The management of canine transfusion reactions reported in some clinics from Transylvania

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Abstract
The transfusion of blood products is an essential and also a common therapeutic procedure used in veterinary medicine. Because blood transfusion is not a completely harmless therapeutic procedure, its usage requires a good amount of knowledge about the possible adverse effects and complications that may occur during this process. This kind of data is exactly what the present study brings to doctors attention, being based upon the management of various forms of transfusions reactions in canines which were given whole blood, erythrocyte concentrate (EC) or blood plasma (P). The main objectives were preventing, monitoring and treatment of this reaction type. The clinics included in this study reported multiple forms of transfusion reactions in canine patients, such as: severe tachycardia (no=5); passing hyperthermia (40°C) (no=5); emesis and melaena during transfusion (no=3); myoclonic head seizures and bruxism (no=1); delayed hemolytic anemia (AHI) (no=1); TRALI type respiratory syndrome (no=1). In most cases, these symptoms subsided after a few minutes from transfusion or stopped completely, except the last two cases, which presented severe reactions, without any response to treatment and resulting in death. This kind of complications resulted due to AHI condition in a patient with chronic renal failure (the diagnosis was based on pollakiuria, haematuria and BUN/creatinine ratio of 28.4) and the evolution of TRALI respiratory syndrome in another patient with malignant multicenter lymphoma (cytologically confirmed). The AHI type of post transfusion reaction diagnosis and management was done by monitoring the decreasing oscillations of the whole blood mass, after 3 transfusions with whole blood. The TRALI syndrome (Transfusion-Related Acute Lung Injury) diagnosis was based upon cytological examination and later, necropsy.
Keywords*: adverse reactions, blood products, dog, transfusion.*

Introduction

Transfusion adverse reactions are the most common complications after therapy with blood products, and in humans, they occur at least once in one hundred cases (Delaney et al., 2016; Ognean, 2017). Similar reactions with those encountered in humans have been frequently reported in pets, without a correct diagnosis and treatment in most of the cases. These kinds of reactions are commonly caused by transfusion incompatibility and sometimes by inappropriate storage or administration of blood products. The majority of the adverse reactions occur during the transfusion process or right after it and they present acute hemolytic, anaphylactic or allergic reactions. Apart from these evident reactions, there are types of complications that cannot be immediately identified, such as: delayed hemolytic reaction, immune and non-immune reactions, hypothermia, citrate intoxication or heart failure (Abrams-Ogg, 2000; Kohn et al., 2000; Hohenhaus, 2006; Ognean, 2017).

Among the principles that stand upon prevention and management of adverse reactions, we mention selection of the best compatible donor patient, proper storage and handling of blood products, patient monitoring and care during transfusion process, immediate stopping of transfusion when adverse reactions occur, immediate treatment of allergic or anaphylactic reactions with antihistaminic or cortisone products; adding adrenergic and antipyretic medication to the treatment protocol in case of fever and intravenous use of calcium in case of citrate intoxication symptoms.

Material and methods

The present paper was designed to present the management of complications and transfusion reactions occurred in canine patients transfused with whole blood, erythrocytes concentrate (EC) or plasma (P) in some veterinary clinics from Transylvania. The major purpose of this research was to evaluate the efficiency of some preventive methods and the adverse transfusion reactions treatment in dogs from clinics and veterinary practices.

The evaluation process was carried out by 3 clinics and 4 veterinary private practices, which reported 16 forms of complications/transfusion reactions, such as: severe tachycardia - 5 cases; passing hyperthermia (40°C) - 5 cases; emesis and/or melaena during transfusion - 3 cases; myoclonic head seizures and bruxism - one case; delayed hemolytic anemia (AHI) - one case; TRALI (Transfusion-Related Acute Lung Injury) type respiratory syndrome - one case. Patients evaluation and decision making of blood transfusion was based upon the correlation between the clinical, haematological and biochemical examination, with automated or semi automated hematology analyzers (Abacus Junior Vet and Idexx QBC Vetautoread) and automated analyzer for clinical chemistry (Arkray Spotchem EZ-SP-4430 Refurbished and MINDRAY BA-88ª). In some cases of severe illness, the diagnosis confirmation was carried out at SinevoVet or other diagnostic centers.

The transfusion of blood products was made, in most of the cases, after establishing the donor-patient compatibility with blood type tests (Rapid Vet®H-DMS Laboratories and Rapid DMEVET-Alvedia) and/or Crossmatch. We observed that 4 of the patients were transfused without compatibility evaluation, the reason invoked being the emergency of the procedure and the absence of transfusion risk. The blood products were administered exclusively intravenously (cephalic vein, saphenous vein and jugular vein), by using closed circuit IV (intravenous) set tubing and catheters, which were previously selected according to patients size; in some cases, blood filters were used.

The blood product dose was set after the correlation between body weight and anemia severity and erythrocytes mass values (RBC, HCT and Hb). Some of the doctors evaluated even the lost blood volume, using the well known formula: "Given blood volume (mL) = 80 x kg x (desired Ht - receiving patients Ht)/donors Ht" (Abrams-Ogg, 2000). Taking into account the aforementioned factors, the calculated doses for whole blood and EC varied between 10 and 20 mL/kg. Two of the patients were treated with CE, diluted with saline (3:1), for the administration ease. The transfusion rate, in the first 30 minutes was of 0.3-3 mL/kg/h, upping the dose to 10 mL/kg/h. Moreover, during the entire transfusion process, some of the basic physiological parameters were monitored, namely respiratory rate and internal temperature in every patient.

The values obtained from blood chemistry tests were statically and graphically analyzed, by using GraphPad Instat, Excel, Prism 4th version and OriginLab 8.5 programs. Because most complications subsided after a few minutes of pausing the transfusion or completely stopped, we continue with two exceptions, resulting due to AHI and TRALI type transfusion reactions, characterized by severe evolution, unfavourable therapeutic response and patients death.

AHI type transfusion reaction was observed in a patient (6 years old, unneutered, female), suffering from chronic renal failure, with BUN (71 mg/dl) and creatinine values (28.4 mg/dl), complicated with severe non regenerative chronic anemia, low values of RBC (1.46 T/L), HCT (6.3%), Hb (2.1 mg/dl) and VEM (42mg/dl), critical clinical status and unfavourable prognosis. Based on the severity of the anemia, the patient received an immediate first transfusion with whole blood (one unit - 450 mg, on CPAD1), collected from a donor (male, half-breed, vaccinated and dewormed), without any blood compatibility tests done prior. This patient received in previous other 2 compatible transfusions, a unit of whole blood, in order to correct the mild increase of

erythrocyte indices, 24 hours after transfusion, and to correct the decreasing trend of these values in the next 30 days.

TRALI respiratory syndrome was diagnosed in a patient (male, Rottweiler breed, 7 years old, DEA 1.1 positive), suffering from a multicenter lymphoma, cythologically confirmed. This patient was undergoing treatment for arthritis and renal failure 1st grade, and it was brought to the clinic because he presented loss of appetite, listlessness, hypersalivation, mass weight loss and dysphagia. Clinical examination revealed fever, hypertrophy of the prescapular and popliteus lymph nodes and tonsils hypertrophy. Blood tests were performed and biopsy was performed form prescapular and popliteal lymph nodes, in order to establish a diagnosis certainty. Based upon the conducted tests and the obtained results, a complex therapeutic protocol was elaborated, focusing on chemotherapy (cyclophosphamide, doxorubicin, vincristine and prednisone), symptomatic treatment and whole blood transfusion. Unfortunately, this patient developed an extremely severe form of TRALI reaction, 72 hours after the second transfusion (with one whole blood unit), which was followed by increased values of Ht (19%) and Hb (6,9 mg/dl) and finally, a third transfusion with whole blood was necessary.

Results and disscussions

During the whole blood, EC and P transfusions, in all the canine patients subjected to this study, no severe adverse reactions, such as acute intravascular hemolysis or anaphylactic shock were observed. However, some mild intensity transfusional reactions were recorded in 5 cases, namely the increasing pulse and heart rate. The main measure chosen for subsidising these complications was to interrupt, even for a few minutes the transfusion, until the heart rate normalized - in 3 cases and in another 2 cases - the solution was to completely stop the transfusion. We have also noticed an increased sensitivity in peripheral veins, punctured for administration of whole blood and EC, characterized by patients agitation, without causing phlebitis or thrombophlebitis. In case of plasma administration, no side effects were registered. In 5 of the cases, temporary transfusion reactions were observed, such as hyperthermia (40°C), which occurred 24 hours after the transfusion, but without any complications.

The emesis and melaena symptoms during transfusion, in 3 patients with parvovirosis, were considered to be complications of this intensive treatment procedure. Another post transfusion reaction consisting of myoclonic head seizures and bruxism was present in one patient, aged 3 months, after the second transfusion with whole blood. According to this patient's anamnesis, we must recall three surgical procedures, namely an osteosynthesis for treating a fractured injury resulted after a car accident and two enterectomy procedures after an episode of recurrent intestinal volvulus. Additionally, the patient also developed a parasitic infestation which hastened its death. As expected, important increases in serum total bilirubin were reported in most patients, exceeding the maximum allowable limits (3.6 mmol/L) only in the first 24 hours after whole blood, EC and even P transfusion, but these values normalized shortly after.

Regarding the patient with AHI, we mention that 24 hours after the first transfusion with one unit of whole blood, it presented mild increases of the erythrocyte indices, which determined the requirement of another two transfusions, each of them performed with one unit of whole blood, during two months. The post- transfusion data, presented in table 1, revealed that RBC values increased the following day to 2.33 T/L and the HCT values to 13.5%; on the third day after transfusion we observed a decrease in values to 1.81 T/L, for RBC and 10.2%, for HCT. The evolution of erythrocyte indices included mild increases of RBC (4.7 T/L) and HCT (27.2%) within the 4-30 days interval, followed by an important decrease of these values, two months post transfusion, when the patient health worsened considerably. Based on this evolution, we consider

that the investigated patient developed a delayed hemolytic reaction, because the transfusion with whole blood was done without testing the donor-patient compatibility prior to transfusion and it was not followed by an important increase of erythrocyte indices, which may indicate a possible rejection of the administered red blood cells. Furthermore, the data presented in table 1 indicate that after the last transfusion, the decreasing trend of erythrocyte values amplified and the low values of RBC (1.55 T/L) and HCT (11.2%) were associated with a worsening health state. Under these conditions, intensive care measures were taken, with hydration intravenous (IV) infusion and glucocorticoids to lower creatinine levels. Furthermore, it is noteworthy to mention the severe chronic renal failure evolution, which also led to important changes in blood leukocyte and biochemical indices. Therefore, we can only mention the very high values of leukocytosis (21-42 G/L) and granulocytosis (78.8-84.4%), maintained in the first 30 days of the survival period.

Equally important were metabolic indices changes, such as blood sugar fluctuations (101-228 mg / dL), associated with increased values of BUN (40-155 mg / dL) and creatinine (2.5-11.2 mg / dL) (Table 1). Finally, all the undergoing measures proved to be inefficient, because the patient went into cardio respiratory arrest and died.

Table 1.
Haemato-biochemical parameters evolution in a patient with AHI condition

Parameter/Day	References	1	2	3	4	5	6	8	12	33	00	660
RBC (T/L)	5-7.9	1.49	2.33	1.81	2.08	2.2	2.5	3.18	4.8	4.7	1.55	
HCT (%)	35-57	6.3	13.5	10.2	11.02	11.9	13.7	17.8	21.7	27.2	11.2	
HGB (g/dL)	12-19	2.1	4.7	3.7	3.9	4	4.6	6.2	6.7	9.6	4.4	
MCV (µm3)	66-77	42	58	56	54	54	55	56	53	58	72	
MCH (pg)	21-26.2	13.9	20.1	20.5	18.9	18.4	18.3	19.5	16.4	20.5	28.2	
MCHC (g/dL)	32-36.3	32.9	34.6	36.5	34.8	34	33.4	34.9	30.9	35.5	38.9	
RDW (%)	14-17	17.9	22.4	22.2	24.7	25.6	24.4	22.5	23.4	21.9	13	
WBC (G/L)	5-14.1	33.8	33.9	30.1	25.4	28.9	39.6	-	42	29.5	9	
GR(%)	58-88	71.7	84.2	84.4	82.1	83	80.6	-	78.8	84.2	87.4	
LYM (%)	8-21	21	10.5	10.5	14	13.2	14	-	13.4	11.3	8.2	
MONO (%)	2-10	7.3	5.3	5.1	3.9	3.8	5.4	-	7.8	4.5	4.4	
PLT (x10^9/L)	211-621	280	355	250	460	697	675	642	571	616	425	
Glu. (mg/dL)	76-119	76-119	228	114	152	101	118	141	115	115	144	
BUN (mg/dL)	8-28	8-28	71	55	57	45	40	49	53	43	155	
Tbili. (mg/dL)	0-0.3	0-0.3	0.3	0.5	-	-	1.1	1.2	1.0	0.9	-	
Ca (mg/dL)	9.1-11.7	9.1-11	10.5	-	-	-	-	-	12.7	-	-	
Tprot. (g/dL)	6.0-7.5	5.4-7.5	7.9	8.1	8.6	8.6	9	8.5	8.9	7.3	6.3	
Alb. (g/dL)	2.3-3.1	2.3-3.1	2.1	2.2	-	-	-	-	2.6	-	1.9	
ALT (UI/L)	22-47	10-109	26	-	11	11	15	8	6	6	118	
ALP (UI/L)	1-114	1-114	108	146	204	190	224	218	216	190	77	
Crea. (mg/dL)	0.5-1.7	0.5-1.7	2.5	2.9	4.7	4.7	4.4	3.9	3.1	2.5	11.2	

Legend: **RBC**-Red blood cells; **HCT**-Haematocrit; **HGB**-Haemoglobin; **MCV**-Mean corpuscular volume; **MCH**-Mean corpuscular hemoglobin; **MCHC**-Mean corpuscular hemoglobin concentration; **RDW**-Red cell distribution width; **WBC**-White blood cells; **GR**-Granulocytes; **LYM-**Lympocytes; **MONO**-Monocytes; **PLT**-Platelets; **Glu**-Glucose; **BUN**- Blood urea nitrogen; **Tbili**.-Total bilirubin; **Ca**-Calcium; **Tprot**.- Total serum protein; **Alb**.- Albumin; **ALT**-Alanine aminotransferase; **ALP**-Alkaline phosphatase; **Crea**.-Creatinine.

Regarding TRALI respiratory syndrome evolution, we have observed that this post transfusion reaction manifested in an extremely severe form, although the patient presented slight

improvements of the health state and hematologic parameters right after the transfusion with whole blood (Table 2). 72 hours after the second transfusion, with one unit of whole blood, the values of HTC (19%) and Hb (6,9 mg/dl) decreased (Table 2), which determined the transfusion of another unit of whole blood. 24 hours after the last transfusion, the health state worsened, the patient presenting emesis, severe dyspnea, fever, pale mucosa, tachycardia, decubitus position. Under these conditions, the emergency therapeutic protocol was supplemented with oxygen therapy, intravenous rehydration, vitamins, furosemid, antacids, antiemetics, and liver protection. Despite the implemented measures, the onset of decompensated shock was inevitable, with cardio-circulatory arrest and patient death.

The necropsy examination emphasized specific changes for TRALI syndrome, due to the presence of a foamy fluid in the trachea and in the entire bronchial tree, and dense formations the size of a millet, onto the entire surface of the lung.

Most of the adverse transfusion reactions reported in this study are undesirable metabolic or immunological disturbances that may occur frequently during or after administration of blood products (Mcdevitt et al., 2011; Ognean, 2017). Moreover, they were of mild intensity and did not present any threat to the patients life.

Table 2.
Main haematological parameters evolution in a patient with TRALI syndrome

Parameter	Evolution of haematological parameters prior and post transfusion						References
	First transfusion		Second transfusion		Third transfusion		
	Prior	Post	Prior	Post	Prior	Post	
HTC (%)	21.5	21.9	20.8	24.3	19	23.2	37-55
HGB (g/dL)	7	7.9	6.9	8.3	6.9	8.3	12-18
WBC (G/L)	26	26.7	17.7	16.1	24.1	11.5	6-16.9
GR (G/L)	25.5	25.1	16.3	14.1	22.7	7.9	3.3-12
PLT ($x10^9$/L)	476	323	547	694	272	170	175-500

Legend: **HCT**-Haematocrit; **HGB**-Haemoglobin; **WBC**-White blood cells; **GR**-Granulocytes; **PLT**-Platelets.

The early administration of citrate, in an weakened patient, with an underlying condition of hypocalcemia, can result in the so called citrate intoxication (Lucas et al., 2004; Ognean, 2017); therein, we can explain the existence of myoclonic seizures and bruxism in one case. Other signs that can occur in these situations are tetany, hyperreflexia, epileptic form seizures, laryngeal spasms and even respiratory arrest (Giger et al., 1990). None of the patients presented any clinical signs of an acute hemolytic reaction.

Generally, in the case of an acute hemolytic reaction, serum and urinary levels of hemoglobin increase in a matter of minutes after transfusion and the incompatible cells are removed from the circulatory system flow in less than 2 hours (Capon et al., 1995). Concerning the investigated patients included in this study, the post transfusion decrease of the total serum bilirubin levels sustained the positive evolution and not at all the onset of intravascular hemolysis, which had to be represented by hyperbilirubinemia (Weingart et al., 2004). The existence of emesis and melaena symptoms in 3 cases, could be attributed to parvovirosis and not to transfusion complications, although some authors claim that acute hemolytic reaction may go undetected or even falsely attributed to an underlying disease (Kessler et al., 2010). Hyperthermia presented by

some patients receiving compatible blood, was due to the platelets or leukocytes amount brought by the administered blood. We mention that the non hemolytic fever is frequently associated with increased anti leukocytes antibodies to receptors. Moreover, one analysis based upon 348 transfusion cases in dogs at the Berlin small animals clinic described the evolution of some transfusion reactions caused by the administration of CE, in 4 patients and the administration of P in 2 patients, these aspects representing only 1.7% of the cases (Kohn et al., 2000). Reitemeyer et al. (2000) identified 2.2% of temporary transfusion reactions, during the procedure or immediately after the administration of red blood cells products in 186 dog patients. In general, the frequency of transfusion reactions, detected in this study, as well as by other researchers in the field is decreased, these being controlled by pre-transfusion testing of partner compatibility. On the other hand, we mention that only a small percentage of patients needed to repeat the transfusion with one of the blood products, which means that the number of previously sensitized dogs that had the opportunity to show an undesirable post-transfusion reaction was low. However, special attention should be paid to the identification of compatible blood when repeating the transfusion in any canine patient (Ognean, 2015). Concerning the evolution of the delayed hemolytic reaction, we remind that the symptoms have developed only after 9 days from the first incompatible transfusion, linked with increased levels of antibodies. Such a transfusion incompatibility can be caused by red blood cells bearing DEA 3, 5 and 7 antigens observed with a frequency of 10% in dogs that are DEA 3 negative and 20% in dogs that are DEA 5 negative (Ognean, 2017). Furthermore, AHI type reactions have been reported in DEA 7 negative dogs, after transfusion with DEA 7 positive blood (Ognean, 2017). It is also important to highlight the general tendency of labeling any undesirable effect of blood products transfusion as an immunological or non immunological reaction, with immediate response or later onset. In this regard, a major importance must be attributed to preventive measures designed to decrease the risk of developing any post transfusion reactions, with closer monitoring of the donor, the conditions in which the blood is collected, prepared, stored and administered.

It is well known that the most worrying form of hemolytic transfusion reaction is the acute one, observed in canine patients DEA 1.1 negative that received DEA 1.1 positive blood, that were sensitized prior with red blood cells carrying DEA 1.1 antigen. The symptoms in this acute hemolytic reaction are fever, tachycardia, dyspnea, muscular tremor, emesis, apathy, low levels of hemoglobin and hemoglobinuria. As opposed to the acute form, the delayed hemolytic reaction has an extravascular form, with similar symptoms, but not with the same severity. This kind of reaction may occur from day two until the 21th day after transfusion.

TRALI syndrome is one of the most severe forms of post transfusion reactions, due to its high rate of morbidity and mortality (Kopko et al., 1999; Toy et al., 2005). Confusions may sometimes appear because this syndrome has been known under different names, such as pulmonary hypersensitivity reaction, allergic pulmonary oedema, non cardiogenic oedema. In addition to this, it is unanimously accepted that this pathological entity is still hard to recognize, because it is not yet completely understood and described, due to diagnostic errors and due to lack of awareness about its importance (http://www.mymed.ro/injuria-pulmonara-acuta-post-transfuzionala-trali.html).

Regarding the evolution of the patients subjected to the present study, we regard the TRALI syndrome as the main factor that caused the patient death. The early onset of pulmonary symptoms, occurring during the transfusion, followed by late symptoms after transfusion (dyspnea, cyanosis, fever) described a characteristic clinical picture of this respiratory syndrome. This was well argued by morphopathological changes, focused on the predominance of tracheal and bronchial infiltrate. It should be noted, however, that the patient did not show symptoms of Acute

Pulmonary Injury prior to transfusion. Another observation that could support the existence of TRALI in this patient is the persistence of thrombocytosis (Kohn et al., 2006). Platelets are thought to secrete numerous proinflammatory factors, often involved in the mechanism of TRALI syndrome, such as chemokines, which attract and activate neutrophils causing endothelial layer permeability. (Toy et al., 2005; Marik et al., 2008).

Conclusions

Most of the reported transfusion reactions had a minor clinical impact and ensured a high level of recovery of the transfused patients, which was also supported by the implementation of adequate measures for the preparation and monitoring the transfusion therapy. However, we also encountered severe forms of transfusion reactions, which progressively worsened, ending with the patients death. In this regard, we attributed major clinical interest to delayed hemolytic anemia and TRALI respiratory syndrome, which caused serious complications in two patients, transfused 3 times with whole blood, as palliative treatment in a severe form of chronic renal failure and malignant lymphoma, respectively. We consider that the patient with chronic renal failure developed an AHI-type transfusion reaction, because it was transfused with large volumes of whole blood, without prior testing of patient-donor compatibility, which did not cause a rapid increase in erythrocyte indices, but their significant decrease in the first three days, with a slight remission at 30 days, followed by a decreasing trend. The results of the three blood transfusions indicated a possible rejection of red blood cells administered to this patient. The evolution of TRALI syndrome in a patient suffering from malignant lymphoma was based on the major risk conferred by repeated transfusions and the relevant changes detected at necropsy.

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