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IMMUNE-MEDIATED HEMOLYTIC ANEMIA IN A DOG – A CLINICAL CASE

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ABSTRACT

The article describes a case of Immune-mediated hemolytic anemia in a dog. There is a description of the dynamic of the disease before and after the beginning of treatment with immunosuppressive drugs (Prednisolone and Azathioprine). The laboratory study shows significant improvement, the patient has no clinical complaints. There is a discussion for the approach to such clinical presentation and the application of a successful therapeutic strategy.

Key words: immune-mediated, hemolytic anemia, immunosuppressive drugs.

Introduction

Immune-mediated hemolytic anemia (IMHA) is one of the most common immunological diseases in the dog. It can be a primary disorder with unknown etiology or secondary to different infectious, parasytic and neoplastic disorders (Mackin, 2014). It is characterized by IgM and/or IgG production against RBCs antigens which leads to intravascular RBC destruction mediated through complement activation or with extravascular lysis mediated through phagocytosis of the erythrocytes from the monocyte-macrophage system (Balch and Mackin, 2007). Both mechanisms result in severe anemia accompanied with varying degree of systemic hypoxia. Complications can be observed – like disseminated intravascular coagulation syndrome (DIC), pulmonary thromboembolism (PTE) (Pedersen, 1999).

Diagnosis is based on laboratory findings, detection of self-agglutination in vitro and some typical RBC alterations detected on a blood smear – spherocytosis, polychromasia and anisocytosis (Thrall et al., 2012). Primary IMHA is a diagnosis of exclusion – all of the possible causes have to be dismissed.

First line treatment regimen includes immunosuppressive doses of corticosteroids and afterward combination with azathioprine or cyclosporine (Piek, 2011).

Materials and methods

The object of this study was a 1 year old female, mix breed dog (10 kg body weight). At the time of hospitalization a medical examination was performed and a blood sample was collected from v. cephalica antebrachii using 22G needle. The CBC was done on Mindray BC-288 Vet automatic blood counting analyzer. Biochemical profile included total protein, albumin, alanine amino transferase (ALAT), aspartate amino transferase (ASAT), alkaline phosphatase (AP), total bilirubin (TB), creatinin, urea and potassium level on semiautomatic analyzer Mindray BA-88A using reagents by Giesse Diagnostics, Italy.

Blood self-agglutination assay was performed ex tempore via the standard technique with washed RBCs from the patient which are mixed with saline on a slide and read macro- and microscopically for agglutination. Blood smears were prepared from an EDTA sample and stained by

Romanowsky-Giemsa technique. The microscopic examination was performed under oil emersion with Olympus CX-31.

Quick immunological tests for detection of antibodies were used (AnigenR).

Urinanalysis was performed through standard test strips and microscopicically.

Therapy included licensed veterinary and human drugs in appropriate doses and regiment.

Results

The dog originated from Sandanski, south-west Bulgaria. The dog was vaccinated 6 months before the occurrence of clinical signs. It was regularly dewormed. There was no medical history of previous usage of other drugs. Anamnesis included severe weakness and anorexia from few days. The clinical examination showed pale mucous membranes, tachycardia and a moderate hepatomegaly on palpation. Body temperature was within the normal reference range (38.6 ^{o}C). The hematological profile at Day 1 (D1) is showed on Tabl. 1.

Table 1: Hematological parameters at the moment of presentation

Test	Result	Unit	Reference range dog
WBC	15.9	x 10^9/L	6.0–16.9
LY	2.9	x 10^9/L	1.1–6.3
Mon	0.6	x 10^9/L	0–0.84
GR	12.4	x 10^9/L	3.3–12.0
Ly %	18.1		12–30
Mon %	3.8		3–10
Gr %	78.1		60–77
RBC	**1.80**	x 10^{12}/L	5.6–8.7
Hgb	**44**	g/L	120–180
Hct	**14.4**	%	37.0–55.0
MCV	80.1		58–79
MCH	24.4		19–28
MCHC	305	g/ L	30.0–36.9
Plt	**147**	x 10^9/L	175–500
Eos %	1.6		2–10

The laboratory data showed severe anemia and moderate thrombocytopenia. The leukocyte profile at the day of presentation indicated values at the upper reference range. Biochemical parameters determined hyperproteinemia (TP-80.1 g/l), hypoalbuminemia (21.5 g/l) with alteration in albumin:globulin ratio and hyperkaliemia (8.3 mmol/l).

The immunogical tests for some infectious and parasitic diseases (Ehrlichiosis, Anaplasmosis, Babesiosis, Leishmaniasis, Lyme disease, Dirofilariosis) were all negative.

The self-agglutination assay determined macro- and microscopically RBCs agglutination. Blood smears showed marked anisocytosis, polychromasia and spherocytosis.

The urinanalysis established no hematuria, bilirubinuria or other alterations.

On the basis of the clinical presentation and laboratory findings we diagnosed a **primary immune-mediated hemolytic anemia** with extravascular mechanism of hemolysis.

The treatment protocol in dynamics was the following:

- ✓ Day 1 to day 3: saline solution – 15 ml/kg i.v. slow constant infusion; colloid solution (Haes 6%) – 5 ml/kg i.v.; Dexamethason – 0.5 mg/kg i.v. q24 h; Omeprazole – 1 mg/kg i.v. q24 h, Enrofloxacin – 5 mg/kg i.v. q24 h.

- ✓ Day 4 to Day 6 – Prednisolon – 1 mg/kg p.o. q12 h, Famotidine – 0.5 mg/kg p.o. q12 h, Enrofloxacin – 5 mg/kg s.c. q24 h.
- ✓ Day 7 to Day 9 – Prednisolon – 1.5 mg/kg p.o. q12 h, Famotidine – 0.5 mg/kg p.o. q12 h, Enrofloxacin – 5 mg/kg s.c. q24 h, Legaphyton^{R} 200 mg – ½ tabl. q 24 h.
- ✓ Day 10 to Day 30 – Prednisolon – 1.5 mg/kg p.o. q12 h, Azathioprine (Imuran^{R}) 50 mg– 2 mg/kg, p.o. q24 h., Famotidine – 0,5 mg/kg p.o. q12 h, Legaphyton^{R} 200 mg – ½ tabl. q24 h.
- ✓ Day 30 to Day 50 – Prednisolon – 1 mg/kg p.o. q12 h, Azathioprine (Imuran^{R}) 50 mg – 2 mg/kg, p.o. q24 h., Famotidine – 0.5 mg/kg p.o. q24 h, Legaphyton^{R} 200 mg – ½ tabl. q 24 h.
- ✓ Day 50 to Day 60 – Prednisolon – 0.5 mg/kg p.o. q12 h, Azathioprine (Imuran^{R}) 50 mg– 2 mg/kg, p.o. q24 h., Legaphyton^{R} 200 mg – ½ tabl. q 24 h.
- ✓ After Day 60 – Prednisolon – 0.5 mg/kg p.o. q12 h, Azathioprine (Imuran^{R}) 50 mg – 2 mg/kg, p.o. q48 h., Legaphyton^{R} 200 mg – ½ tabl.q 24 h.

The main erythron parameters in dynamics are demonstrated at Fig. 1, 2 and 3.

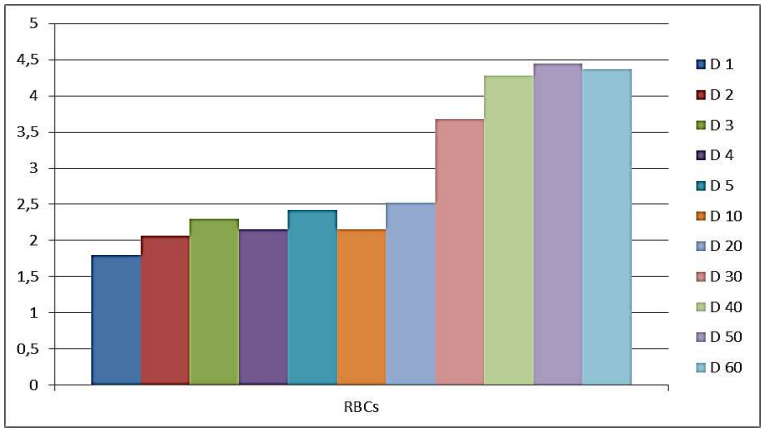

Figure 1: Red blood cells count in dynamics (10^{12}/l).

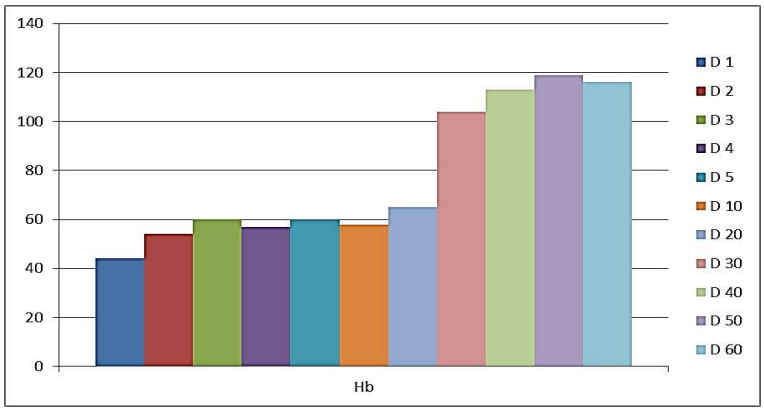

Figure 2: Hemoglobin concentration in dynamics (g/l).

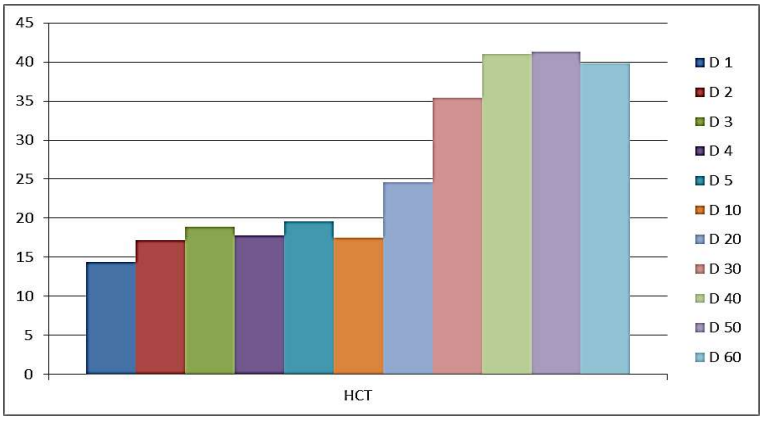

Figure 3: Hematocrit value in dynamics (%).

The leukocyte profile ascertained a progressive elevation of the total WBC count due to neutrophilia. At D2 it was 24.7×10^9/l, at D3 – 33.5×10^9/l and 42.4×10^9/l at D5. There was a moderate thrombocytopenia with maximum at D2 (44×10^9/l) There was normalization of the trombocyte's count after D4 (360×10^9/l).

Liver enzymes showed raise on the control tests. At D5 we determined ALAT, ASAT, AP, TB. Alterations were found in ALAT (144 U/l), AP (831 U/l) and TB (56.9 mcmol/l). The blood protein concentration still demonstrated hyperproteinemia, dysproteinemia and hyperglobulinemia (TP – 82.4 g/l, Alb – 28.4 g/l).

At Day 60 AP had extremely high value – 3018 U/l, ALAT – 211 U/l. Serum proteins still exhibited elevation.

Discussion

The diagnosis is based on the clinical manifestations, laboratory findings (severe anemia), strong self-agglutination reaction and detection of spherocytes on blood smear. The type of anemia is macrocytic, normo- to polychromic with appropriate regenerative response. Lack of hematuria indicates extravascular type of RBC destruction which is mediated by opsonization with immunoglobulin molecules on the RBC surface which induces the phagocytosis of the target cells through Fc receptors of the macrophages (Barcellini, 2015).

Development of DIC is a common clinical finding in patients with severe immune-mediated disorders and have to be suspected in case of low thrombocyte count even without symptoms (Scott-Moncrieff et al., 2001). Another possible complication can be pulmonary thromboembolism, which is one of the main causes for lethality.

Extreme leukocytosis in IMHA is a common but transient accompanying sign and is based on the functional reactivity of the bone marrow. Also it can be caused by the corticosteroid treatment (Cohn 1991).

The treatment protocol started with immunosuppressive doses of glucocorticoids which aims the rapid cessation of erythrocyte lysis (Al-Ghazlat 2009). Combination with other chemotherapeutic drugs helps to restrict the common side effects of the corticosteroids and to induce faster cessation of the immune-mediated disorder. Azathioprine have to be the preferential choice since some retrospective studies show a positive effect on outcome when azathioprine and

prednisolone are used in combination versus prednisolone therapy alone (Weinke et al., 2005). Still we established the development of an exogenous Cushing's syndrome (hypercortisolism) a month after the beginning of treatment which was characterized by polyuria, polydipsia, polyphagia, weight gain and progressively elevated AP level.

The treatment effects included slow but progressive elevation of the RBC count, hemoglobin concentration and hematocrit. The aim of therapy was to achieve a long-term remission in order to allow the reduction of the immunosuppressive drug doses and eventually their discontinuation.

Conclusions

Immune-mediated hemolytic anemia is a therapeutic challenge. Due to lack of definitive healing is important to achieve clinical and laboratory remission and at the same time to diminish the adverse effects of the treatment.

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