRIT study, had comparable findings.\(^2\) They enrolled 4,892 people in 284 ICUs. They found that length of hospitalisation and mortality was associated with the number of transfusions received. These studies used illness severity scoring to account for the likelihood that people who are more critically ill are more likely to receive blood transfusions, and therefore have a poorer outcome.

There are few studies in dogs that assess the relationship between blood transfusion, morbidity, and mortality. The studies that have been performed are largely observational and retrospective in nature. The published studies include dogs undergoing adrenalectomy, splenectomy, and trauma patients, and all found an association between blood transfusion and short-term survival.\(^3-5\) Many of these studies however did not compare disease severity between dogs that were transfused and dogs that were not. It is very likely that dogs with more severe injury or disease were more likely to require transfusion, and therefore had a lower chance of survival. Contrary to these findings, a retrospective study of 110 dogs with immune mediated haemolytic anaemia, a common condition in dogs requiring transfusion, found no association between administration of a transfusion and survival to discharge.\(^6\)

Transfusion Trigger
Many large, multi-centre studies in humans have investigated liberal versus restrictive transfusion strategies, to try and determine an appropriate ‘transfusion trigger’. These studies have consistently shown that a restrictive transfusion strategy is associated with lower mortality and fewer adverse effects.\(^6-11\) A large multicentre, randomised, controlled clinical trial of Transfusion Requirements In Critical Care, the TRICC study, compared a restrictive red blood cell (RBC) transfusion strategy with a liberal RBC transfusion strategy.\(^7\) The restrictive group were transfused when their haemoglobin concentration dropped below 7.0g/dL, whereas the liberal group received a transfusion when their haemoglobin dropped below 10.0g/dL. They found no significant difference in mortality between the two groups, 18.7 % for the restrictive group, and 23.3 % for the liberal group. A sub-group analysis found a significant reduction in mortality in the restrictive group of patients that were less than 55 years of age (5.7 % vs. 13.0 %) and those with an Acute Physiology and Chronic Health Evaluation II score of 20 or less (8.7 % vs. 16.1 %). Overall, patients in the restrictive group received 50% fewer transfusions than patients in the liberal group. There are several other studies in people investigating restrictive versus liberal transfusion strategies, with these studies often finding a reduction in mortality with restrictive transfusion strategies.\(^10-13\) The vast majority of evidence suggests that restrictive transfusion strategies are safe in the majority of clinical settings, reduce the number of RBC transfusions, reduce adverse effects associated with transfusion, and are more cost-effective.\(^13\)

The packed cell volume (PCV) at which transfusion is deemed necessary in dogs, the transfusion trigger, is dependent not only on the severity of anaemia, but also on history, clinical signs, underlying cause, and clinical judgement.\(^14\) Acute haemorrhage causing a decrease in PCV to 20% or less, equivalent to a haemoglobin concentration of <7.0g/dL, is usually considered an indication for RBC transfusion.\(^14\) Dogs that have chronic blood loss generally tolerate a lower PCV and therefore the transfusion trigger is lower.\(^14\)

Non-immunologic Transfusion Reactions
Non-immunologic transfusion reactions are usually associated with the collection, storage and administration of blood products. Blood donors should be an appropriate age, deemed healthy, and screened for infectious diseases. Transmission of infectious diseases via canine blood transfusion has been reported, but only rarely.\(^15\) A recent consensus statement has been published in the Journal of Veterinary Internal Medicine.\(^16\) This statement
recommends routine screening of many vector-borne pathogens. Due to the low prevalence or absence of many vector-borne diseases in Australia, the risk of disease transmission is low, and donors are not routinely tested.

The collection of blood from the donor and administration to the transfusion recipient must be performed in an aseptic manner to try and prevent contamination of blood products with bacteria. The administration of blood contaminated with bacteria can lead to vomiting, pyrexia, septic shock, collapse, and death.\textsuperscript{17} Blood products should be monitored during storage for signs of discoloration that may indicate bacterial contamination. Inappropriate storage of blood products can also affect the quality of blood products. A recent report described 4 dogs that developed acute, life-threatening transfusion reactions after transfusion with packed red blood cells (PRBC) that were lysed during storage. It was thought that cyclic temperatures during storage were the cause of in vitro lysis.\textsuperscript{18}

During storage blood products undergo various physical and metabolic changes. The concentration of 2,3-diphosphoglycerate (2,3-DPG), and ATP declines,\textsuperscript{19-21} ammonia accumulates,\textsuperscript{22} and haemolysis results in the accumulation of free haemoglobin.\textsuperscript{23} Leukocytes within the stored blood product also release cytokines and other inflammatory mediators.\textsuperscript{24,25} The end result is a decline in the post transfusion viability of RBCs, and the transfusion of various bioactive mediators that can lead to recipient inflammation and transfusion reactions.\textsuperscript{26-30}

Declining levels of glucose throughout storage decrease ATP production and 2,3-DPG.\textsuperscript{19} Adenosine triphosphate and 2,3-DPG concentrations help to maintain sodium and potassium ionic composition of RBC which is important for maintenance of deformability.\textsuperscript{31} The shape of RBCs allows for a large surface area to volume ratio which maximises the area for gas exchange, whilst minimising the distance over which gas exchange must occur.\textsuperscript{11} With declining ATP and 2,3-DPG levels, RBCs become crenated, forming echinocytes, spheronechocytes and ultimately spherocytes.\textsuperscript{27} These changes result in increased osmotic fragility, and loss of deformability which can impede microvascular flow.\textsuperscript{19,21,25,27} Declining levels of 2,3-DPG throughout storage reduces the ability of RBCs to release oxygen to the tissues.\textsuperscript{33-36}

Stored PRBC accumulate ammonia secondary to the deamination of proteins within these products.\textsuperscript{22} Stored canine PRBC were shown to have increased ammonia during storage, increasing from 23 +/- 8 mmol/L on day 0, to 562 +/- 27mmol/L on day 35.\textsuperscript{22} The clinical implication of transfusing higher concentrations of ammonia is unclear, however it would likely be detrimental in patients with liver dysfunction or failure, or in patients receiving massive transfusions.\textsuperscript{22}

Haemolysis of RBCs occurs progressively throughout storage which results in the accumulation of free haemoglobin.\textsuperscript{21,37} The administration of high concentrations of iron-rich haemoglobin overwhelms the binding capacity of the major physiologic iron carrier transferrin, and there is an increase in free non-transferrin-bound iron.\textsuperscript{35,38} Elevations in circulating iron levels can enhance bacterial pathogenicity.\textsuperscript{18,39}

Leukocytes within blood products release bioactive mediators throughout storage into the storage medium.\textsuperscript{24,25,40} These bioactive mediators can affect the viability of RBCs and may have a negative impact on the transfusion recipient.\textsuperscript{26,25,40} Bioactive mediators such as cytokines incite an inflammatory response in the recipient,\textsuperscript{20,41} and contribute to the occurrence of transfusion reactions such as febrile non-haemolytic transfusion reactions (FNHTR)\textsuperscript{24,25} and transfusion related acute lung injury (TRALI).\textsuperscript{24,25}

Administration of blood products has the potential to cause Transfusion Associated Circulatory Overload, or TACO. Clinical signs of TACO include dyspnoea, cyanosis, hypertension, and the development of congestive heart failure during or within 6 hours of transfusion.\textsuperscript{42} The necessity of transfusion should be carefully evaluated in at-risk patients. The transfusion of specific blood components rather than whole blood should also be considered wherever possible. Citrate toxicity is a potential risk for patients receiving multiple transfusions. Citrate is an anti-coagulant that binds to ionised calcium and magnesium, and can lead to hypocalcaemia and hypomagnesaemia. Treatment is only required for dogs that develop clinical signs such as hypotension, muscle tremors, and arrhythmias.\textsuperscript{43}

Immunologic Transfusion Reactions
Blood typing and cross-matching are methods used to help prevent haemolytic transfusion reactions. Donors and recipients are typically typed for dog erythrocyte antigen 1.1 (DEA 1.1), as this antigen is the most likely to cause acute haemolytic reactions.\textsuperscript{44} As dogs are not born with natural antibodies to DEA 1.1, acute haemolytic transfusion reactions to an initial transfusion are rare. However there are many other RBC antigens that have the potential to reduce the life-span of transfused RBCs, therefore blood typing and cross-matching are always recommended.\textsuperscript{45}
Acute hypersensitivity reactions are not uncommon, particularly with plasma transfusions. These reactions result from soluble mediators in donor plasma binding to IgE antibodies on recipient mast cells, leading to the release of inflammatory mediators such as histamine. Common clinical signs include facial swelling and hives, which are treated by slowing the rate of infusion and the administration of anti-histamines.

Transfusions have also been shown to cause post transfusion inflammation in people and in dogs. In 76 critically ill non-septic people, 76% developed leukocytosis post transfusion, which was positively correlated with increasing interleukin-8 (IL-8) concentrations in the stored RBC products. Similar findings were reported in 50 critically ill people, with 90% of these patients developing a post-transfusion leukocytosis. Other human studies have also shown elevations in cytokine concentrations following transfusion. Transfusion administration in dogs has also been linked to the development of an inflammatory response. In one study, thirteen dogs were randomised to receive leukoreduced (LR) or non-leukoreduced (NLR) autologous PRBC that had been stored for 21 days. The leukocyte count, plasma fibrinogen and C-reactive protein (CRP) concentrations significantly increased from baseline after transfusion of autologous NLR PRBC. These elevations were not seen in dogs that received LR PRBC. Another study, in healthy dogs, found that transfusion of blood stored for 28 days induced production of monocyte chemoattractant protein-1, and an increased neutrophil count. These studies identify the presence of bioactive mediators within transfusion products which trigger the endogenous release of inflammatory mediators, and may contribute to transfusion reactions such as FNHTR and TRALI.

An FNHTR is a febrile response defined as a rise in temperature of at least 1°C, during or shortly after blood transfusion. In humans, the frequency of FNHTR is reported to occur in 1-6.8% of RBC transfusions and 18-37.5% of platelet transfusions. Patients suffering from FNHTR experience fever, chills, and discomfort. There are two mechanisms by which FNHTR are thought to occur. The first mechanism is associated with the production of leukocyte antibodies by the recipient, which react with donor leukocytes to cause the release of cytokines. A second mechanism involves the presence of inflammatory cytokines within the stored blood product. During storage, leukocytes continue to produce inflammatory cytokines via normal metabolic processes and also release cytokines and other bioactive mediators during apoptosis. These cytokines cause pyrexia by inducing the synthesis of prostaglandin E2 in the hypothalamus, resulting in elevation of the thermostatic set point. In 60 people experiencing FNHTR, there was a significant increase in interleukin-6 (IL-6) and IL-8 post transfusion.

The frequency of FNHTR in veterinary patients is not well reported. A recent retrospective study of 211 PRBC transfusions in dogs reported an incidence of FNHTR of 24%. The criterion to diagnose an FNHTR in this study was a rectal temperature greater than 39°C, therefore this study may have over-reported the occurrence of febrile reactions, as some of the dogs may have had an elevated temperature prior to transfusion. Determining the frequency of FNHTR in animals can be difficult as not all transfusion recipients have their temperatures closely monitored. It has also been demonstrated that dogs can develop an inflammatory response after transfusion that is not always associated with a febrile response. Similarly in people, a study of 41 patients receiving 117 RBC transfusions and 65 platelet transfusions, found that 28 patients had reactions associated with chills, cold and discomfort, consistent with FNHTR, but only three of these people were febrile.

Transfusion related acute lung injury is widely recognised in people following blood transfusion. The syndrome is defined by development of acute lung injury (ALI) within 6 hours of transfusion that is not associated with an alternative risk factor for ALI. Acute lung injury is defined as an acute onset of hypoxaemia with bilateral infiltrates on radiographs and no evidence of left-sided heart failure. Transfusion related acute lung injury is thought to be caused by the presence of antibodies within the donor plasma that react with leukocytes in the recipient. Alternatively, administration of lipids and cytokines within the transfusion product may directly activate leukocytes within the pulmonary capillaries. These activated leukocytes release inflammatory mediators, increasing capillary permeability and ultimately causing pulmonary oedema. The incidence of TRALI in people varies with the blood product administered and is reported to be between 0.04% and 0.16% of transfused patients. Although there has been a low incidence, TRALI is responsible for up to 16.3% of deaths associated with transfusion. The incidence of TRALI in dogs is not known and there is little published on this syndrome in dogs. There is one prospective observational study that assessed 54 dogs that received transfusions for various clinical conditions. The incidence of TRALI in this study was 3.7%. The incidence of TRALI in dogs and people is likely under-reported, as clinical signs seen with TRALI are often attributed to the underlying disease process.

Transfusion related immunomodulation (TRIM) has been extensively reported in humans transfusion recipients. In opposition to post transfusion inflammation, TRIM results from down-regulation of the immune system.
Some of the effects of TRIM include more frequent tumour recurrence, prevention of transplant rejection, and increased risk of post-operative infections. Little is known about these effects in dogs.

Cytokines and Transfusion
There are four major pro-inflammatory cytokines that have been associated with transfusion reactions in people: tumour necrosis factor-α (TNF-α), interleukin-1β (IL-1β), IL-6, and IL-8. Tumour necrosis factor-α and IL-1β have a pro-inflammatory influence on nearby cells, causing the classical signs of inflammation: redness, heat, swelling and pain. Their effects include enhancing the adhesiveness of vascular endothelial cells to neutrophils, promoting migration and activation of neutrophils, and enhancing the ability of neutrophils and macrophages to kill microbes. IL-6 is a mediator of the acute phase response promoting inflammation. Interleukin-1β, TNF-α, and IL-6 are all endogenous pyrogens that stimulate the release of PGE2 from the hypothalamus. Interleukin-8 is a unique type of cytokine called a chemokine. Interleukin-8 induces chemotaxis of neutrophils, promoting adherence to endothelial cells, degranulation and stimulation of the respiratory burst.

Many studies have measured cytokine concentrations in human stored RBC products. Although study results vary, the overall trend is that IL-1β, IL-8 and TNF-α accumulated in blood products during storage, and IL-6 concentrations either did not increase or were not detectable throughout storage. We recently confirmed the accumulation of IL-8 in canine PRBC during storage. A similar study in dogs that was also recently published, had similar findings.

Haemorrhagic shock in blood donors may induce cytokine production
Blood can be sourced from community blood donation programs or from surrendered retired racing greyhounds. These greyhounds are anaesthetised and two to three units of blood are collected from each dog prior to euthanasia. There is concern about the quality of the blood products collected from terminal donors, as these dogs are anaesthetised, often mechanically ventilated, and the last one to two units of blood are collected during the onset of haemorrhagic shock. All of these factors may have implications on the activity of leukocytes, and the concentration of bioactive mediators within the blood units collected, and therefore may have a detrimental effect on the transfusion recipient.

During haemorrhagic shock decreased perfusion to tissues such as the gastrointestinal tract can lead to mucosal sloughing, translocation of bacteria and release of inflammatory mediators. Studies in rats, pigs and people with acute haemorrhage have shown rapid elevations in inflammatory cytokines. Blood collected from anaesthetised dogs with haemorrhagic shock may contain more inflammatory mediators than blood collected from healthy conscious dogs. Therefore, in addition to measuring cytokines in the first units collected from anaesthetised greyhounds, we also measured cytokines in the third units collected from these same donors. We found that the concentration of IL-8 was not significantly higher in the third units collected compared with the first units collected. However, we did find marked variation in IL-8 concentrations in the third units collected, with some individual units having markedly elevated IL-8 concentrations.

Leukoreduction
Leukoreduction prevents the accumulation of bioactive mediators in blood transfusion products and therefore reduces the likelihood of transfusion reactions. In dogs, leukoreduction has been found to prevent the accumulation of IL-8, inflammatory microparticles, and vascular endothelial growth factor. The clinical benefit of leukoreduction has been well reported in people with a reduction in FNHTR, reduced mortality, decreased incidence of TRALI, decreased incidence of post-operative infections and a reduction in the transmission of infectious diseases such as cytomegalovirus. Since the observed benefit of leukoreduction, many countries have instituted universal leukoreduction schemes, and have reported a reduction in transfusion reactions. There are currently no prospective clinical studies in dogs that have investigated the use of LR blood. A study in dogs demonstrated an inflammatory response in healthy dogs receiving stored NLR PRBC units, indicated by elevated leukocyte counts, CRP and fibrinogen levels. In this study, a second group of dogs were given LR stored PRBC units and no inflammatory response was reported.

Leukoreduction increases the cost and preparation time of producing PRBC. However, in light of preventing the accumulation of bioactive mediators, and a likely reduction in patient morbidity and mortality, leukoreduction of canine blood products may be found to be very cost effective. Further large scale clinical studies are needed to determine the role of bioactive mediators on transfusion morbidity and mortality in dogs, and the effects of leukoreduction.
REFERENCES