

## CANINE IMMUNE-MEDIATED HEMOLYTIC ANEMIA – BRIEF REVIEW

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### ABSTRACT

Immune-mediated hemolytic anemia (IMHA) is a common autoimmune disorder in dogs. It affects both sexes but occurs more often in female, middle-aged animals. IMHA can be idiopathic (primary) or secondary to infectious, neoplastic and autoimmune disorders. There is an acute regenerative anemia with accompanying hypoxia. Destruction of erythrocytes can be intravascular (as a result of complement system activation) or extravascular (removal of antibody-coated red blood cells by the macrophages in the liver and spleen). Diagnosis is based on the presence of anemia, in vitro autoagglutination, positive direct antiglobulin test (Coomb's test), detection of spherocytes. It is crucial to exclude possible secondary causes. The treatment protocol aims to cease cell destruction by high doses of corticosteroids, aggressive supportive care and long-term application of immunosuppressive drug combinations. Still lethality is high because of complications (pulmonary thromboembolism, DIC), medication resistance, relapses.

**Key words:** immune-mediated, anemia, canine, hemolysis, immunosuppressive drugs.

Immune-mediated hemolytic anemia is one of the commonly diagnosed canine autoimmune diseases and a model of acute and clinically relevant anemia. Impaired immune tolerance leads to premature destruction of red blood cells (RBCs).

IMHA can be divided in two types – primary (idiopathic) and secondary as a result of some underlying condition. The primary is more common in canine population (Barker et al., 1991) and represents an autoimmune disorder with the formation of antierythrocyte antibodies mostly directed against glycophorins – a sialoglycoprotein of the RBCs membrane which enables them to circulate without adhering to other cells or vessel endothelium (Barker and Elson, 1995; Day, 1999).

The affected animals are usually neutered, female dogs (Weinkle et al., 2005). Some breeds are overrepresented – most commonly Cocker Spaniels and Miniature Schnauzers (Piek 2011).

### Etiology

Current etiological factors in Europe for the secondary canine IMHA can be divided in the following groups:

- ✓ Infectious – Ehrlichiosis, Anaplasmosis, Leptospirosis.
- ✓ Parasitic – Dirofilariosis, Babesiosis.
- ✓ Neoplastic – lymphosarcoma, hemangiosarcoma, lymphocytic leukemia, pulmonary carcinoma, sarcoma.
- ✓ Drugs – trimethoprim-sulfonamide, penicillins, cephalosporins, levamisole.
- ✓ Other immune-mediated disorders – Lupus erythematosus, Immune-mediated thrombocytopenia (IMT).
- ✓ Miscellaneous – garlic, onion, bee venom, vaccination.

In secondary IMHA exogenous antigens adhere to and modify the RBC membrane structures. In some cases there are hemolysins which directly destruct RBCs, therefore a non-immune hemolysis is present. Several drugs can act as haptens and induce immunological reaction after

interaction with bigger molecules. There is an assumption that a previous vaccination can induce IMHA (Duval and Giger, 1996; Klotins et al., 2003).

### **Pathophysiology**

IMHA represents a type II hypersensitivity reaction with the formation of autoantibodies which get fixed on the RBCs surface. The synthesis of antibodies (predominantly IgM) activates the complement system by the classical pathway which leads to generation of membrane-attacking complex and subsequent intravascular hemolysis (Barcellini, 2015). In this case hemoglobinemia and hemoglobinuria are important diagnostic markers. Red blood cells opsonization with IgG molecules induces extravascular hemolysis. The mechanism includes recognition of Fc part of the immunoglobulin molecules from macrophages in spleen and liver and phagocytosis of the target cells (Mackin, 2014). The stimulated bilirubin synthesis results in icterus and bilirubinemia. Mixed intra- and extravascular erythrocyte destruction can be presumptive. Macrophage activation leads to the synthesis of pro-inflammatory mediators. Antibodies coated RBCs interact with the Fc receptors of other cells of the immune system which can result in antibody-dependent cell-mediated cytotoxicity.

Hemostatic abnormalities can be expected (Scott-Moncrieff et al., 2001). Impaired coagulation and high intravascular expression of tissue factor induce thrombogenesis or disseminated intravascular coagulation syndrome (Piek et al., 2011a).

Fulminant or acute hemolysis results in severe hypoxia.

### **Clinical manifestations**

Clinical presentation is often nonspecific with signs attributed to anemia and compensatory reactions. Anamnesis includes weakness, lethargy, anorexia, dyspnea. Clinical examination shows pale mucous membranes, tachypnea, tachycardia, systolic murmur, hepatomegaly and/or splenomegaly, lymphadenomegaly, fever (Mackin, 2014). Icterus is an important clinical finding in intravascular hemolysis. Concurrent thrombocytopenia (Evan`s syndrome) can cause petechiae, ecchymoses and melena (Orcutt et al., 2010).

### **Diagnosis**

There is no pathognomonic sign. It is crucial to differentiate primary from secondary IMHA to achieve a successful treatment.

Blood examination determines normocytic, normochromic anemia with low hematocrit (<30%). Half of the patients demonstrate strong regenerative response (Day et al., 1996). Concurrent finding can include a transient thrombocytopenia or rarely a combination of IMHA and IMT (Evans syndrome) (Goggs et al, 2008; Orcutt et al., 2010). Leukocytosis with neutrophilia represents the altered bone marrow reactivity as a result of inflammatory mediators influence (McManus and Craig, 2001).

Polychromasia, anisocytosis and spherocytosis can be seen microscopically on a stained blood smear. The most relevant diagnostic finding is the presence of spherocytes which are shown in 89% to 95% of IMHA cases (Scott-Moncrieff et al., 2001). Such red blood cells lack their bi-concaved shape due to a partial destruction of membrane and cytoskeleton from phagocytes in spleen and liver.

A biochemical profile shows hyperbilirubinemia as a result of intense bilirubin metabolism, increased liver transaminases due to hepatocyte hypoxic damage and hyperproteinemia because of hyperglobulinemia (Mackin, 2014).

A coagulation profile completes the diagnostic panel. Possible alterations are prolonged prothrombin time, prolonged partial thromboplastin time, low fibrinogen level, positive D-dimer test. They indicate an impaired hemocoagulation and a prothrombotic tendency (Klein et al., 1989).

The goal of the immunological assays is to determine the presence of antierythrocyte antibodies fixed on RBCs surface. A sensitive, technically simple and rapid assay is the self-agglutination test. A mixture of washed RBCs of the patient with saline on a slide can show agglutination macro and microscopically – then it is called a positive self-agglutination test. It is crucial to distinguish true RBCs agglutination which is the result of antigen/antibody reactions from rouleaux formation in which the cells appear as "stacked coins."

A specific immunological assay for the detection of antierythrocyte antibodies is the direct antiglobulin test (Coombs' test) (Jones et al., 1992; Warman et al., 2008). Erythrocytes are incubated with polyclonal human serum which contains antibodies against IgM, IgG and C3b of the complement system. Antiglobulin antibodies attach to the antierythrocyte antibodies and results in RBCs agglutination. Some patients with IMHA can have a false negative Coombs' test because of low numbers of immunoglobulin molecules (Rochant, 1980).

## **Treatment**

Recommendations are based on the registered drugs in Bulgaria (2017).

The goal of treatment is the rapid suppression of RBCs destruction and reduction of immunoglobulin synthesis. At the same time supportive care is important for the outcome. Undoubtedly the first line drugs are corticosteroids (Rhen and Cidlowski, 2005). Their mechanism of action is characterized by the rapid alteration of Fc receptors which diminishes the RBCs destruction (Al-Ghazlat, 2009). The recommended starting dose is 2 mg/kg twice daily; however there is no certain data if the dose regimen or the type of corticosteroid chosen (Prednisolone vs. Methylprednisolon vs. Dexamethason) can influence outcome (Grundy and Barton, 2001). A positive result can be expected in 3–4 days which is manifested by the stabilization of hematocrit values. But the long-term use of immunosuppressive doses of prednisolone inevitably will cause exogenous hyperadrenocorticism (Cushing syndrome). Other common side effects include gastrointestinal irritation, recurrent infections, sepsis. To reduce these negative effects and improve the outcome a combination between corticosteroids and other immunosuppressive drugs is sought. The aim is to progressively reduce the dose of corticosteroids and finally stop them.

Azathioprine is a purine antagonist which inhibits lymphocyte activation and proliferation. It also suppresses phagocytosis and macrophage synthesis of cytokines. Several retrospective studies recommend its use in combination with steroids. The initial dose is 2 mg/kg once daily following a gradual reduction to 2 mg/kg q 48 h. It has a slow onset of action. The therapeutic effects can be expected after 2–3 weeks so azathioprine cannot be used as a single drug for the induction of remission. Common side effects include gastrointestinal disorders, acute pancreatitis and myelosuppression, indicated by the development of leukopenia (Houston and Taylor, 1991). Retrospective studies show a positive effect on outcome when azathioprine and prednisolone are used in combination versus prednisolone therapy alone (Weinke et al., 2005). However the conclusions of another trial showed lack of evidence for this claim (Piek et al., 2011b).

Cyclophosphamide is an alkylating agent which suppresses DNA formation. It was widely used in veterinary and human patients with IMHA, but retrospective studies demonstrated no positive effect in dogs when given alone or in combination with prednisolone (Mason et al. 1997; Burgess et al., 2000; Mason et al., 2003). On the contrary, it can augment immune responses and have a negative influence (Grundy and Barton, 2001). The drug possesses a long list of serious side effects – gastroenteritis, myelosuppression, hemorrhagic cystitis, secondary neoplasia.

Splenectomy can be performed in patients refractory to drug treatment or those which need high-dose therapy (Toll and Aronsohn, 2003; Horgan and Roberts, 2009). The procedure removes one of the major sites for RBCs destruction. An important presurgical condition is the lack of infection and a stable hematological and hemostatic status therefore it rarely can be performed in newly diagnosed patients with IMHA.

Aggressive supportive therapy is crucial for short term survival. As a result of acute intravascular hemolysis the patient is endangered by shock and acute renal failure so fluid therapy is mandatory. Low hematocrit level (<15%) and severe hypoxia are indications for hemotransfusion. It is important to remember that dogs with IMHA may have a false positive cross-matching test.

Thromboembolism prevention presumably has positive effects and is an important aspect (Kidd and Mackman, 2013). Ultralow-doses of aspirin (0.5 mg/kg sid) successfully suppresses platelets adhesion with no side effects. Alternatively clopidogrel (2 mg/kg sid) can be used which blocks the binding of ADP to its platelet receptors. A small prospective study showed no benefit of clopidogrel used alone or in combination with aspirin in comparison to aspirin alone for prevention of thrombogenesis (Mellett et al., 2011).

### **Complications and prognosis**

Expected complications are DIC and thromboembolism, more often manifested as pulmonary embolism (Klein et al., 1989; Carr et al., 2002). Prognosis is guarded although intensive therapy (Piek 2012). The main predictors for mortality in dogs with idiopathic IMHA are the presence of increased plasma urea and bilirubin concentration, thrombocytopenia, and petechiae at the time of diagnosis (Ishihara et al. 2010; Goggs et al., 2015). The estimated half-year survival for dogs treated with prednisolone and azathioprine that survived the first 2 weeks was 92.5% and the 1-year survival was 69% (Piek et al., 2008).

### **Conclusions**

Future research have to be focused on understanding the intimate pathogenesis of primary IMHA and the development of alternative protocols for control of IMHA with fewer side effects. Nowadays most of the trials are retrospective, mono-centered, with low number of patients and the results are not definitive and conclusive. Still the physician assessment for the approach to every single patient is determinant.

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