

# Triple Immunosuppressive Therapy of Immune-mediated Hemolytic Anemia in a Dog: Case-report

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## Abstract

Immune mediated hemolytic anemia (IMHA) is a disease in which the body's own immune system attacks and destroys the erythrocytes causing anemia. This may be primary or secondary as the result of an underlying illness such as neoplasia or intracellular parasites like babesiosis. In most cases the underlying trigger of the anemia may never be found, in which case the disease is called idiopathic immune-mediated anemia.

A double immunosuppressive therapy including prednisolone and cyclosporine as well as various blood transfusions has been administered to the patient, without any results. Mycophenolate mofetil was introduced as the third immunosuppressive drug on the eight day after admission (Park *et al.*, 2012; Wang *et al.*, 2013; West *et al.*, 2014)

The diagnosis of immune-mediated hemolytic anemia has been made after the autoagglutination test of the patient was positive after three RBC washes and negative results for *Ehrlichia*, *Babesia*, *Leishmania* and *Borrelia* antibodies. Also, tumors have not been found. The patient received in the first eight days of treatment four type specific blood transfusions with erythrocyte concentrate and two immunosuppressive drugs, but still manifested post-transfusion reactions after each transfusion with the hematocrit (Hct) dropping back to initial levels after two days. After the addition of mycophenolate mofetil the Hct stayed stable at a higher level and the patient was discharged two weeks after with a stable Hct of 27%.

Mycophenolate mofetil can be used in severe cases of IMHA when other immunosuppressive agents might not be enough. However due to the frequently encountered side effects it is recommended to be used mostly as a last resort therapeutic agent.

**Keywords:** *blood transfusion, IMHA, immunosuppressive therapy, mycophenolate mofetil, prednisolone.*

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## INTRODUCTION

Immune mediated hemolytic anemia (IMHA) is one of the most common immune mediated diseases in dogs. The most commonly affected individuals are middle-aged female dogs. Some breed predispositions are also known, with the Cocker Spaniel being one of the most commonly inflicted (Balch and Mackin, 2007).

Hemolysis can be intravascular or extravascular (in the liver or spleen), with the immunopathological process being the same in both cases. Erythrocytes are marked as non-self by the attachment of IgG and IgM and destroyed by macrophages. Red blood cell extravascular

destruction occurs in the liver, but mostly in the spleen and is mediated by IgG, whereas intravascular hemolysis is mainly mediated by IgM (Piek *et al.*, 2009). The degree of autoagglutination and spherocytosis is higher when both immunoglobulin isotypes are involved (Harkin *et al.*, 2012). This mechanism can be initiated by a variety of triggers such as vaccines, neoplasia, certain medication, infectious diseases and other immune mediated illnesses. In this case the hemolytic anemia is secondary to an underlying disease process or an administered substance.

Primary immune mediated hemolytic anemia is rare in veterinary patients, but idiopathic IMHA

is one of the most common forms of the disease, where the triggering factor is unknown (Piek *et al.*, 2009).

## MATERIALS AND METHODS

A nine year old neutered female mixed breed dog was presented to the emergency service in the Small Animal Internal Medicine department of the Justus-Liebig University in Giessen with lethargy, inapetence and oligodipsy. The symptoms started 4 days earlier with lethargy, weakness and vomiting. A foreign object or poison ingestion could not be excluded by the owner. From the start of the symptoms the patient was treated in a private practice with antipyretic, antiemetic and antibiotic (Metamizol, Emeprid, Cerenia and Enrofloxacin) medication, without any clinical improvement. Hematologic and biochemical findings included: WBC 6.7 G/l, RBC 2.0 T/l, Hb 5.0 g/l, Hct 17%, absolute reticulocyte count 89.400 MCV 84 fl, PLT 369 G/l, total bilirubin 3.4mg/dl, total proteins 6.2 g/dl and LDH 226 U/l. The blood smear showed a slight anisocytosis and polychromasia. Due to these findings, on the day before presentation in the clinic the patient received a blood transfusion from an unknown donor that did not have any positive effects.

In the clinical examination the dog was anxious but cooperant, with a good general condition, pale and dry mucous membranes with a capillary refill time less than 2 seconds. The pulse was regular and symmetric of medium speed and strength. Pulse rate was 120/minute, respiratory rate 24/minute and rectal temperature 39.3 C°. A 2x3x2 cm large mammary tumor has been found on the left cranial mammary complex. The thorax and abdomen were normal in the physical examination and radiographic imaging. Given the medical history of vomiting unresponsive to treatment, an ultrasound of the abdomen has been performed. Except for a slightly enlarged spleen and gall bladder nothing has been found.

A quick hematological as well as a blood-gas analysis and blood smear has been done in the intensive care unit of the clinic with the following results: RBC 2.03 T/l, Hct 14.8%, Hb 4.3 mmol/l, MCV 72.9 fl, absolute reticulocyte count 171.1 G/l, reticulocyte percentage 8.4 %, WBC 32.03 G/l, Neutrophiles 19.92 M/l, Lymphocytes 6.38 M/l, Monocytes 5.67 M/l and PLT 156 G/l. Blood-gas analysis results were all within reference

range. On the blood smear an anisocytosis and polychromasia with 10-15% spherocytes and 15-20% toxic band neutrophils were observed. An autoagglutination test was performed before the blood transfusion, which resulted in a positive reaction even after the third erythrocyte wash. Together with the spherocytosis seen on the blood smear a preliminary diagnosis of immune mediated hemolytic anemia has been made (Harkin *et al.*, 2012; Hodges and Christopher, 2011).

The patient received a type specific blood transfusion of erythrocyte concentrate in addition to the antithrombotic, anti-inflammatory and gastro-intestinal protection therapy. Immunosuppressive therapy was started with 3.6 mg/kg prednisolone. During the transfusion the patient developed hyperthermia, a painful abdomen and vomiting. Hematocrit 6 hours post-transfusion was 19% and the patient improved clinically but developed hematuria. An abdominal ultrasound showed signs of cholecystitis, enteritis and blood clots in the urinary bladder.

From the third day after admission the prednisolone dose was reduced to 2 mg/kg, but the next day the patient's clinical state deteriorated with RBC dropping to 1.41 T/l and the Hct to 12%. A blood transfusion of 270 ml type specific erythrocyte concentrate and cyclosporine as a second immunosuppressive medicine has been started at a dose of 5.3 mg/kg. Transfusion reactions have been observed this time as well and 8 hours post-transfusion the patient developed signs of hemolysis, but improved clinically. The Hct and RBC remained at pre-transfusion levels. Another type specific erythrocyte concentrate was given that raised the Hct to 22% 6 hours after transfusion.

Three days after the last transfusion RBC dropped to 1.18 T/l and Hct to 12% making the administration of another type specific erythrocyte concentrate necessary. Post-transfusion reactions haven't been as strong this time, but the disease progression justified the introduction of a third immunosuppressive drug (Wang *et al.*, 2014; Park *et al.*, 2012). From the eighth day after admission mycophenolat mofetil has been added to the treatment protocol in a dose of 10 mg/kg.

After the initiation of the triple immunosuppressive therapy, the patient improved every day clinically, it's appetite came back, urine and stool were normal. Hematocrit and RBC levels

rose slowly over the next week. As the patients gastro-intestinal symptoms ceased, the therapy was changed to oral administration.

Due to the progressive clinical improvement and stable hematological parameters, the patient was discharged after 18 days with a Hct of 27%. The triple immunosuppressive therapy, along with the antithrombotic and gastrointestinal protective medication was given for another week as an outpatient treatment, until the next control examination.

## RESULTS AND DISCUSSIONS

Canine IMHA patients often require the use of more than one immunosuppressive medication as treatment can last for several months and combination of these drugs allows the physician to use a lower dose of each one, thus reducing the side-effects (Whitley and Day, 2011).

Still, a triple immunosuppressive therapy is used quite rarely in veterinary medicine, but the severity of the hemolysis in this particular case and the frequent relapses to a critical state of the patient, justified the usage of all three drugs. Treatment has been initiated with a glucocorticoid drug with the later addition of cyclosporine and mycophenolate mofetil, to the treatment protocol until the desired effect has been reached (Tab. ).

The patient was closely monitored after discharge, with follow-up hematological tests every 2 weeks. At the time of the first follow-up control the patient has been under a triple immunosuppressive therapy for 24 days. Hematological parameters showed a very good recovery with RBC at 4.74 T/l, Hct 38% and spherocytosis below 4%. Due to this evolution the prednisolone dose has been reduced by 25% and mycophenolate mofetil was taken out entirely. The

first two follow-up examinations were conducted at 2 weeks apart, after which the patient was called in every 4 weeks for the following 3 months.

The owner reported severe glucocorticoid induced side-effects on the first follow-up visit, manifested thru massive polyuria/polydipsia and polyphagia.

Hematologic parameters steadily increased at each follow-up control, allowing the reduction by 25% of the prednisolone dose. Side-effects produced by the glucocorticoid medication slowly reduced as the dose was lowered.

On the last recorded visit the patient's hematological parameters were all within normal reference range, RBC 6.11 T/l, Hct 40% and spherocytosis 0%. The immunosuppressive therapy has been reduced at this point to 0.5 mg/kg prednisolone and cyclosporine remained unchanged at 5.3 mg/kg (Tab. 2)

Further follow-up examinations were done in a private practice from which the only feedback given was that the patient is doing very well clinically, the hematologic parameters are within normal reference range and any signs of glucocorticoid induced side-effects have ceased.

Mycophenolate mofetil is an immunosuppressive medication used mostly in human medicine on transplant patients. The immunomodulating effect of the drug consists in the prevention of T and B cell proliferation and differentiation by reversible inhibition of the key enzyme in the de novo synthesis of purine (Whitley and Day, 2011). Side effects of the medication include mostly gastro-intestinal symptoms.

Published data on the use of mycophenolate mofetil in veterinary medicine is contradictory, some authors reporting severe gastrointestinal symptoms in canine patients that had to lead

**Tab. 1.** RBC, Hct and spherocytosis evolution under the immunosuppressive therapy during hospitalization

Hematological parameters	3.6 mg/kg prednisolone		2 mg/kg prednisolone + 5.3 mg/kg cyclosporine		2 mg/kg prednisolone + 5.3 mg/kg cyclosporine + 10 mg/kg mycophenolate mofetil	
	Day 1	Day 2	Day 3	Day 7	Day 10	Day 15
RBC (T/l)	2.03	2.66	1.41	1.18	1.86	2.73
Hct (%)	14.8	19	12	12	19	27
Spherocytosis (%)	5-10	40-50	4	5-10	5-10	<5

**Tab. 2.** Evolution of hematological parameters after discharge

Hematological parameters	2 mg/kg prednisolone + 5.3 mg/kg cyclosporine + 10 mg/kg mycophenolat mofetil	1.6 mg/kg prednisolone + 5.3 mg/kg cyclosporine	1.3 mg/kg prednisolone + 5.3 mg/kg cyclosporine	1 mg/kg prednisolone + 5.3 mg/kg cyclosporine
	14 days after discharge	26 days after discharge	55 days after discharge	86 days after discharge
RBC (T/l)	4.74	5.08	4.86	6.11
Hct (%)	38	38	34	40
Spherocytosis (%)	<4	<4	-	0

to the drugs discontinuation from the therapy (West and Hart, 2014), while other researchers encountered no or very mild side-effects and very good therapeutic results. (Wang *et al.*, 2013, Park *et al.*, 2012)

In this particular case clinical and hematological improvement of the patient has been observed only after the introduction of mycophenolate mofetil in the therapeutic protocol. Severe hemolysis has been observed with the use for the first three days of treatment with only prednisolone in an immunosuppressive dose of 3.6 mg/kg as well as the following 5 days when cyclosporine was also added.

The patient was already manifesting gastro-intestinal symptoms on the day of presentation. These were later attributed to the enteritis and cholecystitis suspected in the abdominal ultrasound examination. The gastro-intestinal symptoms persisted though the first eight days of hospitalization and began to fade only after the overall clinical improvement and stabilization of the hematological parameters of the patient on the triple immunosuppressive treatment protocol.

## CONCLUSIONS

Immune mediated hemolytic anemia in dogs is a difficult to treat disease that may require the usage of multiple immunosuppressive medications. The patient required multiple blood transfusions during its hospital stay and although every erythrocyte concentrate has been type specific, it triggered immediate transfusion reactions. Setbacks in form of hemolysis were observed after every blood transfusion, except the last one, when the third immunosuppressive

medication, mycophenolate mofetil has been introduced.

The patient did not show any symptoms that could have been related to the potential toxic effects of mycophenolate mofetil manifested mostly through gastro-intestinal symptoms. Given the fact that the patient had a cholecystitis and enteritis diagnosed via ultrasound, these did not worsen after the addition of the mycophenolate mofetil.

Clinical as well as hematological parameter improvement and stabilization occurred in a relatively short time, the patient recovered well enough to be released after 10 days on a triple immunosuppressive treatment.

Glucocorticoid induced symptoms represented by polyuria/polydipsia and polyphagia persisted for another 86 days after discharge, when the prednisolone dose could be safely reduced to less than 1 mg/kg. Severity of these symptoms gradually reduced as the prednisolone dose has been steadily lowered.

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