



Evaluation of Blood Lactate Concentration in Dogs Receiving Whole Blood Transfusion

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ABSTRACT

Background: A blood transfusion is a routine, life-saving procedure used to replace blood cells or blood products. The current study was aimed to assess whether the blood lactate concentration has a prognostic value of successful blood transfusion.

Methods: During the period 2018-2019 group I dogs (n=19) received whole blood transfusion and twenty dogs were assigned to the control group (group II). Blood samples in group I were obtained from cephalic vein before blood transfusion (T0), then after it was finished (4hr±20 min) (T1) and 24hr±20 min after the T0 (T2).

Results: The level of blood lactate reached the normal level after blood transfusion in T1 and T2. The clearance of lactate had a moderate negative correlation with packed cell volume (PCV) and red blood cells (RBC). There was no significant correlation between survival rate and blood lactate level. The survivals 24hr after transfusion (T2) had five times higher count of reticulocytes (RETIC) than non-survivals. Serial blood lactate measurement can improve the prediction of successful blood transfusion and it is useful in monitoring the patient status 24hr post blood transfusion, but it doesn't have the predictive value of survival.

Key words: Anemia, Dog, Lactate, Transfusion.

INTRODUCTION

Blood transfusion is an important tool in the management of severe anemia in dogs. Transfusion of blood products restores tissue perfusion by increasing blood oxygen carrying capacity and improving oxygen delivery to the tissues (Kisielewicz *et al.* 2014). Blood transfusion has been used as an emergency and life saving step, since many years in human as well as animal medicine. It is the most common practice to save the critically ill patient having low blood parameters (Davidow, 2013). Whole blood and packed red cell transfusions are indicated in a variety of conditions including tissue hypoxia from acute/chronic blood loss or infection, immune mediated hemolytic anemia, bleeding disorders, poisonings like warfarin, hypoproteinemia, burns or decreased erythrocyte production due to bone marrow diseases (Bhikane and Kawitkar, 2002; Godinho-Cunha *et al.* 2011). Acute blood loss can lead to shock or even death if hypovolemia that develops is not treated. Studies in human emergency settings have shown, that beginning the transfusion before the arrival to the trauma center reduce the mortality rate of the patients (Brown *et al.* 2014; Brown *et al.* 2015).

Estimating the depth of shock and adequacy of resuscitation is at the highest importance in the care of badly injured patients (Basel *et al.* 2007; Dezman *et al.* 2015). Vital signs are often used as an initial measure of hemodynamic stability in both humans and animals, but studies prove that they might grossly underestimate the depth of shock (Basel *et al.* 2007; Scalea *et al.* 1998). There are numerous studies, which investigate central venous oxygen saturation, base deficit and lactate changes being more accurate than vital signs (Basel *et al.* 2007; Davis *et al.* 1996; Fukuma *et al.* 2019; Scalea *et al.* 1998).

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Although the transfusion manner is strict, but there are no universal, standardized, objective guidelines established for assessing whether people or dogs could benefit from transfusion (Kisielewicz *et al.* 2014). According to Kisielewicz *et al.* (2014) a critical PCV, HGB, RBC concentration, a level below which cellular metabolism becomes compromised, signifying that transfusion would be beneficial, remains undetermined despite clinical and experimental studies in people and an experimental study in dogs.

Blood lactate level represents global tissue oxygenation, anaerobic metabolism and lactic acidosis. Indications for measuring serum lactate include assessment of tissue perfusion and oxygenation, predicting outcome or response to therapy in critically ill patients and evaluation of metabolic acidosis (Vernon and LeTourneau, 2010). Several studies in veterinary medicine indicate that lactate concentration may have implications for prognosis (Beer *et al.* 2013; Di Mauro and Schoeffler, 2016; Sharkey and Wellman, 2013). Studies investigating hyperlactatemia in dogs with pyometra

and gastric dilatation-volvulus have shown a negative correlation between blood lactate concentration and survival rate (Beer *et al.* 2013; Hagman *et al.* 2009). Nel *et al.* (2004) concluded that serial blood lactate measurements are useful in predicting survival in dogs with severe and complicated canine babesiosis. However, during previous studies the authors did not investigate the predictive algorithm and correlation of lactate level and complete blood count during whole blood transfusion.

The main purpose of this study was to assess whether adding the serial blood lactate measurement to combination of clinical examination and blood analysis can improve the prediction of successful blood transfusion and whether it is useful in monitoring the patient status 24hr post blood transfusion and can be used as a biomarker of patient survival. Our study focused on determining whether blood lactate has a predictive algorithm of successful blood transfusion and survival of dogs.

MATERIALS AND METHODS

Thirty-nine client-owned dogs admitted to the Veterinary Teaching Hospital at the Lithuanian University of Health Sciences between June 2018 and July 2019 were assessed in this study. Nineteen dogs – 7 females and 12 males, weighing 21.68 ± 2.58 kg, 7.47 ± 0.7 years, were assigned to blood transfusion group (group I) and twenty dogs – 11 females and 9 males, weighing 24.3 ± 2.18 kg, 3.5 ± 0.4 years, were assigned to the control group (group II).

The study was carried out in the compliance with the EU legislation. The procedures complied with the criteria given by the Lithuanian animal welfare regulations (No. B1-866, 2012; No. X/i-2271, 2012) and the decree of the director of the State Food and Veterinary service, the Republic of Lithuania (No. B6-(1.9.)-1274, 2018). All dog-owners signed owner consent from prior to inclusion of their pet.

The criteria for selection of group I included dogs that were deemed by the attending clinician to require the whole blood transfusion. The decision to perform whole blood transfusion was determined by clinical signs and results of physical and special examination methods, not only by pre-selected PCV levels, which is PCV <15% (Choudhary *et al.* 2017). For blood transfusion Polymed (Poly Medicure Ltd., India) blood bag systems were used. According to Udegbumam *et al.* (2020) canine blood can be preserved with anticoagulant citrate phosphate dextrose adenine solution for 21 days and still remain viable for transfusion.

Dogs with complicated and uncomplicated hemolytic and non-hemolytic anemia were used in this study. All 19 dogs primarily were diagnosed with *Babesia spp.*, but after additional tests in ICU one of them was diagnosed with splenic mass and one with bone marrow suppression. The diagnosis was based on a case history, physical examination, blood analysis, blood smear cytology and additional examination methods. Physiological parameters (capillary refill time (CRT s), mucous membrane color (mm) (slightly pink, hyperemic, moderately pale, severely pale),

heart rate (HR beats/min), respiratory rate (RR breaths/min), rectal temperature (RT °C) were observed during clinical examination, also hydration status, body mass index (BMI (scale from 1 to 5) and general attitude were evaluated at the time of admission. Anemic dogs were not included in this study if they had received intravenous fluids previous 48 hr, previously has been treated with the whole blood transfusion or during the time of blood transfusion underwent surgery and anesthesia. Dogs that were in need for the second blood transfusion were not involved in the study.

Before blood transfusion group I dogs were blood typed as DEA-1.1. positive or negative by canine blood typing kit Rapid Vet-H (The RapidVet Company®, DMS laboratories, USA) and type compatible whole blood units were administered. The whole blood products were designated regarding the economical restrains presented by the owners and limited availability of other blood products in the area.

The volume of donor blood to be administered to the recipient was calculated using the following formula (Choudhary *et al.* 2017):

$$\text{Amount of donor whole blood} = \frac{\text{Body wt. (kg)} \times 90 \text{ ml} \times (\text{Desired PCV} - \text{PCV of recipient})}{\text{PCV of Donor}}$$

Healthy adult dogs that were staff-owned (n=8) and client owned (n=12) were included in the group II and admitted for prophylactic check-up and routine blood tests. A history questionnaire was completed to confirm that the owners considered the animals healthy for at least six months and did not have intensive training 48 hr prior to the examination. CRT, mm color, HR, RR, temperature and BMI were recorded. None of the control dogs had previously been treated with blood transfusion or had been used as a blood donor.

Approximately 2 ml of blood was collected from the distal cephalic vein into EDTA (IDEXX LaserCyte CBC5R Test Kit) tubes for blood hematological analysis. Blood samples were obtained after physical examination in control group (C0) and immediately after physical examination in blood transfusion group (T0), and then just after the blood transfusion was over 4-hr $\pm$ 20 min (T1). Follow-up sample (T2) was obtained 24-hr $\pm$ 20 min after the T0.

After filling of the blood collection tubes and release of the stasis, a drop of the fresh whole blood without preservative was applied on a Lactate Pro2 test strip (Arkray Inc., Kyoto, Japan) and analyzed immediately by the lactate analyzer Lactate Pro2® LT-1710 (Arkray Inc., Kyoto, Japan), according to the manufacturer's instruction. Morphological blood evaluation was conducted by IDEXX Lasercyte® hematology analyzer (IDEXX Laboratories, Inc., Westbrook, Maine, USA) within 5 min of samples collection. Owners of dogs discharged from the hospital were contacted 4 weeks after the discharge to obtain information regarding the survival of the patient. After period of 4 weeks dogs were classified as survivors and non-survivors.

All dogs with complications were treated for the specific complication as deemed necessary by the attending

clinician. Before, during and after blood transfusion capillary refill time, mucous membrane color, heart rate, respiratory rate and temperature were recorded. Hospitalization was considered necessary until the patient was stable.

Data analysis was performed using IBM SPSS Statistics® software program (Statistical Package for Social Sciences 20 for Windows). Averaged experimental results were expressed as means $\pm$ standard error of the mean (SEM). Data between group I at T0, T1, T2 and group II were processed using a non-parametric statistical Mann-Whitney test. The significance of differences between T0, T1, T2 data in group I was evaluated by non-parametric Friedman test. A P value <0.05 was considered to be significant. The association between lactate and blood parameters was measured by Spearman's rank correlation.

RESULTS AND DISCUSSION

Eight dogs of group I were admitted to the clinic with severely pale mucous membranes, nine with moderately pale mucous membranes, one with slightly pink and one with hyperemic mucous membranes. The difference with group II of which all were presented with salmon pink mucous membranes was highly significant ($P<0.05$). There were no significant difference concerning the body temperature before blood transfusion in group I (38.0 ± 0.23) and group II (38.45 ± 0.11) ($P<0.05$). Heart rate in group I patients before blood transfusion (T0) was 130.11 ± 38.65 beats/min and decreased highly significantly ($P<0.05$) to 106.42 ± 21.60 beats/min (T1), while in group II the heart rate was 82.44 ± 12.1 beats/min. In case of a blood loss and the decrease in oxygen delivery to the tissues the body activates the sympathetic and adrenergic nervous systems, which increases heart rate and cardiac output (Carson and Hébert, 2009). When these patients receive a blood transfusion, blood volume and oxygen-carrying capacity are re-established; therefore this normalizes the heart rate and decreases the pulse rate (Godinho-Cunha *et al.* 2011). The breathing rate was within normal range in group I (49.68 ± 7.39 breaths/min) (T0) and above normal range in group II (82.4 ± 12.1 breaths/min) that could be due to stress factor. Experimental studies have shown that careful monitoring of a patient, a strict transfusion policy, blood typing and cross matching procedures minimize the risk of an adverse reaction and maximize the benefits of blood transfusion (Godinho-Cunha *et al.* 2011; McDevitt *et al.* 2011). The results of this study have shown that the color of mucous membranes and the heart rate will improve significantly after blood transfusion in anemic dogs and it is helpful in clinical decisions and monitoring of the patient status, however it is recommended that every anemic dog status would be followed by complete clinical examination and recorded before, during and after transfusion (Kisielewicz *et al.* 2014).

Lactate concentration is widely used as an indicator of tissue hypoperfusion and hypoxia in critically ill patients and as a biomarker to determine response of the therapy or to predict outcome of the disease (Fukuma *et al.* 2019; Shapiro

et al. 2005, Sharkey and Wellman, 2013, Vandromme *et al.* 2010). As the end product lactate forms primarily during anaerobic glycolysis. Lactic acidosis occurs most commonly with tissue hypoperfusion and hypoxia, often as a consequence of systemic or regional hypoperfusion, severe anemia, or hypermetabolic states (Di Mauro and Schoeffler, 2016; Sharkey and Wellman, 2013). Increased oxygen demand or decreased oxygen delivery causes type A lactic acidosis. Shock and anemia induce hypoperfusion and decrease oxygen delivery and increase anaerobic glycolysis and accumulation of pyruvate causing type A lactic acidosis.

In our study before blood transfusion (T0) the mean blood lactate level was above normal range (4.42 ± 1.19 mmol/l) and indicated highly significant difference ($P<0.05$) compared with T1 (2.23 ± 0.3 mmol/l) and T2 (1.75 ± 0.27 mmol/l). In the 20 control dogs the lactate levels ranged between 1.1 - 2.4 mmol/l, with a mean level of 1.51 ± 0.1 mmol/l not reaching the upper limit of a normal range (<2.5 mmol/l) and was significantly lower compared to T1 ($P<0.05$). In eleven dogs in group I (T0) before blood transfusion blood lactate level was in a normal range (<2.5 mmol/l) and in eight dogs were above normal range (>2.5 mmol/l). After blood transfusion (T1) twelve dogs had the level of lactate in a normal range and six dogs still had it above normal range (one dog was excluded from the study because of aggressive behavior). On the last sampling (T2) fourteen dogs maintained or reached the normal level of blood lactate and one presented the level of 5.0 mmol/l (two dogs were excluded from the study due to revised diagnosis and one due to impaired venous access). When lactate levels decrease it suggest an improvement, whereas prolonged increase in lactate concentration is a sign of a poor prognosis (Regnier *et al.* 2012, Sharkey and Wellman, 2013).

Blood transfusion was effective in inducing measurable increase in PCV ($P<0.05$), RBC ($P<0.05$) and HGB ($P<0.05$) in T1 and T2, although the platelet count decreased significantly in T1 ($P<0.05$). The mechanism by which the decrease in platelets develops was described by Jutkowitz *et al.* (2002) in a retrospective study on massive transfusions in dogs. The most important causes seem to be hemodilution caused by intensive fluid therapy and infusion of platelet-poor blood (stored for over 6 hr). Blood lactate, PCV, RBC, HGB and PLT levels are presented in Table 1. Present study concluded that clearance of lactate had a significant ($P<0.05$) moderate negative correlation with PCV and RBC. A large retrospective study of dogs with immune-mediated hemolytic anemia found that blood lactate was inversely correlated with PCV and non-survivors had higher median blood lactate concentrations at presentation than survivors (4.8 mmol/l vs 2.9 mmol/l) (Holahan *et al.* 2010). According to that study all dogs in which blood lactate normalized by 6 hr survived, whereas 71% of dogs with persistent hyperlactatemia survived, implying that serial monitoring may improve the predictive value of lactate evaluation. In one cohort of 90 dogs with babesiosis Nel *et al.* (2004) concluded that blood lactate concentrations can serve as a predictor of outcome

Table 1: Blood lactate, PCV, RBC, HGB and PLT levels changes in dogs during blood transfusion.

Parameters	Group I			Group II
	T0 (n=19) (before blood transfusion)	T1 (n=18) (after blood transfusion)	T2 (n=15) (24 hr after T0)	C0 (n=20)
Blood lactate (mmol/l)	4.42±1.19 a)	2.23±0.3	1.75±0.27	1.51±0.1
PCV (%)	19.87±2.47 a)	24.62±2.2 a)	26.18±3.12 a)	51.3±1.79
RBC (10 ¹² /l)	2.85±0.35 a)	3.67±0.36 a)	3.90 ±0.46 a)	7.16±0.25
HGB (g/dl)	7.45±0.87 a)	9.26±0.83 a)	9.43±0.87 a)	15.9±0.34
PLT (K/μl)	253.92±50.47	171.77±40.6 a)	180.85±53.44 a)	274.15±11.6
P value b)				
Parameters	P T0-T1	P T1-T2	P T0-T2	P T0-T1-T2
Blood lactate (mmol/l)	P>0.05	P>0.05	P<0.05	P<0.05
PCV (%)	P<0.05	P>0.05	P<0.05	P<0.05
RBC (10 ¹² /l)	P<0.05	P>0.05	P<0.05	P<0.05
HGB (g/dl)	P<0.05	P>0.05	P<0.05	P>0.05
PLT (K/μl)	P<0.05	P>0.05	P>0.05	P<0.05

The values represent the means ± standard error. a) Statistically significant difference between Group I (T0, T1, T2) and Group II ($p<0.05$); b) Significance of differences between T0, T1, T2 data in group I.

in dogs suffering from severe or complicated canine babesiosis. Hagman *et al.* (2009) in their study concerning blood lactate levels in dog with pyometra found out that a single preoperative lactate measurement was not indicative of outcome as determined by presence of SIRS or increased hospitalization. Although pretreatment hyperlactatemia indicates a poorer prognosis, subsequent serial lactate concentrations show a much stronger association with mortality (Nel *et al.* 2004; Sharkey and Wellman, 2013).

In group I four dogs died in the period of 4 weeks after blood transfusion. We did not find significant correlation between survival rate and blood lactate level. According to Eichenberger *et al.* (2016) study in acute *Babesia canis* infection they had opposite results and non-survivors showed significantly higher concentrations of lactate, triglycerides and phosphate and lower PCV and PLT counts when compared to survivors, but in our study we found out that the concentration of lactate, PCV, RBC and HGB doesn't have a prognostic value for the survival of a patient. There are various studies published concerning blood lactate level changes and prognostic values of it. Beer *et al.* (2013) in their study have shown that in dogs admitted to the hospital with gastric dilatation volvulus plasma lactate concentration was a good predictor of gastric necrosis and outcome. Vandromme *et al.* (2010) concluded that in human emergency department blood lactate level is an important adjunct in characterizing shock and is a better predictor than systolic blood pressure in identifying patients requiring blood transfusion and suggested that point-of-care blood lactate measurements could improve trauma triage and better identify patient for enrollment in interventional trials.

In our study the survivals 24 hr after transfusion (T2) had five times ($P<0.05$) higher count of reticulocytes (170.78 ± 44.22 K/μl) then non-survivals (29.55 ± 9.03 K/μl) and two times more than group II patients (74.52 ± 6.34 K/μl). Decrease or ineffective erythropoiesis is associated with various specific mechanisms – impaired hormonal

stimulation, diminished availability of nutrients or other reasons. Ineffective erythropoiesis is a relatively uncommon condition (Grimes and Fry, 2015), so more precise attention could be given in the more detailed study to indicate the reasons of ineffective erythropoiesis.

CONCLUSION

Clinical examination of the patient was an important tool for making a decision to perform blood transfusion and to monitor the patient status during and after the procedure. Blood transfusion was effective in inducing measurable significant increase in PCV, RBC and HGB, accordingly blood analysis as suspected was useful in evaluating the success of blood transfusion but did not show a prognostic algorithm. The level of blood lactate reached the normal level after blood transfusion in T1 and T2. The clearance of lactate had a moderate negative correlation with PCV and RBC. In group I four dogs died in the period of 4 weeks after blood transfusion. There was no significant correlation between survival rate and blood lactate level. The survivals 24 hr after transfusion (T2) had five times higher counts of reticulocytes then non-survivals. We conclude that adding the serial blood lactate measurement to combination of physiological variables and blood analysis can significantly improve the prediction of successful blood transfusions and it is useful in monitoring the patient status 24 hr post blood transfusion, but it doesn't have the predictive value of survival.

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