UNIVERSITY OF VETERINARY MEDICINE BUDAPEST
DEPARTMENT OF PATHOLOGY

Literature review of Immune Mediated Haemolytic anaemia and four case examples

By Guro Ølmheim

Supervisor: Dr. Mándoki, Mira

Budapest, Hungary

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LIST OF ABBREVIATIONS

CBC - Complete blood count

CRP - C-reactive protein

CRT - Capillary refill time

DLA - Dog leucocyte antigen system

HCT - Hematocrit

HLA - Human leukocyte antigen

hIVIG - Human intravenous immunoglobulin

IMHA - Immune mediated haemolytic anaemia

MAC - Membrane attack complex

MMF - Mycophenolate-mofetil

MPS - Mononuclear phagocyte system

PCV - Packed cell volume

RBC - Red blood cell

1. ABSTRACT

Immune mediated haemolytic anaemia (IMHA) is one of the most common forms of anaemia seen in small animal medicine practices. It can occur primary or secondary to an underlying disease. IMHA occurs to antibodies and/or a compliment being attached to the red blood cell membrane causing them to be phagocytosed earlier than normal. The destruction of RBCs may occur intravascularly or extravascularly. Clinical signs of IMHA includes lethargy, pale mucous membranes, anorexia, dypsnoea and in intravascular cases, icterus and pigmenturia. The disease is diagnosed based on clinical signs and laboratory findings. On the laboratory findings, the presence of spherocytes, autoagglutination or a positive Coomb's test is regarded as a conclusive in the diagnosis of IMHA. The treatment involved immunosuppression, where glucocorticoids are the most commonly used group of drugs. Azathioprine, Mycophenolate-mofetil and human intravenous immunoglobulin are also drugs that are being used as immunosuppression in animals diagnosed with IMHA. Supportive treatment to prevent thromboembolisms and gastric ulcers are being used. In severe cases, blood transfusions and splenectomy may be indicated. More research is needed on the field of treatment, as of now the mortality of IMHA patients are high and range from 22-80%. It is believed that most patients die from pulmonary thromboembolisms.

2. INTRODUCTION

Immune mediated haemolytic anaemia (IMHA) is a very common type of anaemia and is one of the most seen anaemias in small animal medicine practices. The disease can occur in both dogs and cats but is most frequently seen in dogs. The morbidity of IMHA is high and the mortality rate is according to various articles between 20-80% (Orcutt et al., 2010, Scott-Moncrieff et al., 2001). IMHA can develop as a primary or secondary disease. Primary IMHA is also called idiopathic IMHA and is seen in animals with no underlying diseases and is responsible for 60-75% of the cases of canine IMHA (McCollough, 2003). Secondary IMHA is seen in animals with underlying diseases. Neoplasia, infections, drugs, toxins and parasites can be some of the causes for secondary IMHA (Wang et al., 2013).

A French physician was the first one to describe the disease in the early 20th century. With the first case described auto-hemagglutination of red blood cells (RBCs) was detected in a patient with icterus (Johnson, 2002). In veterinary science, IMHA was first described in the 1960s by Miller et al. (Piek, 2011) at the University of Rochester. This was the first reported case of autoimmune haemolytic disease in any other species than human. It was detected agglutination of RBCs following exposure to rabbit anti-dog serum in a young cocker spaniel (Johnson, 2002). The report included 19 dogs, out of these, 11 of the dogs did not survive (Piek, 2011). From then on, IMHA has been a current topic for research, and new drugs and treatment approaches are on the market (Balch and Mackin, 2007).

Previously IMHA was also called autoimmune haemolytic anaemia and is a classic example of an autoimmune disorder (Balch and Mackin, 2007). Autoimmune diseases involve intolerance to a self-antigen or a primary immune response. In IMHA, erythrocytes are coated with antibodies and are destroyed by an immune destruction (Scott-Moncieff et al., 2001), and production of auto-reactive antibodies against the antigens on the erythrocyte membrane is also characteristic the disease (Andres et al., 2019). Because of this, the term IMHA is a more well-described term and has replaced the term autoimmune haemolytic anaemia (McCollough, 2003). The RBCs are destroyed by phagocytosis or direct destruction and cause a rapid decrease in the total RBC count mass. The RBC destruction can be due to intravascular or extravascular haemolysis,

meaning it happens within or outside the vascular system. With extravascular haemolysis the destruction of the RBCs happens in the liver or in the spleen (McCollough, 2003).

Clinically, patients with IMHA usually present non-specific clinical signs related to anaemia, including lethargy, weakness, dyspnoea and anorexia. Pale mucous membranes, tachypnoea, tachycardia and a systolic murmur are also frequently seen/heard in patients with IMHA. Patients with intravascular haemolysis are also showing icterus and pigmenturia (Manev and Marincheva, 2018). Many of the patients with IMHA are also diagnosed with thrombocytopenia and shows signs of bleeding, like petechiae, ecchymosis and melena (McCollough, 2003).

IMHA is diagnosed mainly based on the history, clinical signs and on exclusion of any other diseases. Presence of spherocytes is one of the most important diagnostic signs of IMHA, RBC agglutination and a positive Coomb's test is additionally supporting the diagnosis (Balch and Mackin, 2007). A low haematocrit and a strong regenerative response are diagnostically valuable in IMHA (Manev and Marincheva, 2018). Abdominal ultrasonography and thoracic x-rays are diagnostic procedures performed in patients suspected with IMHA, but with a debated significance (Andres et al., 2019).

A fair amount of different therapeutic procedures is described in the literature. Immunosuppressive doses of glucocorticoids are, however the most frequent and well-known treatment protocol (Whitley and Day, 2011). Intensive supportive therapy with blood transfusion, gastric protectants and fluid therapy is important to ensure the short-term survival of patients with IMHA (Manev and Marincheva, 2018). For long term survival, some patients may also require more invasive procedures, like splenectomy. As well as treating the anaemia, it is just as important to treat any other underlying causes of the IMHA (McCollough, 2003).

3. MATERIALS AND METHODS

This thesis is a literature review regarding immune mediated haemolytic anaemia. A wide selection of previously published articles and case studies have been obtained from scientific databases such as google scholar, pubmed and science direct. Veterinary journals and books dated back to 1985 until today were collected. Cases from Fredrikstad Dyrehospital (Norway) recorded in the journal programme Sanimalis were additionally used to complete this thesis.

4. AIMS AND OBJECTIVES

The aim of this thesis is to obtain an in-depth understanding of immune mediated haemolytic anaemia. The topic of the thesis has been wisely chosen as a veterinary student, in the respect of further interests in internal medicine and clinical work with companion animals. Previously published articles and studies are compared to gain a broad knowledge of the topic which is highly relevant in following clinical work as a practicing veterinarian.

5. PATHOGENESIS

IMHA is one of the most common autoimmune diseases in canine medicine. Normally RBC lives in the circulation for 100-120 days. The mononuclear phagocyte system (MPS) usually removes these aged RBCs as antibodies directed against aged membrane antigens are identified and removed from the circulation. Patients with IMHA, have a much shorter lifespan of their RBCs because premature RBCs are destructed due to an immune response that targets the RBC either directly or indirectly (Balch and Mackin, 2007). RBCs becomes coated with either compliments or most commonly antibodies including IgG, IgM and IgA causing haemolysis. This is an example of a type II hypersensitivity or antibody dependent cell cytotoxicity (Manev and Marincheva, 2018).

IMHA is divided into primary or secondary IMHA. The primary IMHA is also called idiopathic and was previously called autoimmune haemolytic anaemia as autoantibodies are formed from a type II hypersensitivity reaction against the antigens of the animal's own RBCs (Andres et al., 2019). The autoantibodies are targeting a protein or a glycoprotein, most commonly glycophorin which is a part of the RBC membrane. In healthy animals, suppressor T cells prevents these autoantibodies from reacting with the hosts own tissue. In animals affected with IMHA, it is suggested that the suppressor T-cell function of these animals are not well enough regulated or they have an overstimulated immune system allowing these autoantibodies to bind to healthy RBC and provoke RBC destruction (Balch and Mackin, 2007; Day and Kohn, 2012). Secondary IMHA is caused by a foreign antigen causing an immunological reaction modifying the normal RBC membrane structure (Balch and Mackin, 2007)

It is important to know whether the haemolysis is intravascular or extravascular, as the prognosis may differ. Intravascular haemolysis occurs within the bloodstream and have a worse prognosis than extravascular haemolysis which occurs by macrophages in the spleen, liver, lymph nodes or bone marrow. Haemolysis may also occur both intra- and extravascular simultaneously (Balch and Mackin, 2007). In severe immune reactions, many antibodies are being attached to the RBC membrane causing an activation of the complement cascade (Figure 1). This complement activation may induce immediate intravascular haemolysis or extravascular haemolysis may be encouraged (McCollough, 2003).

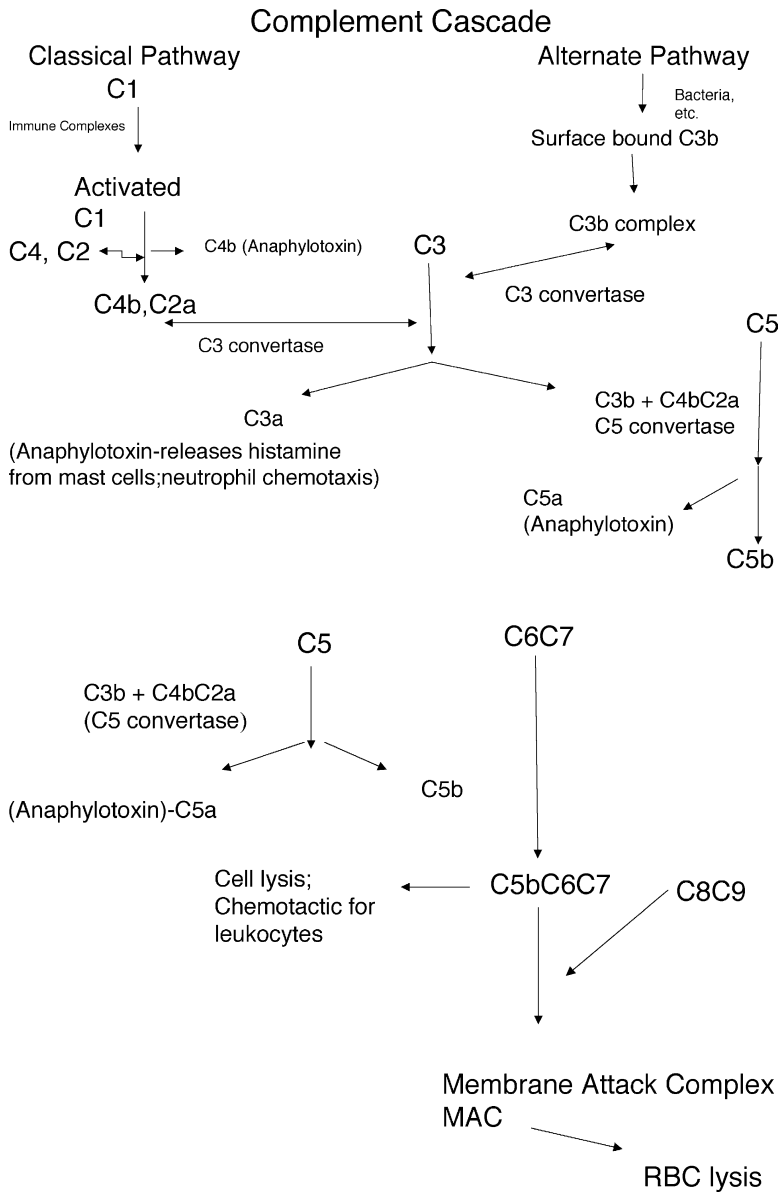


Figure 1. Activation of the compliment cascade leading to hemolysis (McCollough, 2003)

Intravascular haemolysis is the less common form (Villiers and Ristić, 2016) and occur when RBC are being ruptured while still in the bloodstream causing a release of free haemoglobin to the circulation as well as to the urine if the concentration of the haemoglobin increases the capability of renal destruction. There might also be seen an increased amount of bilirubin as the destruction of RBCs are higher than the livers capability to process it. This causes poor oxygenation as well as reduced liver function (Day and Kohn, 2012). Intravascular haemolysis is more likely to occur with IgM-mediated diseases as IgM have a better capability at fixing complement than IgG. The activation of this complement system results in the formation of the membrane attack

complex (MAC) which is causing the intravascular haemolysis (Balch and Mackin, 2007, Manev and Marincheva, 2018).

Extravascular haemolysis is the most commonly seen form and is causing a moderate to severe anaemia. With extravascular haemolysis, it is usually IgG that is bound to the surface of the RBC. These antibody-coated RBCs are being removed by the MPS by Fc receptors on the macrophages in the spleen and liver. These Fc receptors binds to the Fc components found on the antibody coated RBCs resulting in phagocytosis and destruction of the RBC (Balch and Mackin, 2007). Spherocyte formation (Figure 2) is seen if the RBC membrane is only partly phagocytosed, as the cell content remaining is squeezed within a smaller surface area (Villiers and Ristić, 2016).

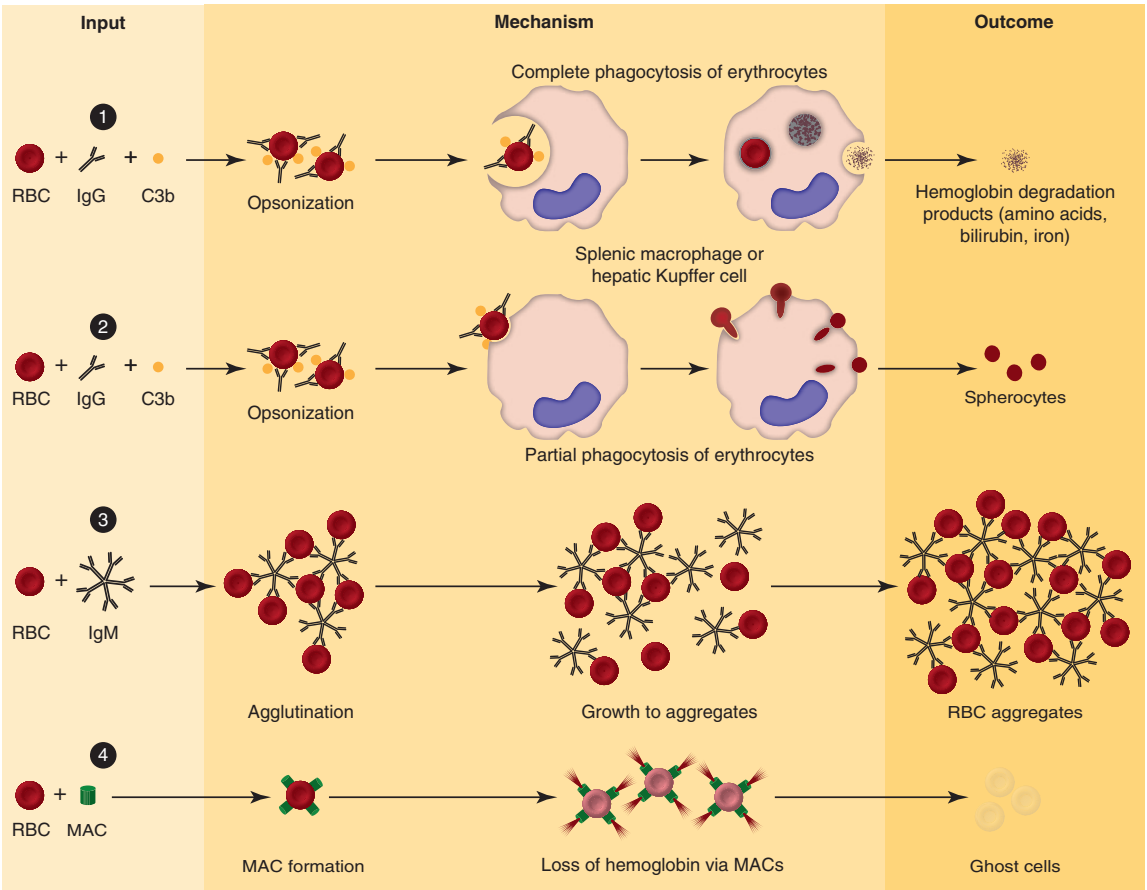


Figure 2. Different morphological changes of the RBCs occurring in IMHA and the mechanism behind it (Zachary, 2017).

Intrinsic or extrinsic defects of the RBCs may result in haemolysis. Because these RBCs are destroyed at an earlier stage than normal, they will undergo a morphological change. These changes may provide information about the reason behind the haemolysis

(Day and Kohn, 2012). There are several morphological changes observed regarding IMHA (Figure 2). RBC degradation occurs when RBC surface antigens are bound by anti-erythrocytic antibodies. The immunoglobulin and complement opsonize the RBC causing phagocytosis and digestion by the sinusoidal macrophages. Spherocytes are the result of RBC being partly removed from the circulation. Macrophages are phagocyting RBC that have immunoglobulins, complements or both bound to its membrane. Another morphological change seen is agglutination. This occurs when several erythrocytes are being bound at the same time by anti-erythrocyte immunoglobulins. Ghost cells may also occur. This occur when the RBCs are ruptured because the MAC are formed by the activation of complement due to anti-erythrocytes binding antigens to the RBC membrane. MAC causes pores in the membrane which results in free haemoglobin being released to the circulation as well as to the urine. RBC remnants lacking cytoplasm or hemoglobulin are these ghost cells (Zachary, 2017).

6. AETIOLOGY

1.1. Predispositions

IMHA can occur in both dogs and cats but are most frequently seen in dogs. Some breeds have been reported to be more prone to the disease than others. Earlier studies done on humans have suggested that different ethnic groups can have different human leukocyte antigen (HLA) system, making them more susceptible to different immune-mediated diseases. A similar observation has been made with dogs, different major histocompatibility complexes (MHC) have been observed in different dog breeds, making them more susceptible to IMHA (Kennedy et al., 2006).

With feline IMHA, there has been proven that younger cats are more susceptible than older cats. In a study done for predispositions in cats, the highest percentage of incidences happened at the age group ranging from two to six years old. There is no breed or gender related predispositions proven for feline IMHA (Swann et al., 2015).

Breed

Cocker spaniels, poodles, old English sheep dogs, English Springer spaniels, Irish setters and collies are breeds that have a greater risk of getting IMHA (Balch and Mackin, 2007). American cocker spaniel is the breed with most reported cases and is especially predisposed for IMHA (McCollough, 2003). These breeds have a combination of alleles of genes of the major histocompatibility complex that have been recognized to have a role in some of these breeds regarding primary IMHA (Day and Kohn, 2012). Cocker spaniels lack the erythrocyte antigen number 7 and is one of the causes that have been associated with the increased risks (Villiers and Ristić, 2016). In a study done by Threlfall et al. (2015), it was evident that Cocker spaniels have a more limited genetic diversity compared to other breeds, making them more susceptible to autoimmune diseases in general, including IMHA, keratoconjunctivitis sicca, hypothyroidism and chronic pancreatitis. When IMHA is diagnosed in young animals, hereditary diseases may be valuable to consider. Some of the hereditary diseases that can cause IMHA in dogs are also breed specific. Pyruvate kinase deficiency is found in basenjis, phosphofructokinase deficiency is found in English springer spaniels and American cocker spaniels and hereditary RBC osmotic fragility is found in Alaskan malamutes and miniature

schnauzers. These are all intrinsic RBC defects that cause haemolysis (Balch and Mackin, 2007; Day, 1998).

Sex and age

Females are more disposed to obtain IMHA than males. Neutering has also been suggested to increase the risk of IMHA (Carr et al., 2002). Reproductive cycle abnormalities as well as increased oestrogen levels in intact bitches has been reported as a predisposing factor, even though most of the cases of IMHA is seen in spayed bitches (McCollough, 2003). IMHA is most commonly seen in young to middle aged animals but can occur from the age of 1 to 13 (Balch and Mackin, 2007, McCollough, 2003). It has been implied in a few studies that IMHA is more frequently seen in the Spring and Summer. The reason for that has not been clarified, but an undiagnosed infectious cause has been suggested to be the cause, like tick-borne diseases, which is likely to occur in those seasons (Balch and Mackin, 2007).

6.2. Causes

Sixty to seventyfive % of the cases of IMHA in dogs are primary (Villiers and Ristić, 2016, Andres et al., 2019), also called idiopathic or autoimmune haemolytic anaemia. Primary IMHA is occurring without any underlying diseases. Genetic reasons can be a part of the cause of primary IMHA. The dog leucocyte antigen (DLA) system and different haplotypes have been investigated in a study and it has been proven that there is an underlying DLA association in dogs with primary IMHA, indicating an essential immunological risk factor (Kennedy et al., 2006). However, the cause of primary IMHA remains unknown.

Secondary IMHA

Secondary IMHA is occurring in patients with an underlying disease causing the anaemia. A general mechanism explaining this is that exogenous antigens bind to and modifies the RBC membrane structure. Haemolysins may destruct the RBCs directly causing a non-immune haemolysis (Manev and Marincheva, 2018). Animals that do not fit into the predisposal groups for primary IMHA are often suspected to have a secondary

form of the disease. This including cats and geriatric animals (Balch and Mackin, 2007) Most of the research done for secondary IMHA is done with humans, however, a fair amount of various causes for secondary IMHA in dogs has been identified as well.

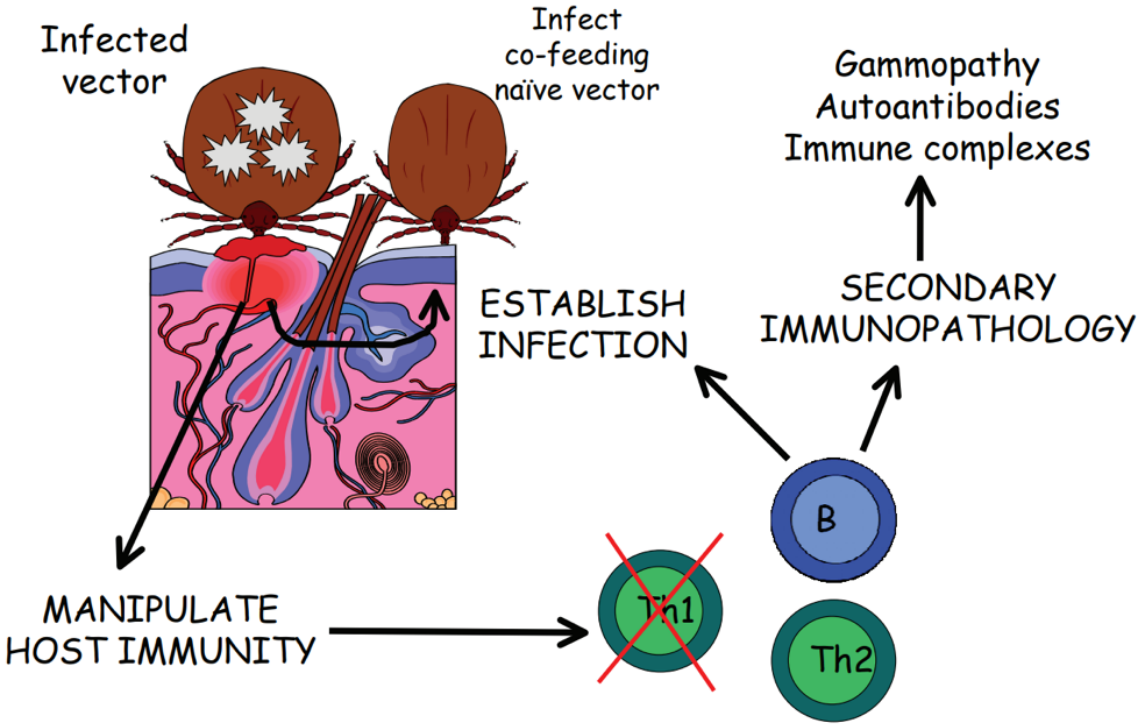


Figure 3. The mechanism behind ectoparasites causing secondary IMHA (Day, 2011)

Infections and ectoparasites

Infections can cause secondary IMHA, especially systemic lupus erythematosus and infections including ehrlichiosis, babesiosis, anaplasmosis, leptospirosis, dirofilariasis and histoplasmosis (Manev and Marincheva, 2018). Grey hounds and American Pit Bull terriers are predisposed to blood born parasites and are therefore at a greater risk of obtaining secondary IMHA (Balch and Mackin, 2007). Immune mediated diseases can occur in dogs with vector-borne diseases because toxins bind to the membrane surface of the erythrocytes (Furlanello and Reale, 2019). The protective Th-1 regulated cellular immune response is promoted below the Th2-regulated humoral response. Persistence of the infection is kept, and the development of unfavourable secondary immunopathology is encouraged. This secondary immunopathology is characterized by hypergammaglobulinemia, autoantibody and immune complex formation (Figure 3) (Day, 2011). Localized bacterial infections such as subacute

endocarditis may also cause secondary IMHA (Villiers and Ristić, 2016), due to the fact that infections can cause the immune-mediated erythrocyte destruction (Garden et al., 2019)

Neoplasia

In humans, IMHA has been proven to be a paraneoplastic syndrome, however, there is limited studies regarding neoplasm causing IMHA in dogs and the mechanism behind it (Garden et al., 2019). Lymphangiosarcoma, hemangiosarcoma, lymphocytic leukaemia, lung and gastric carcinoma and other diffuse sarcomas have been diagnosed as a cause of secondary IMHA (Balch and Mackin, 2007). Neoplasia stimulates autoantibodies which can lead to IMHA. These autoantibodies can be created by the neoplasia itself, by a large amount of tumour-related antigens that stimulates cross-reacting antibody formation or by activation of helper T-cells or inhibition of suppressor T-cells caused by chemotherapeutic immunomodulation (Keller, 1992)

Drug induced

IMHA can be a side effect of certain drugs. There are a few drugs that act as haptens which provokes an immunological reaction (Manev and Marincheva, 2018). Especially antibiotics have been proven to cause secondary IMHA including trimethoprim-sulphonamides, tetracyclines, penicillin, cephalosporins and levamisole (Villiers and Ristić, 2016, Manev and Marincheva, 2018). Phenylbutazone, dipyrone and chlorpromazine have also been reported to cause IMHA. Different drugs have different mechanisms of how IMHA is occurring. With IMHA caused by penicillin and cephalosporins the drug or the breakdown products are bound to the RBC membrane, inducing the complement attack or cell destruction. IgM antibody production may be produced by sulphonamides and tetracyclines. Intravascular haemolysis will occur when the drug-antibody complex is bound to the RBC membrane and complement activation is initiated (Balch and Mackin, 2007).

Vaccination

Vaccinations has been a debatable possible cause of secondary IMHA (McCollough, 2003). It has been proven to occur, but there is a low frequency of cases reported. (Villiers and Ristić, 2016). In a study done by Duval and Giger (1996), one

quarter of the dogs that was diagnosed in the study was vaccinated within a month. This has also been proven to occur in children vaccinated with diphtheria-pertussis-tetanus vaccine. The type of vaccine causing IMHA in dogs has not been identified (Balch and Mackin, 2007). The vaccines used in the study were the most common dog vaccines, including DHLPP and rabies (Duval and Giger, 1996). Another study done by Carr et al. (2002), did not show a significant link between vaccines and IMHA. Only 10% of the animals in that study was vaccinated within one month. The exact mechanism of how vaccines are causing IMHA has not yet been concluded. It has been suggested to have the same mechanism as drug induced IMHA with autoantibody production or it has been suggested that vaccines may activate macrophages, causing an inflammatory condition or depress the immune system stimulating an already present IMHA (Balch and Mackin, 2007, Duval and Giger, 1996). When relating the mechanism of drug induced IMHA to vaccine induced IMHA, pre-existing erythrocytes with autoantibodies on the surface may be destroyed due to activation of macrophages and the immune system after vaccination (Duval and Giger, 1996).

Toxins

Toxins from allium species like onions and garlic can cause IMHA (Manev and Marincheva, 2018). When chewed or crushed the sulphur compounds in the onion and garlic are hydrolysed to thiosulphinate and then decomposed to disulphides. Disulphides causes haemolysis due to the fact that they are oxidating agents. This haemolysis causes the production of Heinz bodies, methemoglobinaemia and anaemia. (Poppenga and Gwaltney-Brant, 2011). Japanese breeds like Akita Inus and Shiba Inus have a genetic disorder which cause a deficiency of an enzyme called glucose-6-phosphate dehydrogenase (G6PD). This condition causes a lower glutathione level, a high potassium and low sodium concentration, which is vice versa of what is normal and makes them more susceptible to allium toxicosis. Because cats' haemoglobin is more susceptible to oxidative damage, they are also more sensitive to allium toxicosis (Simmons, 2001). Zinc toxication has can also be a cause of secondary IMHA and is mostly found in patients that have swallowed a US penny or zinc oxide ointment (Balch and Mackin, 2007).

Bee sting envenomation

There have been a few cases reporting secondary IMHA related to bee stings. In a study done by Noble and Armstrong (1999), two dogs were diagnosed with IMHA secondary to a bee sting envenomation. A more frequent case was published by Nair et al. in 2019 with a Belgian Malinois having all the typical signs of IMHA after being exposed to a massive bee envenomation. This dog was presented with spherocytosis, haemolysis, and thrombocytopenia. The primary components of the bee venom, melittin and phospholipase A2 may be the cause of these effects. Haemolysis is caused by the melittin due to fact that large pores in the erythrocytes are formed causing leakage of haemoglobin and spectrin stiffening, resulting in echinocytosis and spherocytosis (Nair et al., 2019).

Commercial dog food

Smith et al. published an article in 2006 about an outbreak of dogs eating commercial natural dog food as a cause of hepatic disease and IMHA. This has not been described previously and the cause of the outbreak is unknown. The mechanism of how this occurred was not found. There was no exposure to any hepatic toxins, and there were no chemical or nutritional components causing haemolysis in the discussed feed. A possible theory could be that an immune-mediated haemolysis was caused by a primary immune response to the RBCs stimulated by a cofactor or a hapten in this particular food (Smith et al., 2006). No other cases describing a relation between a commercial food has been found.

Pregnancy

In pregnant patients with anaemia, IMHA should be considered. It has not been done much research on pregnancy as a cause of secondary IMHA, but a few recent cases have been seen. A 6-year-old Bichon Frise was present with anaemia with spherocytosis, drug induces IMHA, parasites and infections were all ruled out. The anaemia resolved after the dog gave birth, and did not relapse (Fernandez and Sharman, 2020). Pregnancy was also suggested to be the cause of IMHA in a 7-year-old cat. The kittens were taken out and the cat had an ovarioehystectomy. The IMHA resolved1.5 month after the intervention (Kopke et al., 2019). Pregnancy as a cause of IMHA has been identified in

humans, but the mechanism behind this has not yet been described. In humans, IMHA has been reported to occur four times more than in the non-pregnant population, but even in human medicine, the pathogenesis is poorly understood (Kopke et al., 2019).

Feline patients

Regarding feline patients, it is debatable whether primary or secondary IMHA is most common. It has been reported that secondary is most common and seen together with Feline Leukaemia virus (FeLV) infections, mycoplasma infections or drug reactions (McCollough, 2003). It has also been suggested that primary IMHA is the most common, like in dogs and are responsible for 83% of the cases (Swann et al., 2015). Another study stated that primary IMHA is fairly uncommon in cats and was only responsible for 4% of the anaemia cases seen in the study (Villiers and Ristić, 2016).

7. DIAGNOSIS

There are no pathognomic signs of IMHA, due to this the diagnosis is mainly based on exclusion of any other immune-mediated diseases as well as by the clinical signs. It is important to diagnose whether the IMHA is primary or secondary as this will affect the treatment. Even though there are no specific pathognomic signs, a collection of tests may be performed which can give a strong indication of the diagnosis (Balch and Mackin, 2007). Blood samples including blood count, biochemistry and blood smear, urine sample and diagnostic imaging are among the main diagnostic tools valuable for diagnosing IMHA (Andres et al., 2019)

It is also important to diagnose whether the anaemia is immune-mediated or not. It may be difficult to distinguish secondary IMHA from a non-immune mediated anaemia as the differential diagnoses may be the same as the causes of secondary IMHA. Parasitic diseases like babesiosis have been reported to cause secondary IMHA, but it may be challenging to say whether the haemolysis is caused by that or by direct parasite-associated RBC damage (Balch and Mackin, 2007). Similarly, in the study done by Noble and Armstrong (1999), where two dogs were diagnosed with secondary IMHA after bee sting envenomation. There are haemolysins in bee venom, so it is difficult to know if the haemolytic anaemia occurs from antibody-mediated destruction or from direct toxin-induced haemolysis (Balch and Mackin, 2007).

7.1. Clinical signs

The clinical signs of IMHA are usually non-specific and are associated with compensatory reactions as well as anaemia (Manev and Marincheva, 2018). Some clinical signs are due to hypoxia on different organ systems such as the liver, kidneys, intestines, heart and lungs (McCollough, 2003). Animals with acute and severe IMHA typically shows more defined signs than chronically affected animals. These animals tend to only show mild signs, even though there is a marked anaemia present (Archer and Mackin, 2013; Manev and Marincheva, 2018). In cases where the anaemia is slowly developing, the clinical signs may be minimal or absent until the anaemia reaches a significant level (Balch and Mackin, 2007).

Animals affected with IMHA, have been reported to have a history including lethargy, collapse, weakness, exercise intolerance, anorexia, vomiting, diarrhoea, tachypnoea, dyspnoea and sometimes polyuria and polydipsia. Compensatory reactions like tachypnoea, tachycardia and increased cardiac output may be helpful at the initiation of the anaemia but will cause decompensation as the anaemia progresses (Balch and Mackin, 2007; Carr et al., 2002). In some cases, acute death has occurred (Smith et al., 2006).

Figure 4. Dog with IMHA showing jaundice. Very clear yellow discoloration in the sclera and on the skin on the inside of the pinna. Picture from Fredrikstad Dyrehospital

Physical examinations may reveal signs like pale mucous membranes, prolonged capillary refill time (CRT), jaundice (Figure 4), abdominal discomfort, hepatomegaly, splenomegaly, lymphadenopathy, pigmenturia, tachypnoea, systolic heart murmur, weak and increased pulse and fever (Balch and Mackin, 2007; McCollough, 2003; Smith et al., 2006). The degree of the findings is concurrent with the severity of the anaemia. In intravascular cases pigmenturia and jaundice may be observed. The pigmenturia can be both haemoglobinuria as well as bilirubinuria. Jaundice, also called icterus, is initially observed as yellow discoloration of the mucous membranes like the sclera (Figure 4), oral cavity and vulva or penis. When the bilirubin level increases the jaundice may also be observed on the skin of the animal (Figure 4). Anaemia-associated blood turbulence is

causing the systolic heart murmur and may occur in various grades from II to VI. This is detected in animals with a packed cell volume (PCV) below 15-20% (Balch and Mackin, 2007).

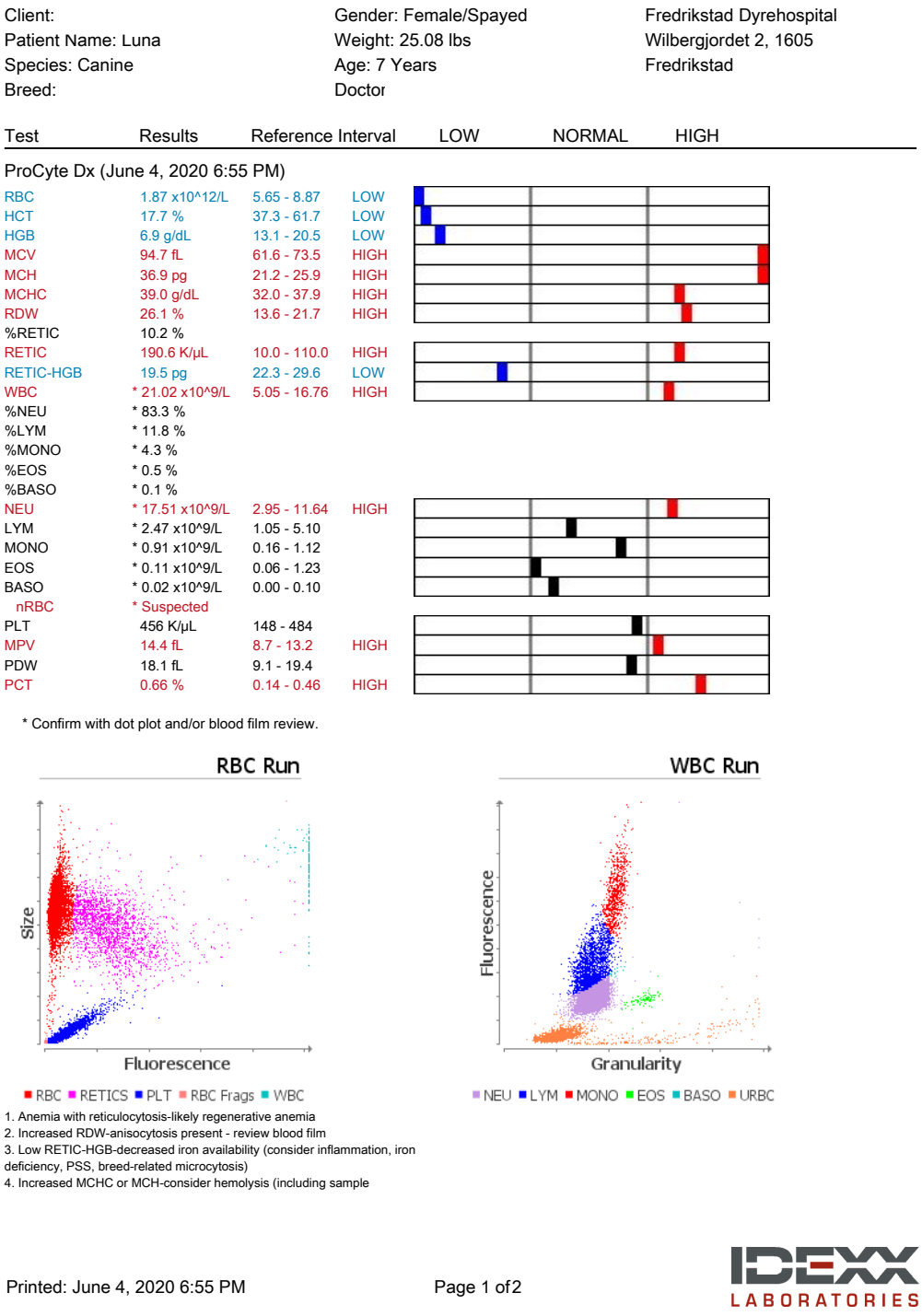
A fair amount of the dogs suffering from IMHA are diagnosed with thrombocytopenia simultaneously. Studies suggests that 50-70% of all the IMHA affected dogs have thrombocytopenia as well (Balch and Mackin, 2007; McCollough, 2003). Out of these, only 20% have a low enough platelet count to show clinical signs. Clinical signs of thrombocytopenia are signs of incomplete coagulation ability and includes petechiae, ecchymosis, melena and epistaxis (Villiers and Ristić, 2016)

7.2. Laboratory findings

Haematology and biochemistry

The typical IMHA patient shows a moderate to severe, regenerative haemolytic anaemia. The anaemia is initially non-regenerative, but the regeneration usually occurs after 3-4 days (Villiers and Ristić, 2016). Doing a complete blood count (cbc) is an important diagnostic tool (Figure 5). The haematocrit (htc) measured is typically below 25-30% (Balch and Mackin, 2007). In a study done by Nassiri et al. (2005) all dogs diagnosed with IMHA had a decreased htc with the mean value of 21.4%. All the dogs additionally showed haemolysis by having a decreased haemoglobin value. As mentioned, the anaemia is usually regenerative. A strong regeneration is seen in half of the diagnosed patients (Manev and Marincheva, 2018). Regeneration is confirmed by reticulocytosis, polychromasia and anisocytosis (Nassiri et al., 2005). Healthy patients should have a reticulocyte count below 1%. Reticulocytes are immature blood cells with extruded nucleus, still containing ribosomes, polyribosomes, and mitochondria. Reticulocyte amount increases in the peripheral blood and is an indication of blood loss, haemolysis or can be elevated due to remission of other types of anaemia. One-third of the animals diagnosed with IMHA does not have a significant regeneration. The reason for that may be due to the fact, that the anaemia is acute and pre-regenerative (Balch and Mackin, 2007). A non-regenerative response may also be seen if the antibodies or complements on the erythrocyte precursors are phagocytosed before being released from

the bone marrow (Nassiri et al., 2005; Villiers and Ristić, 2016), hence why IMHA should not be excluded based on the lack of regeneration.



Client:
Patient Name: Luna
Species: Canine
Breed:

Gender: Female/Spayed
Weight: 25.08 lbs
Age: 7 Years
Doctor

Fredrikstad Dyrehospital
Wilbergjordet 2, 1605
Fredrikstad

ProCyte Dx (June 4, 2020 6:55 PM)

Test	Results	Reference Interval	LOW / NORMAL / HIGH
RBC	1.87 x10^12/L	5.65 - 8.87	LOW
HCT	17.7 %	37.3 - 61.7	LOW
HGB	6.9 g/dL	13.1 - 20.5	LOW
MCV	94.7 fL	61.6 - 73.5	HIGH
MCH	36.9 pg	21.2 - 25.9	HIGH
MCHC	39.0 g/dL	32.0 - 37.9	HIGH
RDW	26.1 %	13.6 - 21.7	HIGH
%RETIC	10.2 %		
RETIC	190.6 K/µL	10.0 - 110.0	HIGH
RETIC-HGB	19.5 pg	22.3 - 29.6	LOW
WBC	* 21.02 x10^9/L	5.05 - 16.76	HIGH
%NEU	* 83.3 %		
%LYM	* 11.8 %		
%MONO	* 4.3 %		
%EOS	* 0.5 %		
%BASO	* 0.1 %		
NEU	* 17.51 x10^9/L	2.95 - 11.64	HIGH
LYM	* 2.47 x10^9/L	1.05 - 5.10	
MONO	* 0.91 x10^9/L	0.16 - 1.12	
EOS	* 0.11 x10^9/L	0.06 - 1.23	
BASO	* 0.02 x10^9/L	0.00 - 0.10	
nRBC	* Suspected		
PLT	456 K/µL	148 - 484	
MPV	14.4 fL	8.7 - 13.2	HIGH
PDW	18.1 fL	9.1 - 19.4	
PCT	0.66 %	0.14 - 0.46	HIGH

* Confirm with dot plot and/or blood film review.

1. Anemia with reticulocytosis-likely regenerative anemia
2. Increased RDW-anisocytosis present - review blood film
3. Low RETIC-HGB-decreased iron availability (consider inflammation, iron deficiency, PSS, breed-related microcytosis)
4. Increased MCHC or MCH-consider hemolysis (including sample

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IDEXX LABORATORIES

Figure 5. A classical haematologic picture of a dog diagnosed with IMHA. The htc is decreased (17.7%), there is a decreased haemoglobin value (6.9g/dL) and an increased reticulocyte count (10.2) suggesting a strong regenerative haemolytic anaemia. Leukocytosis with neutrophilia is also seen. Related to "case 4", from Fredrikstad Dyrehospital.

In feline patients, the anaemia is more commonly non-regenerative (Villiers and Ristić, 2016). Regarding the leukogram, leucocytosis with an increased neutrophile count is commonly seen as a result of an inflammatory reaction causing an altered bone marrow activity (Manev and Marincheva, 2018). Toxic changes of the white blood cells may also occur (Villiers and Ristić, 2016).

On the biochemistry there is commonly seen an increased bilirubin level as well as increased liver enzymes (Archer and Mackin, 2013). Bilirubin levels are almost always elevated in patients with IMHA. As mentioned, when the bilirubin level is severely elevated (>2-3mg/dl), jaundice can be observed (Balch and Mackin, 2007). The increased liver enzymes are due to the hypoxic damage of the liver. Elevated fibrinogen and globulins are also common findings (Manev and Marincheva, 2018; Villiers and Ristić, 2016). Fibrinogen is increasing during systemic inflammation as it is an acute phase protein (Scott-Moncireff et al., 2001). C-reactive protein (CRP) has also been reported to be significantly increased in patients with primary IMHA (Griebsch et al., 2009) Urine analysis would show bilirubinuria in almost all cases of IMHA, haemoglobinuria may be present in the case of intravascular haemolysis (Archer and Mackin, 2013; Villiers and Ristić, 2016).

Blood smear

Blood smear analysis can be quite a certain diagnostic tool for IMHA. As mentioned, regeneration can be diagnosed by reticulocytosis, polychromasia and anisocytosis. All these features may be detected on a blood smear. The anisocytosis is seen due to the large reticulocytes and small spherocytes (Figure 6). The detection of spherocytes on a blood smear is highly valuable as these are considered as diagnostic for IMHA in dogs if seen in moderate to large amount, meaning >10% (Villiers and Ristić, 2016). Spherocytes have been detected in 89-95% of the canine cases (Scott-Moncireff et al., 2001). These spherocytes are seen as small, spherical cells, intensely stained, and lacking the central pallor due to a partial damage of the membrane (Manev and Marincheva, 2018). At the tail of the smear normal RBCs may be flattened and lose their central pallor, this may give a false indication of spherocytes. In feline patients, it is impossible to detect spherocytes, as the normal feline RBCs have a very small or no central pallor and cannot be distinguished from spherocytes (Villiers and Ristić, 2016). In intravascular haemolysis, ghost cells may be detected (Figure 6). These cells are RBC

membranes that are ruptured, lacking the cytoplasmic content. In IMHA where antibodies or compliment are attached to the RBC membrane, pores are formed, releasing the cytoplasmic content into the plasma. Eventually, these ghost cells are cleared from the circulation within the spleen by phagocytic macrophages (Zachary, 2017).

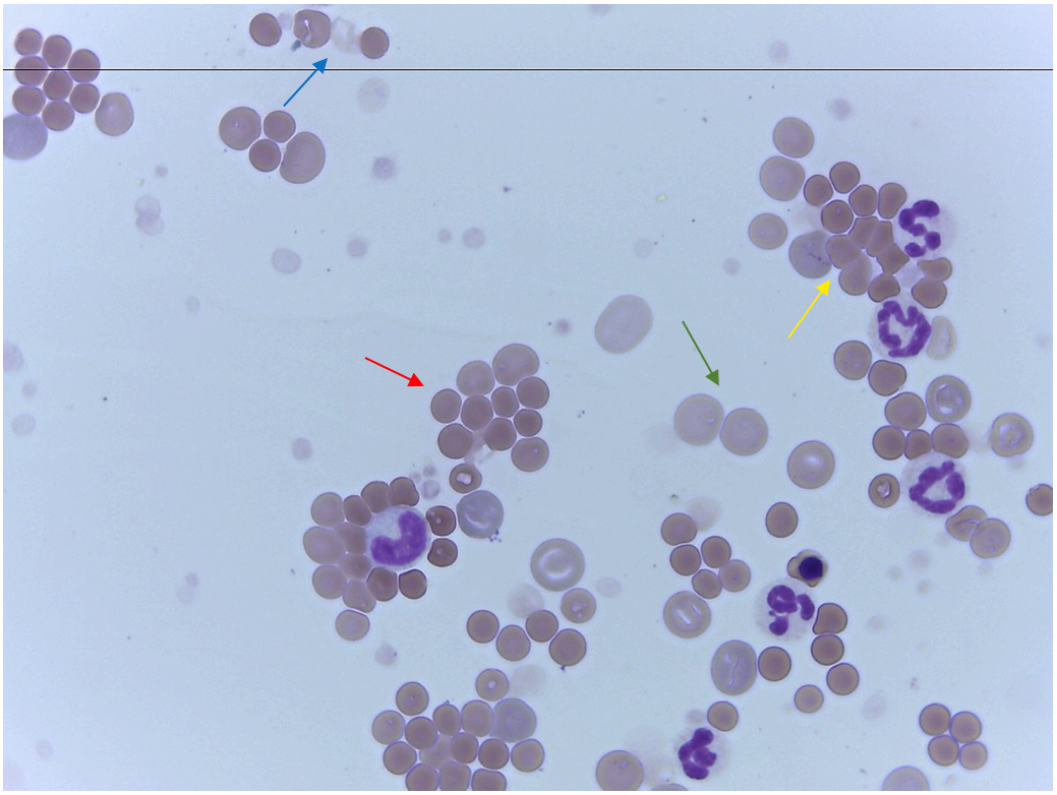

Figure 6. Spherocytes with no central pallor (red arrow) and reticulocytes that are polychromatic cells indicating regeneration (green arrow). Ghost cells are seen (blue arrow) without cytoplasmic content indicating intravascular haemolysis. Agglutination is also seen (yellow arrow) Blood smear related to "Case 2" from Fredrikstad Dyrehospital.

Autoagglutination is another valuable sign of IMHA. This has been reported to be present in 40-89% of dogs with IMHA (Balch and Mackin, 2007). When a high number of antibodies are present, one antibody may attach itself to more than one RBC, this will cause the RBCs to clump together (Figure 6). This clumping of RBC will cause a higher rate of removal of the blood cells, causing a more severe anaemia. Autoagglutination can be seen macroscopically in the sampling tube or microscopically by an agglutination test. For this, equal amount of blood and saline is put on a glass slide and looked at in the microscope (Figure 7). Autoagglutination may also occur in other diseases such as neoplasia, inflammation, or organ failure, hence it is important to perform this test. If the agglutination stays after saline washing, it indicates IMHA rather than the others (Villiers and Ristić, 2016).

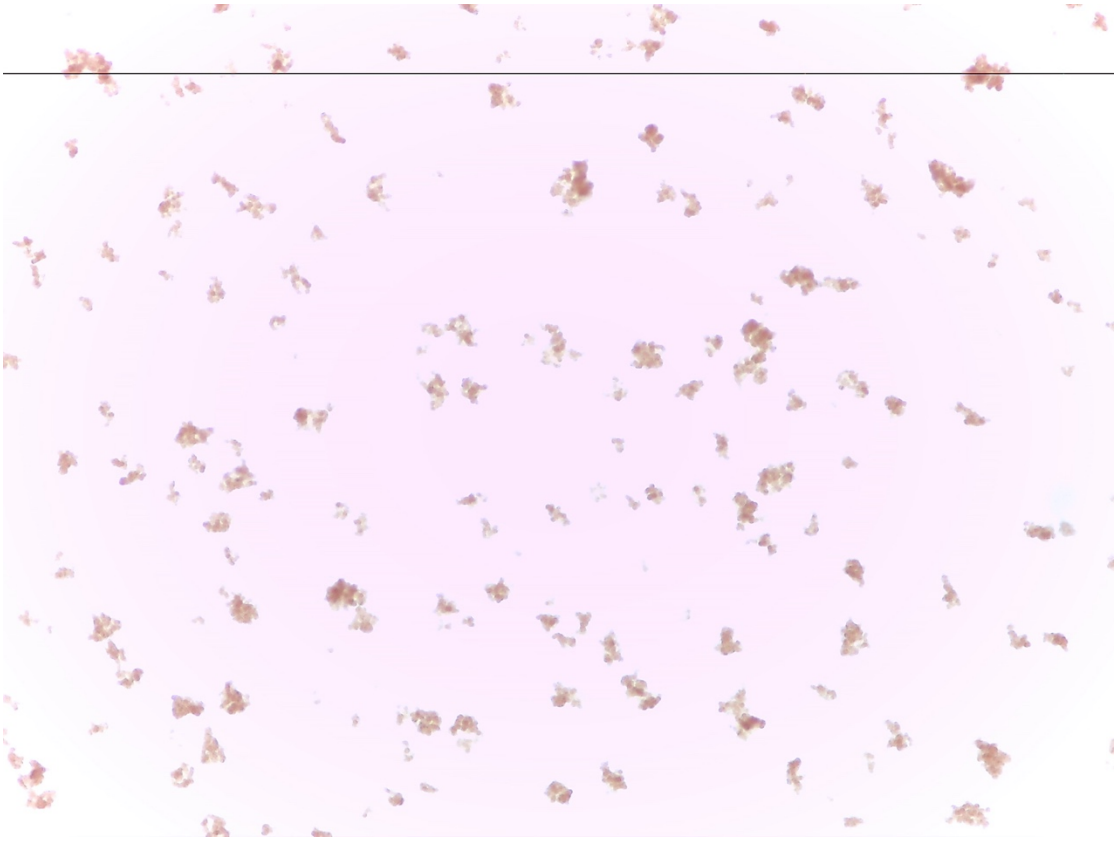

Figure 7. Agglutination seen as clumps of RBC seen microscopically. From a blood smear related to "Case 1" from Fredrikstad Dyrehospital.

Coomb's test

If the agglutination breaks up after doing the agglutination test, Coomb's test is indicated. Coomb's test is regarded as the confirmatory test for IMHA and is also called direct antibody test. The presence of antibody and / or complement on the surface of the RBCs can be detected by this test. Washed RBCs are incubated together with species-specific polyvalent antiglobulin directed towards IgM, IgG and complement showing visual agglutination when positive result (Villiers and Ristić, 2016). Unfortunately, the Coomb's test may provide a great number of false negative due to low titre of immunoglobulins, glucocorticoid therapy, technical problems with reagents or dilutions and human errors (Nassiri et al., 2005). There have been studies done where the Coomb's test has been compared with direct flow cytometry. The sensitivity of the direct flow cytometry was reported to be 90-100%, while for the Coomb's test, the sensitivity was reported to 50-60%. However, the specificity was reported to be 100% for the Coomb's test and 90-100% for the direct flow cytometry (McCollough, 2003).

A diagnosis of IMHA can be supported when there is either spherocytosis present, autoagglutination or a positive Coomb's test (Villiers and Ristić, 2016). These signs may appear individually or in combination with each other.

7.3. Diagnostic imaging

Thoracic x-rays and abdominal ultrasound may be beneficial to exclude or diagnose any underlying diseases or disorders causing the anaemia. As neoplasia is one of the main causes of secondary IMHA, it is important to rule this out to ensure the right treatment (McCollough, 2003). The thoracic x-ray may exclude primary heart disease, causing the murmur that may be heard in patients with IMHA. With the abdominal ultrasound, the size of the liver and spleen should be evaluated as well as excluding any gastrointestinal foreign bodies (Balch and Mackin, 2007). The value of diagnostic imaging is discussed regarding IMHA patients, in a study done by Andres et al. (2019) there were not any abnormalities seen in 68% of the thoracic x-rays and 25% of the abdominal ultrasounds. The treatment would not have changed after the imaging in 76% of the patients for thoracic x-ray and 50% for the abdominal ultrasound. However, diagnostic imaging may be crucial in individual cases, so it should always be considered.

7.4. Bone marrow evaluation

A bone marrow evaluation may be performed if there is detected a persistent non-regenerative anaemia, there is a suspicion of a destruction process towards RBC precursors or there are other cytopenias detected on the blood analysis (Archer and Mackin, 2013; McCollough, 2003). The bone marrow evaluation may reveal hyperplasia of erythroid series in patients with IMHA. In patients with chronic IMHA, the bone marrow may be damaged and secondary myelofibrosis may be detected (Carr et al., 2002). However, as primary bone marrow disorders are fairly uncommon, performing such test is debatable both in human and veterinary medicine (Orcutt et al., 2010).

7.5. Pathology

Most of the pathological findings found at necropsy and histopathology are in relation with the clinical signs found in patients diagnosed with IMHA. The necropsy findings may differ for intravascular and extravascular haemolysis as with the clinical findings.

Generally, pale mucous membranes, low blood viscosity, splenomegaly, hepatomegaly and an enlarged gallbladder is found in acute cases of haematolytic anaemia. With extrahepatic haemolysis, icterus is seen clearly in the fat and mesentery as well as on mucous membranes and skin in severe cases (Zachary, 2017).

Thrombosis have been identified as a common complication of IMHA and have been reported in 60-80% of dogs with IMHA by necropsy (Sinnott and Otto, 2009). The pulmonary and splenic vasculature seems to be the most affected sites regarding thrombosis, but thrombosis in multiple organs have been observed (Klag et al., 1993). In a study done by Carr et al. (2002), 80% of the dogs that had a post-mortem examination performed was diagnosed with pulmonary thromboembolism as well as intrahepatic thrombosis in 16% of the dogs, jaundice was also present in these dogs. Lee et al. (2007) reported a case where a thrombus in the right ventricle was discovered in a canine patient with IMHA. Thromboembolisms is possibly the cause of death of many IMHA patients (Mellett et al., 2010), however it is challenging to diagnose it ante-mortem and could be an underestimated finding in these patients (Klag et al., 1993). The thrombus lyses rapidly after death and disappears in approximately 15% of the cases, especially if the necropsy is delayed. Due to this, necropsy is not a sensitive method of discovering thromboembolisms (Scott-Moncrieff et al., 2001; Zachary, 2017).

The degree of leukocytosis have been described to have a connection to the severity of post-mortem lesions. Ischemic necrosis in patients with IMHA are related to thromboembolism or hypoxia. This necrosis can be found during necropsy in several organs, most importantly in the liver, spleen, kidney, heart and lungs (McManus and Craig, 2001). Centrilobular hepatic necrosis has been observed as one of the most common and important necrotic lesions following IMHA (McCollough, 2003; McManus and Craig, 2001). Ischemic tubular necrosis of the kidneys has also been detected during necropsy due to the haemoglobin concentration. Haemoglobin is not nephrotoxic

originally, but tubular necrosis occurs due to renal ischemia with an increased amount of hemoglobin in the glomerular filtrate (Zachary, 2017).

Myelitis, myelofibrosis and necrosis have been found when looking at the bone marrow of patients with IMHA. This inflammation is seen as edema, fibrin depositions and multifocal neutrophilic infiltrates (Zachary, 2017). Hemorrhages throughout the body have also been reported as a common pathological finding but without any anatomical abnormalities explaining the finding (Orcutt et al., 2010). West and Hard (2013) could also describe ulcerative enterocolitis as post-mortem findings in dogs diagnosed with IMHA.

8. TREATMENT

Several treatment options for IMHA have been described and the most successful treatment is yet to be discovered (West and Hart, 2013). The main target of the treatment is to stop the rapid RBC destruction and reduce the synthesis of immunoglobulins as well as treating any other complications of the disease by supportive therapy. In order to obtain a successful treatment of IMHA, separating primary from secondary IMHA is highly important (Manev and Marincheva, 2018). Primary IMHA usually requires intense immunosuppressive therapy, while secondary IMHA is usually disappearing when treating the underlying disease. By trying to treat a secondary IMHA with immunosuppression, it may even worsen the condition of the animal (Carr et al., 2002).

8.1. Immunosuppression

As mentioned, immunosuppression is the treatment of choice when it comes to primary IMHA. Glucocorticoids, azathioprine, mycophenolate mofetil (MMF) and human intravenous immunoglobulin (hIVIG) are amongst the drugs used as immunosuppressive drugs, alone or in combination with each other or other supportive drugs (Wang et al., 2013).

Glucocorticoids

Glucocorticoids are the first line drugs and act by altering the Fc receptors causing the RBC destruction to slow down (Manev and Marincheva, 2018). They decrease the production of spherocytes and immunoglobulins as well as they inhibit the complement cascade, enhancing regeneration. There are several glucocorticoids available, but there are few studies on which one is the better choice. However, prednisolone and prednisone are probably most frequently used as this can be administered orally. The immunosuppressive dose differs between the various drugs, but for prednisolone and prednisone it is 2mg/kg given twice daily for 2 weeks, or until the htc is stable (McCollough, 2003). Long term use of corticosteroids can give several side effects like gastrointestinal irritation, hyperadrenocorticism, recurrent infections and sepsis (Manev and Marincheva, 2018), as well as weight gain, polyuria / polydipsia, delayed wound

healing and alopecia (Wang et al., 2013). It is important to decrease the dose slowly after stabilizing, as a rapid reduction of the dose may lead to a relapse of the anaemia (McCollough, 2003)

Azathioprine

The effect of azathioprine is discussed in several studies, where in some, the safety is being questioned. Azathioprine inhibits activation and proliferation of lymphocytes and suppresses macrophage synthesis of cytokines and phagocytosis (Manev and Marincheva, 2018). Burgess et al. (2000) did a study where azathioprine was reported to have a significant longer median survival time. The dogs that received azathioprine had a median survival time of 370 days, and the dogs that did not, had a median survival time of only 9 days. Studies have also suggested that azathioprine in combination with prednisolone has a beneficial effect and will prolong the survival time even further (Allyn and Troy, 1997). However, Piek et al. (2011) stated that the drug is not beneficial as it is potentially toxic and has a carcinogenic effect. Other side effects of azathioprine are pancreatitis, myelosuppression and gastrointestinal disorders (Manev and Marincheva, 2018; McCollough, 2003; Wang et al., 2013)

Mycophenolate-mofetil (MMF)

MMF is an immunosuppressant widely used for organ transplants as well as in immune-mediated diseases in human medicine (Whitley and Day, 2011). By inhibiting biosynthesis, the drug blocks the proliferation of lymphocytes (West and Hart, 2013). MMF have a great oral absorption, hence it is an attractive option in small animal cases. It has a rapid onset of action and in the study done by West and Hart (2013), the median time for when the spherocytosis resolved was 13 days, and 43.5 days for the anaemia. Mild clinical signs have been observed following MMF treatment. Mild gastrointestinal disorders have been reported in most patients, while weight loss, papillomatosis and allergic reactions are reported as more uncommon side effects (Wang et al., 2013; Whitley and Day, 2011). Any significant differences have not been proven in the survival rate by the use of MMF in combination with glucocorticoids compared to using azathioprine with glucocorticoids, however it has been observed fewer incidences of adverse side effects with the use of MMF (Wang et al., 2013)

Human intravenous immunoglobulin (hIVIG)

In human medicine, hIVIG are successfully used in the treatment of IMHA as well as several other immune-mediated diseases. However, there are limited studies on the effect of hIVIG on canine IMHA. In a study done by Oggier et al. (2017) a significantly faster recovery of the htc in dogs was observed when treated with hIVIG in combination with glucocorticoids compared with dogs treated with MMF in combination with glucocorticoids. However, the survival rate of the first year was the same for both treatment options. The mechanism of hIVIG is to block the Fc receptor on the macrophage and to decrease phagocytic activity (McCollough, 2003). Side effects if hIVIF include allergic reaction, hypercoagulability and hypertension (Wang et al., 2013). As animals with IMHA has been described to already be in a hypercoagulable state, the risk of using hIVIG is questionable (Whitley and Day, 2011).

8.2. Supportive treatment

As previously stated, thromboembolism may be the cause of death in patients with IMHA and is observed in a great portion of the patients during necropsy. Because of this knowledge, prevention of thromboembolism has been described as an important aspect of the treatment. Several drugs have been used, but there are limited studies on the various effect. Aspirin, clopidogrel, heparin and warfarin are all anticoagulative drugs that prevents thromboembolisms with different mechanisms (Manev and Marincheva, 2018; McCollough, 2003). In human medicine, treatment of such anticoagulative drugs have been reported to decrease the morbidity and mortality in patients prone to thromboembolisms, this has also been proven to have a positive effect on the mortality of canine IMHA (Mellett et al., 2010).

Gastric ulcers have also been described as a relatively common finding in patients with IMHA. It may be due to poor perfusion of the gastrointestinal tract as a result of anorexia as well as long-term use of corticosteroids (Paes et al., 2010). To prevent gastric ulceration gastric protectants such as histamine blockers, proton pump inhibitors and ulcer-coating drugs are used (McCollough, 2003)

8.3. Blood transfusion

The aim of blood transfusions in IMHA patients is the increase the haemoglobin concentration to decrease risk for tissue hypoxia (McCollough, 2003; Piek et al., 2008). However, it is debated whether it should be performed or not. There are several disadvantages with blood transfusions. It has been discussed that an increased amount of transfusions will increase the chance of pulmonary thromboembolisms, however several studies have not been able to prove this theory (Carr et al., 2002). In severe cases of IMHA, where the htc is less than 15%, or where tissue hypoxia is seen with clinical signs such as tachycardia, dyspnea or tachypnoea, blood transfusions are indicated regardless the risk (Balch and Mackin, 2007; Manev and Marincheva, 2018). Blood transfusions are seen as one of the most important supportive therapy for dogs with IMHA and roughly 70 to 90% of animals diagnosed with IMHA requires it (Balch and Mackin, 2007). Before a blood transfusion is performed, a crossmatch test should be done. However, due to the autoagglutination, false positive crossmatching test are frequently seen (Manev and Marincheva, 2018).

8.4. Splenectomy

If all treatment fails, splenectomy may be suggested as a last alternative. The aim of the splenectomy is to remove the site for RBC destruction and decrease the antibody production (Manev and Marincheva, 2018; McCollough, 2003). Splenectomy should only be considered if it is IgG that is bound to the RBC surface, as with complement and IgM involvement, the destruction takes place in the liver, and the splenectomy would not be of any use (Jackson and Kruth, 1985). In a study done by Horgan et al. (2009), it was suggested that splenectomy improved the outcome of dogs diagnosed with IMHA and decreased the need of blood transfusions. Another study suggested that when dogs that were treated with glucocorticoids, azathioprine and had a splenectomy performed within 48 hours of presentation had a shorter recovery time and lower mortality rate compared to those that did not have a splenectomy performed (Balch and Mackin, 2007).

9. PROGNOSIS

There are several factors affecting the prognosis of animals diagnosed with IMHA. Unfortunately, the mortality rate is high and has been reported to range from 22 to 80% in various studies (Jackson and Kruth, 1985; Scott-Moncrieff et al., 2001; Wang et al., 2013). Some difficulties in this statistic are present, as patients have been lost to follow-up as well as the duration of follow-up differs in different studies. It may also not have been distinguished between the patients that were euthanized and the patients that died from the disease. In a study done by McCollough (2003), the mortality rate was 70% when all deaths were included, but only 26% when the euthanized patients were excluded.

Patients with concurrent thrombocytopenia have been reported to have a higher mortality rate and a higher risk of obtaining thromboembolisms (McCollough, 2003). Pulmonary thromboembolism has been reported to be responsible for most of the deaths seen with IMHA (Balch and Mackin, 2007; Mellett et al., 2010). Severe leukocytosis, highly elevated bilirubin levels and patients with intravascular haemolysis have also been reported to have an increased mortality rate (McCollough, 2003). In a study done by Orcutt et al. (2010), younger patients had a higher survival rate than older ones. However, other studies failed to observe any relationship between age and mortality (McCollough, 2003).

10. CASE EXAMPLES

Case 1

A 9-year-old neutered female Pomeranian was referred to the clinic with poor appetite, unstable gait and lethargy. The dog was diagnosed with anaemia and was referred for blood transfusion. Two days prior to the visit the dog had undergone an ovariohystectomy by the referring vet because of a pyometra. Due to this procedure, blood loss after bleeding, haemolytic anaemia secondary to anaphylaxis and immune mediated haemolytic anaemia was suggested as differential diagnoses. Additionally, the dog showed neurological signs such as ataxia, reduced proprioception, head tilt and horizontal nystagmus. With these findings, thrombus was also suggested as a differential diagnosis. The dog was tachycardic (140b/min), had a mild murmur and the mucous membranes were pale. On the haematological examination the htc was decreased to 9.5%, the dog had a normal number of reticulocytes, however the dot plot showed regeneration. The dog showed a severe inflammatory leukogram and macroscopic agglutination was observed in the sample tube. With these findings, immune mediated haemolytic anaemia was given as the definite diagnosis. The dog was given a blood transfusion and it was treated with a high dose prednisolone, dexadresone, clopridogrel and losec. After the blood transfusion the htc elevated to 17%. The htc stayed stable at this level and the neurological symptoms disappeared after the blood transfusion. The dog was hospitalized for one week and the htc continued to elevate up to 20.7%. The dog's htc and general condition was controlled daily after discharge. On day 16, the dog's general condition was poor, the dog had abdominal breathing and there was not seen much progress during the follow-ups. The dog was euthanized after the owners wish.

Case 2

A 13-year-old female chihuahua was referred to the clinic for blood transfusion. The dog had been slightly lethargic for two days but was worse on the day of the referral. During the clinical examination it was detected tachycardia (140b/min), fever (40°C) and the mucous membranes were pale and icteric, as was the pinna and abdomen. Other than that, the dog was clinically fine. The dog had taken blood samples at the referring vet, showing a htc at 13.8% with a significant regeneration. The CRP was above 100. A blood smear was done, and a significant anisocytosis was seen, a lot of spherocytes, a few reticulocytes, mild agglutination and a few ghost cells. No blood parasites were detected. The serum was haemolysed, indicating intravascular haemolysis. It also had an elevated total bilirubin level at 23 μmol/L. The dog was diagnosed with IMHA and was hospitalized for further treatment and observation. The dog was treated with a high dosage prednisolone and omeprazole. During the first night, the heart rate increased to 152b/min and the respiratory rate increased. The dog seemed more lethargic and a microhematocrit was measured less than 10%. With this result, a blood transfusion was performed. The microhematocrit was repeated 24 hours later and was elevated to 32%. After another 24 hours the htc decreased to 13% and the dog was non-responsive and had a heart rate of 160b/min. The dog was given another blood transfusion. During hospitalization, the dog received in total three transfusions, and mycophenolate mofetil treatment were started after the last transfusion. The dog showed a great response with this therapy and was discharged after one week hospitalized. The haematocrit was 24% and the total bilirubin was 6 μmol/L when discharged. One week later the dog came back to the clinic with anorexia. The haematocrit was stable, but the dog was hospitalized for observation. An oesophageal feeding tube was placed in to feed and medicate the dog. The dog went home the day after with the feeding tube for the owners to continue the treatment at home. After a few days the dog developed haemorrhagic diarrhoea and lethargy. The dog was then euthanized due to economic and ethical reasons.

Case 3

A one-year-old female Shiba was referred to the clinic with lethargy and anorexia. Urine looking like coffee was also observed. During the last day, the dog had been vomiting as well as having diarrhoea. The clinical examination showed lethargy, fever (40,4°C) and a slightly icteric sclera. Other than that, there were no other findings. The bloodwork showed a htc at 28.7%, low haemoglobin, elevated reticulocytes as well as increased neutrophils. The dog was dehydrated, so after rehydration, the true htc was 19%. On the biochemistry, the total bilirubin was significantly elevated to 72 µmol/L, the dog showed hypolipidemia and hypokalaemia. The CRP was >200. On the blood smear Heinz bodies, eccentrocytes, spherocytes, reticulocytes and ghost cells were seen. The blood smear showed signs of haemolysis due to oxidative reasons. The anaemia was regenerative but not agglutinating. The colour of the blood was darker than normal, a test was performed where the blood was put on a piece of white paper, as the blood was still brown after two minutes, methemoglobinemia was indicated. The urine also showed signs of severe amount of methaemoglobin (Figure 8). With these findings it was suspected that the dog was poisoned with paracetamol or onion. The dog was given a blood transfusion and started with acetylcysteine in case of paracetamol poisining. It was also given buprenorphine for abdominal pain. During the first night of hospitalization the haematocrit elevated to 30% after the transfusion. The dog was discharged after two days at the clinic and was in very good shape.

Figure 8. Urine (top) and serum (bottom) showing severe methaemoglobinaemia after onion poisoning. Picture taken at Fredrikstad Dyrehospital.

Case 4

An 8-year-old intact female cocker spaniel arrived to the clinic with lethargy, anorexia and very yellow urine. The dog was diagnosed with IMHA 2 years earlier, with a poor prognosis. The dog was in heat 2-3 weeks prior to the visit. On the clinical examination it was detected a normal temperature, normal heart frequency, but the femoralis pulse was very protruding. The mucous membranes were pale, dry and the crt was slow (2s). The abdomen was indolent, but the dog had a slightly swollen vagina. On the haematology, the htc was detected at 17.7% with strong regeneration. The dog also showed a neutrophilia and had a CRP at 172. On the biochemistry the dog had low urea, increased ALP and increased total bilirubin. The urinary analysis also showed bilirubinuria. The dog was hospitalized and started up with a dexadreson injection. The dog was given a blood transfusion the day after arrival and the hct increased to 21% the morning after. The dog started on prednisolone, clopridogrel, omeprazole and sucralfate. When the dog was diagnosed with IMHA previously, it was concurrently diagnosed with a thrombus and ascites. Hence why the clopridogrel was started immediately. An abdominal ultrasound was done on the dog as a pyometra was an additional concern with the elevated crp, timing of heat and the swollen vagina. This diagnosis was excluded, and the rest of the ultrasound looked normal. After two days of hospitalization the hct decreased to 18.2%, the dog was then started on mycophenolate mofetil. The htc kept decreasing and on day four it was 11% and the dog got another blood transfusion. After the transfusion, the htc increased to 19%. The dog was hospitalized for one week and when the dog was discharged the hct was elevated to 25% and the total bilirubin was decreased to 6 μmol/L. The dog came routinely for follow-ups with blood samples the first few weeks after the treatment and remained stable.

11. DISCUSSION

These four cases shows a variety of cases regarding pathogenesis, aetiology, treatment and outcome. In case 1, the dog showed neurological signs such as ataxia, head tilt and nystagmus. These signs may indicate a thrombus formation. The dog was treated with clopidogrel which is known to prevent thromboembolisms, however it has also been suggested that it can dissolve an already present thrombus (Harder and Kinkhardt, 2012). It is believable that this was the case with this patient as it arrived with the clinical signs of a thrombus and improved after the treatments were started.

The second case is a great example of primary IMHA. On the blood smear all signs were visible; spherocytes, reticulocytes, ghost cells and agglutination. Intravascular haemolysis was indicated by the detection of haemolysed serum. As previously stated, this gives a poorer prognosis compared to extravascular haemolysis. The dog seemed to improve before discharge but after starting with MMF treatment it developed haemorrhagic diarrhoea while the htc remained stable. As the most common side effect of MMF is known to be gastrointestinal disorders, it is likely to believe that this dog suffered from severe side effects of MMF.

In the third case, it shows a great example of secondary IMHA. Shiba Inus are known to be susceptible to onion poisoning, suggesting this to be the most likely cause of IMHA in this case. The dog showed clear signs of methemoglobinemia in the blood serum as well as in the urine, indicating poisoning. The dog improved quickly without immunosuppressive treatment, supporting the theory of secondary IMHA.

The fouth case is also an excellent example of primary IMHA. It is a female Cocker Spaniel, which are highly predisposed for obtaining IMHA. This dog was diagnosed with IMHA two years earlier including a thrombus. Starting anticoagulative treatment immediately reduces the chances of thrombus formation and is suggested to be used prophylactically in IMHA to reduce the mortality.

12. CONCLUSION

IMHA is a well-known topic within the veterinary medicine and studies have been published since the 1960s. The pathogenesis, aetiology and diagnosis are well described and understood. However, the prognosis is still poor, and more research is needed on treatment options especially concerning thromboembolisms as it may be the main reason for deaths in patients diagnosed with IMHA. There is limited research on how the thromboembolisms occur following IMHA. In conclusion, the ideal treatment is not yet discovered to be able to decrease the mortality rate of patients diagnosed with IMHA.

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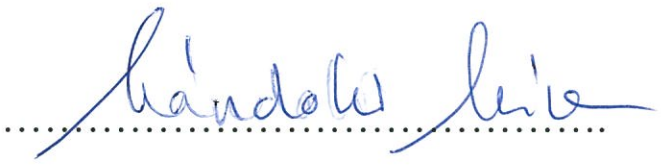

Dr. Mándoki, Míra

Pathology Department

HuVetA

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Name: GURO ØLMHEIM

Contact information (e-mail): guro.olmheim@gmail.com

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