Scientific Works. Series C. Veterinary Medicine. Vol. LXII (2)
ISSN 2065-1295; ISSN 2343-9394 (CD-ROM); ISSN 2067-3663 (Online); ISSN-L 2065-1295

THERAPEUTIC EFFICACY AND SAFETY EVALUATION OF ERYTHROCYTE CONCENTRATE USED IN DOGS

Ildikó BARABÁSI[1], Viorica CHIURCIU[2], Constantin CHIURCIU[2], Laurenţiu OGNEAN[1*]

[1]University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca,
400037, Manastur street, no.3-5, Cluj-Napoca, Romania
[2]Romvac Company, Romania

*Corresponding author: lognean@yahoo.com

Abstract

We studied the clinical and hematological changes of 18 dogs from admission day (T0) until 5 days post-transfusion (5 days after the last administered transfusion), as well as hematocrit changes 6 hours post-transfusion therapy with erythrocyte concentrate. This research took place in a period of 6 months in 2014 in a small animal clinic from Germany. Most of the patients have been diagnosed with immune-mediated hemolytic anemia (n=11), 2 with rodenticide poisoning, 1 with babesiosis, 1 with hemangiosarcoma, 1 with septic peritonitis, 1 with idiopathic hepathopathy and 1 with hypothyroidism. The 18 patients received a total of 30 transfusions with erythrocyte concentrate in a mean dose of 11.46 ml/kg. Of the 30 transfusions, 6 reached the calculated hematocrit rise 6 hours post-transfusion, 2 had a higher than expected value and 22 did not reach the expected value. The hematocrit value 6 hours post-transfusion was statistically extremely significant (p<0.0001). We have observed positive changes in all hematological parameters 5 days after the transfusion therapy of which 3 have been statistically significant. The red blood cell count underwent a statistically very significant (p=0.0052) change, as has the hemoglobin level (p=0.0085). The hematocrit level had a statistically extremely significant change (p=0.0002) from the admission day until day 5 post-transfusion.

Key words: *dogs, erythrocyte concentrate, hematocrit, immune-mediated hemolytic anemia, transfusion therapy.*

INTRODUCTION

The purpose of this study has been the evaluation of the immediate and long term therapeutical efficacy of erythrocyte concentrate in dogs (Kisielewicz et al 2014) with different types of anemia. These objectives have been pursued by measuring the hematocrit level 6 hours after the transfusion therapy has been discontinued and by performing a complete blood count 5 days post-transfusion therapy. We also clinically monitored the patients for any signs of transfusion related adverse reactions.

MATERIALS AND METHODS

This research took place in the Small Animal Clinic of Internal Medicine Department of Justus-Liebing University in Giessen, Germany. The study lasted for 6 months, between February and August of 2014. During this time 18 dogs received transfusion therapy with erythrocyte concentrate. From this patient pool 44.44% (*n=8*) were females and 55.55% (*n=10*) males. Mean age of the patients has been 6.5 years, with the youngest patient being 1 year old and the oldest 11 years old. The patients have been of quite varied breeds: 3 common breeds, 2 Cane Corso Italiano, 2 Labrador Retrievers, 1 Border Collie, 1 Shetland Shepherd, 1 Bearded Collie, 1 Miniature Pinscher, 1 Spitz, 1 Havanese Bichon, 1 Australian Shepherd, 1 Doberman Pinscher, 1 Dachshund, 1 Cocker Spaniel and 1 Belgian Shepherd.

Blood tests have been made with ADIVA hematological analyzer; the 6 hour post-transfusion hematocrit has been determined by performing a micro hematocrit.

Every patient taken into this study has been transferred to the clinics Intensive Care Unit where they were permanently monitored.

On admission every patient received a routine blood test that included 40 hematological parameters and 21 biochemical parameters. In addition, a detailed examination of the blood smears has been also performed by the ADIVA hematological analyzer with 26 parameters that mostly referred to red blood cell and white blood cell morphology.

All erythrocyte concentrate units have been prepared in the clinic, with the help of a special centrifuge designed for blood bags and a plasma separator. This way the obtained erythrocyte concentrate units have been quite similar, with a hematocrit level of about 70%. All units have been stored in a refrigerator used only for blood product storage that has been monitored daily by a technician for the adequate temperature. None of the blood units have been stored for longer than 6 days.

Every patient has been blood typed for DEA 1.1. blood type, using the RapidVet quick test kit. Dogs received only type specific blood and to limit transfusion reaction occurrences, in addition, a crossmatch test has been performed before every transfusion. This crossmatch test served another purpose as well, besides verifying patient-donor compatibility; a positive auto-agglutination test provided an additional proof for the immune-mediated hemolytic anemia diagnosis. The 18 dogs taken into this study received a total of 30 blood transfusions (a *mean of 1.66*). The most transfusions given to one patient has been 4, received in the first 5 days after admission.

The causes of anemia encountered in this patient group has not been very versatile, mostly because the study has been made in a reference clinic where the most difficult to diagnose and to treat patient are being sent and attended. Most patients (61.11%; *no=11*) have been diagnosed with idiopathic immune-mediated hemolytic anemia. Of these 11 patients only in one could the immune-mediated anemia be linked later to a lymphoma. The rest of the group there have been 2 patients (11.11%) diagnosed with rodenticide poisoning, 1 (5.55%) with babesiosis, 1 with a hemangyosarcoma, 1 with adenocarcinoma and septic peritonitis due to complications that occurred after surgery, 1 idiopathic hepatopathy and 1 with hypothyroidism.

Mean dose of administered erythrocyte concentrate has been 11.46 ml/kg, with a minimal dose of 3.4 ml/kg and a maximum dose of 24.7 ml/kg. Mean transfusion rate has been 21.86 ml/kg/h, with a minimum speed of 6ml/kg/h and a maximum speed of 45 ml/kg/h.

Research concerning the long term therapeutically efficacy (5 days post-transfusion) of erythrocyte concentrate used in transfusion therapy has been conducted upon 13 of the 18 initial patients that were used in the starting phases of the study. No data has been available for 4 of these 5 patients that were excluded from the second phase of the study because they have been released from the clinic in less than 5 days.

The 5th patient suffered from an extremely severe form of immune-mediated hemolytic anemia and needed 4 transfusions in the course of 5 days. Among the patients that have been included into the long term therapeutical efficacy study of erythrocyte concentrate 3 have received 2 transfusions in less than 24 hours apart and the other patients received one transfusion each.

Statistical analysis of the transfusion therapy efficacy has been performed with GraphPadInStat 3.0 statistical program and the graphical depiction in form of a box-plot of the obtained results has been made using the Origin8.5. graphics program.

RESULTS AND DISSCUSIONS

In the present study we have followed-up the evolution of the total white blood cell level, the neutrophil level, lymphocyte numbers, the total red blood cell count, the hematocrit level, platelet number, the total number of reticulocytes and the spherocyte percentage from the day of admission (T0) up to the 5th day (T5) after the last administered transfusion therapy with erythrocyte concentrate. In the case of those patients that needed multiple transfusions, T5 has been considered the 5th day after the last administered transfusion therapy.

The decision of starting a transfusion therapy has been made by the attending physician of each patient. Administered doses had been given according to the results obtained from the following equation:

Transfused volume = (desired Ht-patient Ht/donor Ht) x kg b.w x 90

This equation has been chosen according to the results presented in recent studies in this field conducted upon the efficacy of various equations used to determine the ideal transfusion dose for dogs (Short et al, 2012).

The desired hematocrit level that the patient should have reached after the transfusion therapy has been set by the attending physician of each patient. Alongside the transfusion therapy, the patients have been treated according to the their pathology, but none received any kind of intravenous or other type of treatment as long as the transfusion therapy has been administered.

Of the 18 patients that have been monitored for the hematocrit level changes at 6 hours post-transfusion therapy, only in one we have observed transfusion related adverse reactions represented by vomiting, pyrexia, melena, hemoglobinuria and hemoglobinemie. This patient suffered from a very severe form of immune-mediated hemolytic anemia and presented transfusion related adverse reactions after every transfusion therapy.

There have been administered a total of 30 transfusions to the 18 patients taken into the study of the immediate therapeutic efficacy of erythrocyte concentrate in dogs.

Of these 30 transfusions 6 (20%) reached exactly the desired hematocrit level at 6 hours post-transfusion, used in the above presented equation. In 2 occasions (6.66%), both transfusion given to patients with immune-mediated hemolytic anemia, we have observed a higher than expected level of the hematocrit (of 6% and 12%, respectively) at 6 hours after transfusion. The other 22 administered transfusion therapies (73.33%) did not produce the calculated augmentation upon the hematocrit level at 6 hours after transfusion. Besides the 4 transfusions that have been administered to the patient with the very severe form of immune-mediated hemolytic anemia, none could have been related to a potential transfusion related adverse reaction. We did not find any proof of a potential intravascular or extravascular hemolytic reaction in any other patient. Patient-donor compatibility of the administered blood product has been determined by blood typing of both individuals and a crossmatch test performed before every transfusion therapy. The mean difference observed between the desired hematocrit level used in the equation and the actual hematocrit level reached after 6 hours post-transfusion therapy has been 3.59%, with a minimal difference of 2% and a maximum difference of 12%.

The statistical analysis revealed an extremely significant ($p<0.0001$) rise of the hematocrit level changes observed at 6 hours after transfusion. Of the initial 18 patients we could use 13 patients for our study of the long term therapeutic efficacy of erythrocyte concentrate in dogs. Each of these 13 patients survived until discharge.

This study focused upon hematological changes observed between admission day (T0) and the 5^{th} day after the transfusion therapy with erythrocyte concentrate has been discontinued (T5). Table 1 depicts the hematological parameters on admission day that we followed through this study. We have observed major changes in the evolution of every hematological parameter that we followed. The statistical analysis conducted on the obtained results reveal that the total red blood cell count underwent very significant changes ($p=0.0052$); the hemoglobin suffered as well a very significant change ($p=0.0085$). Of all the hematological parameters that we studied, the one with the most significant evolution has been the hematocrit. This suffered an extremely significant ($p=0.0002$) statistical evolution. As far as the other hematological parameters are concerned, none underwent statistically significant evolutions from T0 until T5. After they have been discharged all patients have had outpatient treatments continued at home. In addition to the outpatient treatments, all 13 patients have been called in weekly for the first month after discharge for follow-up examinations and blood tests performed by the same veterinary physician that they were assigned to on admission day. Patients, who could not come to the clinic for the scheduled follow-up examinations, performed the necessary check-ups and blood tests at a local veterinarian office that kept in touch and consulted the next treatment steps with the cases initial physician from the clinic.

According to the patient's evolution, these follow-up examinations developed from weekly to monthly visits. Every case has been followed through until the treatment could be discontinued.

From these periodically performed not only clinical but hematological and biochemical reexaminations, we have learned that none of

the patients included in this study manifested any delayed transfusion related reactions.

Only one of the 18 initial patients suffered a relapse of a severe immune-mediated hemolytic anemia and came back to the clinic in a critical state.

The owners refused treatment and the patient has been consequently euthanized.

Transfusion therapy using erythrocyte concentrate has been proven to be well tolerated by dogs. The safety of this blood product in therapy, even in dogs in critical state has been demonstrated by the few transfusion related adverse reactions we have observed that have been in fact induced by the patients primary pathological process.

Table.1 Hematological parametres on admission day (T0)

Nr	WBC 10^9 /l	N 10^9/l	L 10^9 /l	PLT 10^9 /l	RBC 10^{12}/l	Ht l/l	Hb mmol/l	Reti 10^9/l	Spher %
1.	42.0	7.25	29.67	958	1.33	0.14	3.4	668.5	3
2.	17.08	10.90	4.82	183	1.80	0.14	3.9	82.90	2
3.	73.04	38.53	26.75	359	1.25	0.13	3.2	605.5	2
4.	26.59	18.28	6.51	563	1.57	0.18	2.8	530.2	3
5.	18.03	11.03	1.98	573	4.99	0.26	8.3	156.7	1
6.	8.40	5.57	2.19	16	3.48	0.23	4.7	5.10	3
7.	34.63	26.82	5.32	75	2.11	0.15	3.0	231.1	2
8.	19.53	9.53	8.19	174	1.35	0.10	2.1	85.70	3
9.	17.24	14.38	1.14	70	2.52	0.15	5.2	126.3	2
10.	12.96	7.91	3.51	185	1.96	0.15	4.7	79.2	3
11.	6.44	2.65	3.19	440	1.85	0.12	2.8	10.0	0
12.	8.62	6.48	1.40	81	2.22	0.17	2.9	438.3	1
13.	32.3	19.92	6.38	156	2.03	0.15	3.1	171.1	2
Mean	24.37	13.7	7.77	294.8	2.18	0.15	3.85	245.4	2.1
StD	18.2	9.98	9.33	273.1	1.02	0.04	1.61	232.2	0.95
Min.	6.44	2.65	1.14	16.0	1.25	0.10	2.1	5.1	0
Max.	73.04	38.53	29.67	958	4.99	0.13	8.3	668.5	3
Ref. val.	6.0 -17.0	2.78 -8.73	0.72 –4.71	150 – 500	5.5 – 8.5	0.39 – 0.56	8.06-12.21	0 – 60	<4%

Table.2 Hematological parameters on the 5th day post-transfusion

Nr	WBC 10^9/l	N 10^9/l	L 10^9/l	PLT 10^9/l	RBC 10^{12}/l	Ht l/l	Hb mmol/l	Reti 10^9/l	Spher %
1.	19.59	7.61	10.30	901	3.81	0.34	6.3	724.4	10
2.	35.68	27.48	4.39	18.51	2.37	0.18	5.5	206.50	0
3.	43.9	26.43	12.21	538	3.17	0.29	4.8	974.5	20
4.	30.33	23.53	3.06	905	2.69	0.26	4.5	440.7	3
5.	29.86	27.57	1.17	832	4.26	0.25	4.8	53.5	1
6.	10.22	7.34	2.34	19	4.41	0.30	6.2	7.10	1
7.	10.56	6.92	2.90	129	7.00	0.50	10.1	507.10	10
8.	10.90	3.45	6.54	292	4.01	0.29	5.5	363.90	5
9.	12.02	8.45	2.37	136	4.60	0.33	6.3	227.80	4
10.	10.05	7.75	1.69	464	3.08	0.25	4.4	108.0	7
11.	7.53	3.93	2.80	301	3.39	0.24	4.8	13.70	0
12.	2.76	1.18	1.02	207	3.47	0.26	4.8	42.20	0
13.	76.95	39.11	26.86	110	1.18	0.12	4.9	275.70	7
Mean	23.1	14.67	5.97	373.2	3.64	0.27	5.6	303.4	5.7
StD	20.4	12.3	7.17	327.0	1.37	0.08	1.5	295.5	5.23
Min.	2.76	1.18	1.02	18.51	1.18	0.12	4.4	7.1	0
Max.	76.95	39.11	26.86	905	7.00	0.22	10.1	974.5	20
Ref. val.	6.0 -17.0	2.78 - 8.73	0.72 – 4.71	150 – 500	5.5 – 8.5	0.39 – 0.56	8.06-12.21	0 – 60	<4%

Research conducted upon the hematocrit level changes pre- and 6 hours post-transfusion, revealed a small percentage of cases in which the desired hematocrit rise has been achieved using the blood product dose obtained using the equation. Our study showed a mean difference between the desired hematocrit level used in the equation and the obtained one at 6 hours after transfusion to be 3.59%. This small difference still allows us to stipulate the efficacy of the transfusion therapy. However, a revision of the used equation is needed since in most of the administered transfusions there has been an absence of any transfusion related adverse reactions, but the desires hematocrit level has not been achieved. Also an important fact that must be taken into account is that the transfusion treatment has been initiated shortly after the patient has been admitted. Most of the patients that have been taken into this study were diagnosed with an immune –mediated pathology in which the body destroys its own red blood cells. The treatment for the immune-mediated hemolytic anemia has been started in the same day as has the transfusion therapy. The failure to achieve the calculated heamtocrit rise at 6 hours post-transfusion involves the pathological process from which suffered the patients as well. Taking into account the hematocrit level evolution from the day of admission and 5 days after the transfusion therapy has been discontinued, it can be stated that this therapy has reached the desired effect. In the 5th day after transfusion a rise in the hematocrit level can be observed (Fig.1.).

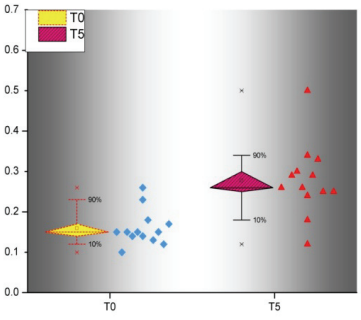

Figure 1. Hematocrit level evolution between day of admission and the 5th day post-transfusion

The same positive effect can be seen in the total number of red blood cells. We could observe a rise of this parameter in each of the 13 patients from the study (Fig. 2.).

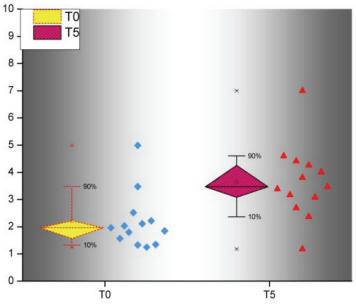

Figure 2. Red blood cell count from admission day and the 5th day post-transfusion

Similar data was found by Gibson and his collaborators (2007), as well as Helm and his collaborators (2010), which made some indications on dosage and good practice protocols following their research. Also Ognean and collaborators (2015) have reached similar conclusions in a study focused on transfused dogsNext to the hematocrit level and the red blood cell count, hemoglobin is the third hematological parameter used to evaluate the severity of the anemia and bloods oxygen carrying capacity (Callan et al, 2010).
Hemoglobin levels have risen in every patient taken into study in the 5 days after the transfusion therapy has been discontinued (Fig.3).

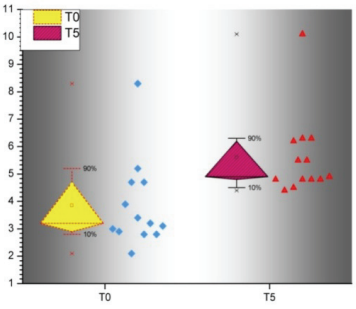

Figure 3. Hemoglobin amount evolution from day of admission and the 5th day post-transfusion

The total number of white blood cells, neutrophils and lymphocytes, has been mostly influenced by the patient's pathology.

The total number of platelets has had a rising tendency between the day of admission and the 5th day post-transfusion, their value being outside of the physiological reference range in only 4 of the patients in the 5th day after transfusion.

The majority of the 13 patients taken into the long term efficacy study of erythrocyte concentrate have reached a point in the 5th day after transfusion therapy in which they were no longer considered in danger of tissue hypoxia. Two patients remained with low red blood cell number and hematocrit level, but a rise of the hematological parameters could be seen anyway in the 5th day post-transfusion comparing to admission day.

Some of the studied values have been increased and influenced by the body's own compensatory mechanisms as well that involve an increased production and release of reticulocytes. These are precursor cells that the body produces in a much faster rate and greater amount to compensate the lack of red blood cells in case of an anemia caused of no matter what. This response of the bone-marrow can be seen in 3 to 5 days after clinical signs of anemia are visible. A greater number of reticulocytes have been observed on admission in comparison to the 5th day after transfusion therapy in 12 of the 13 patients that took part of the study. Production of these cells decreases only if the patient is no longer anemic or if the cell production capacity of the bone-marrow has been suppressed or compromised.

CONCLUSIONS

The erythrocyte concentrate can be used safely even in critically ill patients, immune-suppressed, or in case of an exaggerated immune response.

A clear dosage of this blood product has not been set yet; every administration has to be tailored to the patients needs. The equation used to calculate administered dose, failed to give an amount that would get the desired effect. However, it must be taken into account that most patients in this study have been diagnosed with immune-mediated hemolytic anemia that could have contributed to the failure of reaching the calculated hematocrit level.

ACKNOWLEDGMENT

The authors are thankful to S.C. Romvac Company SA for the financial support of this study.

REFERENCES

Callan, M.B., 2010. Red blood cell transfusion in dogs and cats, In: *Schlam's Veterinatry Hematology, Ed. Douglass J. Weiss, Jane K. Wardrop, ed John Wiley &Sons, chap.* 95, pg. 738-743.

Gibson, G., 2007. Transfusion medicine, In: *Canine and feline emergency and critical care, Ed., Lesley G. King, Amanda Boag,* ed. BSAVA, 2nd ed, chap. 14, pg. 215-227.

Helm, J., Knottenbelt, C., 2010. Blood transfusion in dogs and cats, *In Practice,* vol. 32, pg.184-189.

Kisielewicz, C., Self, I.A., 2014 - Canine and feline blood transfusions: controversies and recent advances in administration practices, *Veterinary Anaesthesia and Analgesia,* vol. 41, pg. 233–242.

Ognean L., Viorica Chiurciu, Cristina Ştefănut, L. Oana, I. Morar, Ildikó Barabási, 2015. Transfusion Triggers and Therapeutic Efficacy in a Group of Dogs That Underwent Whole Blood Therapy. *Agriculture and Agricultural Science Procedia,* 6, pg. 363–369

Short, J.L., Diehl, S., Seshadri, R., Serrano., S., 2012. Accuracy of formulas used to predict post-transfusion packed cell volume rise in anemic dogs, *Journal of Veterinary Emergency and Critical Care,*vol. 22, pg. 428–434.