Universidade de Évora - Escola de Ciências e Tecnologia

Mestrado Integrado em Medicina Veterinária

Dissertação

Canine Immune-mediated Hemolytic Anemia : a clinical challenge

Maria Pereira Esteves

Orientador(es) | Luís Miguel Lourenço Martins

Nuno Jorge Santos da Silva

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A dissertação foi objeto de apreciação e discussão pública pelo seguinte júri nomeado pelo Diretor da Escola de Ciências e Tecnologia:

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Abstract

Immune-mediated hemolytic anemia (IMHA) is one of the most common causes of hemolysis in dogs and it has been associated with high mortality rates. Most patients succumb to the disease in the first weeks of treatment mainly due to thromboembolic complications.

Glucocorticoids remain the mainstay of treatment and protocols which combine them with other immunosuppressive drugs may allow dose reduction and help non-responsive patients. However, reports studying different protocols are difficult to compare due to the small number and variety of the populations enrolled, the difference of the adopted treatment protocols and the lack of long-term follow-up of patients. Besides, these new drugs are often expensive and lack availability in day-to-day clinics.

The aim of this study is to make a literature review of the current available literature in order to try to simplify its diagnostic and therapeutic approach.

Keywords: Anemia; Hemolytic; Immune-mediated; Primary; Glucocorticoids

Resumo

Anemia Hemolítica Imunomediada Canina: um desafio clínico

A anemia hemolítica imunomediada é uma das causas mais comuns de hemólise em cães e está associada a altas taxas de mortalidade. A maioria dos pacientes sucumbe à doença nas primeiras semanas de tratamento sobretudo devido a complicações tromboembólicas. Os glucocorticoides continuam a ser o pilar do tratamento e protocolos que os combinam com outras drogas imunossupressoras permitem-nos reduzir a sua dosagem e ajudam-nos em pacientes que não respondem ao tratamento. No entanto, os estudos acerca dos diferentes protocolos terapêuticos são difíceis de comparar devido á variedade e pequeno número das populações estudadas, à diferença dos protocolos terapêuticos adotados e á falta de acompanhamento a longo prazo dos pacientes.

Além disso estes novos fármacos têm um custo elevado e não estão disponíveis no dia-a-dia das clínicas.

O objetivo deste estudo é fazer uma revisão bibliográfica da literatura disponível de maneira a tentar simplificar o diagnóstico e a abordagem terapêutica.

Palavras-chave: Anemia; Hemolítica; Imunomediada; Primária; Glucocorticoides

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Abbreviation list

- ACVIM – American College of Veterinary Internal Medicine
- ADP - adenosine diphosphate (adenosine difosfato)
- ALP – alkaline phosphatase (fosfatase alcalina)
- ALT – alanine aminotransferase (alanina aminotransferase)
- APTT - activated partial thromboplastin time (tempo de tromboplastina parcial ativado)
- AST - aspartate aminotransferase (aspartato aminotransferase)
- AT – antithrombin (antitrombina)
- BHS – bovine hemoglobin solution (solução de hemoglobina bovina)
- BUN – blood urea nitrogen (nitrogénio ureico no sangue)
- C1-INH - C1-inhibitor (inhibitor de C)
- C3 - Complement component 3 (componente do complemento 3)
- CBCs – complete blood cell counts (hemograma complete)
- COX-1 – cyclooxygenase 1 (ciclooxigenase 1)
- CRT – capillary refill time (tempo de reposição capilar)
- DAT – direct antiglobulin test (teste de antiglobulina direto)
- DEA - dog erythrocyte antigen (antigénios eritrocitários caninos)
- DELAT - direct enzyme-linked antiglobulin test (teste direto de ligação enzimática a antiglobulina)
- DIC – disseminated intravascular coagulation (disseminaçao intravascular disseminada)
- dL – deciliters (decilitros)
- DLA - dog leucocyte antigen system (sistema de antigénicos leucocíticos caninos)
- DNA - deoxyribonucleic acid (ácido desoxirribonucleico)
- EDTA - ethylenediaminetetraacetic acid (ácido etilenodiamino tetra-acético)
- Fc - fragment crystallizable region (proteína do fragment cristalizável)
- FC - flow cytometry (citometria de fluxo)
- FDPs - fibrinogen degradation products (produtos da degradação da trombina)
- GGT - gamma-glutamyl transferase (gamaglutamil transpeptidase)
- hIVIG – human intravenous immunoglobulin (imunoglobulina humana intravenosa)

- IA – immunoadsorption (imunoadsorção)
- Ig – immunoglobulin (imunoglobulina)
- IM – intramuscular
- IMHA – immune-mediated hemolytic anemia (anemia hemolítica imunomediada)
- IV – intravenous (intravenoso)
- kg – kilogram (kilograma)
- LC - liposome clodronate (clodronate liposomal)
- LMWH – low molecular weight heparin (heparina de baixo peso molecular)
- m^2– square meter (metro quadrado)
- MAC – membrane attack complex (complex de ataque à membrana)
- MCV – median corpuscular volume (volume corpuscular médio)
- mg – milligram (miligrama)
- MHC - canine major histocompatibility complex (complex principal de histocompatibilidade canino)
- mL – milliliter (mililitro)
- MMF - mycophenolate mofetil (micofenolato de mofetil)
- MPS - mononuclear phagocyte system (Sistema fagocítico mononuclear)
- NaCl - sodium chloride (cloreto de sódio)
- NO – nitric oxide (oxido nítrico)
- OFT – osmotic fragility test (teste de fragilidade osmótica)
- PCV – packed cell volume (volume da célula embalado)
- pIMHA – primary immune-mediated hemolytic anemia (anemia hemolítica imunomediada primária)
- PLSCR5 - phospholipid scramblase family member 5 (member da família 5 da scramblase fosfolipídica)
- PO – per os (via oral)
- pRBC - packed red blood cells (glóbulos vermelhos empacotados)
- PS – phosphatidylserine (fosfatidilserina)
- PT – prothrombin time (tempo de protrombina)
- PTE – pulmonary thromboembolism (tromboembolismo pulmonar)
- RBC – red blood cells (glóbulos vermelhos)
- RNA – ribonucleic acid (acido ribonucleico)

- RPI - reticulocyte production index (indíce de produção de reticulócitos)
- SAT - saline agglutination test (teste de aglutinação salina)
- SC – subcutaneous (subcutâneo)
- sIMHA – secondary immune-mediated hemolytic anemia (anemia hemolítica imunomediada secundária)
- Tc – cytotoxic T-cells (células T citotóxicas)
- TEG – thromboelastography (tromboelastografia)
- TF – tissue factor (fator tissular)
- TPE – therapeutic plasma exchange (intercâmbio de plasma terapêutico)
- TPMT - thiopurine methyltransferase (tiopurina metiltranferase)
- UFH – unfractionated heparin (heparina não fracionada)
- ULDA – ultra-low dose aspirin (aspirina em dose ultrabaixa)
- μL- microliter (microlitro)

Internship organization

The Master's degree in Veterinary Medicine of the University of Évora includes a final internship. This document is directly associated with the scientific research, and following of the practical activities of the internship.

The Hospital de Referência Veterinária Montenegro was the chosen facility and the internship took place from September 2020 to March 2021. The hospital, which is localized in Oporto city provides several veterinary services: Internal Medicine, Emergency room and Critical care, Anesthesia, Imageology, Physiotherapy, Surgery, Preventive Medicine as well as hospitalization services. The hospital has a 24-hour service.

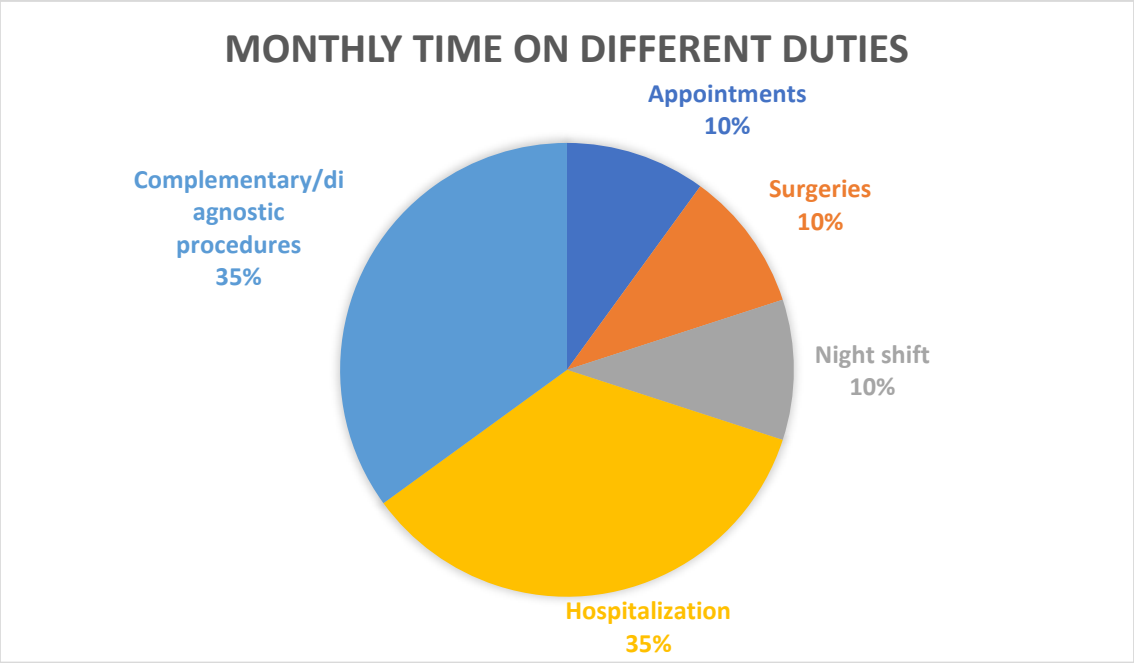
During the internship the student was able to obtain theoretical and practical knowledge throughout the different sectors, with the help from experienced doctors, nurses and veterinary assistants from the hospital staff.

The schedule consisted of a five-day week work with different day shifts (08-16h / 10-18h /14-22h) and a night shift (17-10h).

During each month, on day shifts, there were two days in which the student should follow doctors on their scheduled consultations and two days in which the student should follow doctors on the different surgeries. During the rest of the days the student followed the hospitalized animals and assisted the hospital staff on complementary/diagnostic procedures.

During the night shift the student should monitor all hospitalized animals and inform the doctor on duty for that night if any emergency occurred. On every morning after the night shift the student should inform the morning shift staff of all the hospitalized cases. Night shifts occurred twice a month.

The time spent on each different duty every month is summarized on graphic number one.



Graphic 1: Percentage of time spent every month on different duties.

The student accompanied a mean value of 30 cases every week, which does not reflect the higher number of animals appointed to the hospital. Due to the pandemic situation occurring during the internship, the high number of cases the hospital received, and the rotatory shifts it was impossible to follow every case from the beginning to the end and to register them all. However, a figure of approximately 30 cases a week may be pointed as the number of different cases followed.

From the mean of 30 animals followed every week, 20 were canine patients (*Canis lupus familiaris*) and 10 were feline patients (*Felis silvestris catus*). The different cases can be summarized under three categories:

1. **<u>Clinical Pathology</u>**

   Clinical pathology was the most addressed medical specialty during the internship.

   The student was able to follow different cases of hospitalized animals (including the infectiology sector) as well as help with and perform different medical

procedures in the laboratory, x-ray room, echography room, CT scan/MRI room, pharmacy and physiotherapy room.

The activities developed by the student include a wide range of procedures: animal contention; blood collection; arterial pression measurement; preparation of materials for clinical procedures; preparation and administration of medications; catheter placement; evaluation of the general clinical state of patients among helping the hospital staff with different procedures and veterinary appointments. Basic procedures like feeding/cleaning/walking the animals were also part of the daily routine.

The different specialties regarding clinical pathology were: Gastroenterology, Neurology, Dermatology, Oncology, Infectiology, Hematology, Ophthalmology, Cardiology, Toxicology, Urology, Neonatology, Pneumology, Endocrinology and Traumatology. Unfortunately, a precise number of cases per each specialty cannot be given due to the concerns previously reported.

2. Surgery

During the day shifts dedicated to surgery procedures the student was able to aid the hospital staff on the different phases a surgery patient should undergo: pre/intra/post-operatory care. The pre-operatory care consisted of preparing and monitoring the patient for surgery, calculating the dosages of the pre-anesthetic and emergency drugs, catheter placement, trichotomy and proper asepsis of the surgery site, electrode placement and endotracheal intubation. During surgery the student aid the surgeon as its auxiliary or aid the anesthetist on the anesthesiology monitoring. On the post-operatory the student monitored the patient until it was fully awake.

The different type of surgeries the student was able to accompany were: neurosurgery; soft tissue surgery; skin surgery; orthopedic surgery and minimally invasive surgery. Once again precise numbers of the different surgeries attended cannot be given.

3. Preventive Medicine

Preventive Medicine was the least accompanied specialty as it was mainly performed during veterinary appointments which the student, mostly due to the pandemic situation, could not attend daily.

However, the student was able to aid and perform vaccinations; electronic identifications and internal and external deworming.

1. Introduction

The first cases of immune-mediated hemolytic anemia in dogs were reported in the 1960s, and, until these days it is one of the most common autoimmune diseases in dogs[1,2].Since then numerous other reports have emerged in the intent to understand and better treat the condition, despite this the mortality of the disease remains high with up to 70% of patients succumbing [3–10]. This is related to increasing hemolysis and organ failure accompanied by secondary disseminated intravascular coagulation (DIC), with thromboembolism being the major cause of death[11]. The hopeful prospect being that in recent years mortality numbers have been diminishing specially in patients that survive the first weeks of treatment[12,13].

Clinical manifestations of the disease occur as a consequence of an immune-mediated process against erythrocytes surface molecules, resulting in their destruction. This lysis can be complement-mediated (intravascular hemolysis) or by phagocytosis via cells of the monocyte–phagocyte system located within the liver and spleen (extravascular hemolysis)[2,14]. A regenerative anemia will develop although some cases of non-regenerative anemia have been reported where the immunoglobulins or complement are targeted towards erythrocyte precursor in the bone marrow [2,15].

IMHA can be idiopathic (primary) or secondary to a range of events like infection, neoplasia or drug interaction, among others The majority of cases, up to 75%, are considered to be primary[2,3,15–17].

Several new diagnostic techniques as well as therapeutic approaches have been emerging in an attempt to better treat and comprehend this complex pathology. Treatment still rests on immunosuppressive drugs, with glucocorticoids being the first choice for this matter [18].

The elevated mortality associated with this condition, the recent consensus about diagnosis and treatment and the constant development of new therapeutic approaches induced the realization of this bibliographic review.

A new nomenclature has recently been proposed to properly represent the heterogenicity in the pathomechanisms that trigger IMHA, categorizing the disease as “non-associative” and “associative”. This explanation is necessary since the word “primary” suggests that

all triggers have been absolutely ruled out, while "secondary" indicates causality. Consequently, the term "associative" should be used once a comorbidity is discovered. In some situations, the comorbidity might have triggered the IMHA (secondary IMHA), whereas in others it might be coincidental (primary IMHA). In "non-associative" IMHA cases comorbidities are not identified, this includes primary ("idiopathic") and cryptogenic cases[19]. Although this new nomenclature has been proposed, in this review the terms primary/idiopathic or secondary will be used.

2. Methodology

The bibliographic research for the present review document was made using the databases of PUBMED and Google Scholar and with the aid of scientific books and periodicals.

The key words used were immune-mediated hemolytic anemia (IMHA); canine; diagnosis; treatment; glucocorticoids; thromboprophylaxis and prognosis. Articles from every year from 1945 until 2021 were included.

3. Pathophysiology

The normal lifespan of canine red blood cells (RBC) is 100-120 days[14] .When they age the mononuclear phagocyte system (MPS), composed by macrophages within the liver and spleen, identifies antibodies and complement molecules directed against RBC senescent membrane antigens and clears the erythrocytes attached to them from circulation[14,20] .A pathological condition may occur if RBC of all ages are being prematurely destroyed by an immune response[14].

IMHA is a type II immune reaction that involves the lysis of erythrocytes after being embed in antibody or by complement fixation[2,21,22]. The most common antibody in cases of IMHA is IgG, a tetrameric antibody, that will be recognized and attached by its Fc portion to the MPS membrane receptors so that the RBC can be phagocytized [2,14,20,21,23] . Because this process occurs outside of the circulation, it is known as extravascular hemolysis. Spherocyte formation can occur when only a portion of the membrane is removed, leaving the cell with a reduced surface/volume ratio[209]. The MPS macrophages are found largely in the spleen so an active erythrophagocytosis will lead to splenomegaly and, if this process also occurs within the liver, when immunoglobulin production

increases, hepatomegaly may also occur[23]. IgM also takes part in the process and can also agglutinate red blood cells. Once it's bound to them the complement cascade can be activated or the RBC-IgM complex may be phagocytized by the MPS macrophages [2,23]. This antibody can also detach itself from the RBC after complement activation and attach to different erythrocytes[2]. In some cases, the autoantibodies can target the erythrocyte precursors located within the bone marrow resulting in an acquired pure red cell aplasia [15,22].

If hemolysis takes place within vascular circulation by complement fixation, its activation may cause immediate intravascular hemolysis which will lead to free hemoglobin being released into the bloodstream causing hemoglobinemia and subsequentially hemoglobinuria[2,14]. The activation of the cascade complement will further lead to the generation of a "membrane attack complex," or MAC, which will puncture the RBC membrane, allowing water and electrolytes to enter leading to cell lysis while still in circulation[2,14,20]. Complement fixated to the RBC membrane may as well be recognized by the MPS which will increase extravascular hemolysis[14].

IMHA can be idiopathic, or primary (autoimmune hemolytic anemia, AIHA) when the antibodies are directed against self-antigens of the erythrocytes. Up to seven erythrocyte autoantigens have been identified but the major one is glycophorin, and less frequently band 3 protein [22,24–26].The genes that encode these proteins are overrepresented in animals with IMHA has shown in a recent study[27].The variety of antigens identified by components of the erythrocytes from different individuals suggest that the underlying etiology of the disease may differ [22,24,25]. In healthy individuals autoantibodies are not allowed to react with host tissues by suppressor T-cells, however, apparently, animals with IMHA fail to regulate suppressor T-cell function or have an overstimulated immune system which allows autoantibodies to attack ordinary RBC and the activation of autoreactive T-lymphocytes [2,14,25]. Other evidence has linked the genes of the canine major histocompatibility complex (MHC or dog leucocyte antigen system – DLA) to IMHA, once again supporting the existence of genetic susceptibility to IMHA [28].

Secondary IMHA is originated by an immunologic reaction to nonself antigens which have altered or are attached to normal RBC membranes. The cause may vary between

exposure to drugs or toxins [29–31] ,neoplasia[30,32], infection [30,33–37], intrinsic red blood cell defects[14] , envenomation [38,39] , pregnancy [40] and, controversially, vaccines [4,7,9,41,42].

Alongside the anemia, animals with IMHA often have concurrent thrombocytopenia of variable severity which is associated with poor outcome considering that the most common cause of mortality in dogs with IMHA are thromboembolic events [1,30,43–47]. The thrombocytopenia can be associated with a concurrent immune-mediated destruction of platelets (ITP), a higher consumption resulting from DIC, splenic sequestration of thrombocytes, failure of platelet production, vasculitis or enhanced consumption due to a generalized inflammatory state[5,44].When the concurrent thrombocytopenia is immune-mediated the pathology is termed Evan's syndrome[7,22].

A thromboembolic event may affect both veins and arteries as suggested by various reports[48–50]. Pulmonary thromboembolism (PTE), a venous thrombus, is the most common clinical manifestation of thrombosis[7,15,44,47]. The presence of the two types of thrombi advocates hat dogs with IMHA have both coagulation and platelet dysregulation [51], which should be taken into consideration when deciding the thrombophylactic protocol.

Thromboembolic complications are frequently related to the evolution towards a prothrombotic state, which can cause blood stasis, hypercoagulability, and endothelial damage [52]. Up to 50% of dogs with IMHA are presented in a hypercoagulable state at the time of diagnosis which is in part responsible for the occurrence of thrombosis and can even lead to disseminated intravascular coagulation (DIC)[44,46,53–56]. Studies show that in most dogs with primary IMHA platelets appear to circulate in an activated state[45,54] and due to the inflammatory state of this patients, P-selectin expression values are greater, which will stimulate leucocytes (primarily monocytes) to produce thrombogenic microparticles. Furthermore, along with platelets and endothelial cells, these particles play an important role in thrombogenesis[57,58].

Although thrombogenesis in these patients is an intricate mechanism it gives us more evidence regarding genetic susceptibility to this pathology. Tissue factor (TF), capable of initiating the coagulation cascade via the extrinsic pathway, is not generally found on endothelial cells or cells in contact with blood, but, in IMHA patients it can be induced by inflammatory cytokines, free hemoglobin or platelets[59–62]. Adding to these,

intravascular TF gene expression is upregulated in these animals and may contribute to consumptive coagulopathy [53,62].

In addition to this, phosphatidylserine (PS), which facilitates the assembly of prothrombinase and tenase complexes and is normally located on the inner leaflet of the cell membrane, can be expressed on the outer surface of platelets upon their activation or on RBC when they become senescent or their membrane is disrupted, such as in spherocyte formation, promoting coagulation [59,61,63–65]. Reticulocytes have high levels of PS on their cell surface and so, reticulocytosis has been proposed as a risk factor for thrombosis in hemolytic disease[63]. PS is also exposed on microparticles that derive from activated platelets and erythrocytes which may play a significant role in facilitating thrombosis[59]. Furthermore, free hemoglobin, released by intravascular hemolysis, will bind to nitric oxide predisposing the animal to a hypercoagulable state. Nitric oxide is required for vasodilatation and acts as a powerful inhibitor of platelet aggregation, being bound to free hemoglobin makes it unavailable to counteract coagulation[7,64].

A recent RNA sequencing study in dogs with IMHA found that these animals had increased expression of genes related to neutrophil function, coagulation, cell cycle regulation as well as hematopoiesis, and reduced expression of genes related to lymphocyte function when compared to healthy age- and breed-matched controls[27]. In this study the phospholipase scramblase PLSCR5 gene was found to be the most overrepresented, this gene assists in the externalization of PS on cell membrane surfaces, once again providing evidence that there is a genetic basis for the development of the disease [27].

Besides the excessive coagulation activation in animals with IMHA reduced anticoagulant levels also occur, such as a decrease in AT levels which contributes to the prothrombotic state [59].

4. Patient Presentation

The classical patient presentation for IMHA is reported to be a middle-aged neutered female dog[2,66]. Most reports suggest female dogs have a substantially higher incidence than male dogs [3–5,7,9,16,67–69] although some suggest no predisposition in any sex [15,30,70]. Despite this, being neutered seems to be an important factor for the development of the

disease [3,5,7,43] . The mean age is around 6 years, but it can vary from 7 months to 16 years [2,3,7,9,13,15,16,30,53,67,68,71].

Some breeds seem to be predisposed to develop IMHA but the most reported one is Cocker Spaniel [3–5,7,13,15,30,44,54,69,70,72] though it may be influenced by the population available for the study [9], despite this, there is enough evidence to suspect that there is a genetic predisposition in this breed. IMHA has even been linked with the blood type in one study where the absence of DEA 7 in Cocker Spaniels was related to an increased risk of developing the disease[73].

Other common canine breeds are described in Table 1.

Table 1 - Other common canine breeds	
Old English Sheepdogs [71]	Collies [3,15]
Doberman Pinchers [2,30]	Miniature Schnauzer [3,5,44]
Irish Setters [2,13]	English Springer Spaniels [3,15]
Poodles [3]	German Shepherds [13,30]
Dobermanns [43]	Maltese-breed [9]
Hungarian Vizslas [9]	Airedale Terriers [9,43,44]

Although a correlation between the time of the year and the incidence of IMHA has been proposed [15] most studies fail to demonstrate any association with the development of the disease [3,9,16] .However, the probability of greater rates of IMHA during the warmer months may imply an underdiagnosed infectious trigger, including tick-borne disorders or simply a higher number of owners scheduling routine visits in this time of the year[2,5,74].

5. Clinical Presentation

Signs can vary between patients but lethargy/anorexia, pale mucous membranes and icterus appear more frequently[3,4,15,69]. When serum bilirubin levels surpass 2-3 mg/dL jaundice will be observed first on the mucous membranes and, when bilirubin concentrations become higher as the disease develops it will affect the patients skin[14].

Clinical findings consistent with anemia as tachycardia; tachypnoea; prolonged CRT and a systolic murmur associated with blood turbulence are commonly reported[2,14,33,43,58,68,75]. Due to the hemolysis the patient may present yellow to orange coloration of the feces and pigmenturia (hemoglobinuria or bilirubinuria). Splenomegaly and hepatomegaly are also common[2,14,58,75].

Fever and lymphadenopathy have been reported[2,3,8,14,37,58], as well as abdominal discomfort, vomiting and diarrhea [2,33,58,68].

Signs related to thrombocytopenia like petechiae and epistaxis may occur but are less common[2,4,8,14,58,71,75].

6. Diagnosis

After collecting the patient general history and performing a clinical evaluation a differentiation must be made among primary and secondary IMHA. This is of vital importance because the treatment protocol must be adequate, considering that patients with secondary IMHA respond poorly to immunosuppressive treatment, unless the underlying cause is eliminated [14]. Most studies suggest primary IMHA as being more common than the secondary form of the disease, with up to 75% of the reported cases. Although these numbers may be accurate, they could also propose the fact that in some situations there is an incapacity to detect the underlying cause, which contributes to the overall poor prognosis associated with the disease[3,8,14,16,75].

Standard and more specific analysis/tests must be performed considering that there is no golden standard. The diagnostic tests should be interpreted in combination with the response to immunosuppression[19].

For the confirmation of the diagnosis there must be proof of:

- anemia (regenerative or not),
- signs of immune-mediated RBC destruction (spherocytosis/positive saline agglutination test/demonstration of anti-erythrocyte antibodies)
- evidence of hemolysis (spherocytosis/hyperbilirubinemia/ hemoglobinemia or hemoglobinuria/ erythrocyte ghosts) [19]

6.1. Complete Blood Cell Counts (CBC)

A CBC is one of the most simple and beneficial tests in diagnosing IMHA considering that most of the dogs are presented with a mild to severe regenerative anemia, although animals with the chronic form of the disease can present a higher value of PCV [75]. Severe macrocytic anemia is present in IMHA dogs, with hemoglobin levels as little as 2.5g/100mL [1].

Individual reticulocyte volumes are typically greater than mature erythrocyte volumes and so MCV values are expected to be higher as well. However, in dogs with regenerative anemia, these values are normally within the normal range. This occurs because larger cells must constitute a significant portion of the total erythrocytes in order to raise the MCV above the reference range[66].

An inflammatory leukogram is present in almost all dogs at the time of diagnosis or develops during the first days of hospitalization [58,76]. A pronounced leukocytosis with a left shift is present in up to 85% of patients [4,67,72]. The potent regenerative erythroid response by the marrow during the disease progression could be one of the causes for the rise in leucocyte numbers, as well as a myeloid hyperplasia stimulated by cytokines, a decreased migration into poorly perfused tissues that are necrotic or because of neutrophil demargination[14]. A necropsy-based study demonstrated that leukocytosis may serve as a prognostic factor for clinicians seen that it is linked to the possibility of a moderate to severe tissue necrosis[72]. During complement activation, some of the components released are also involved in stimulating neutrophil discharge from the bone marrow[2].

Decreased platelet counts are common with as many as 70% of dogs with IMHA having counts <200,000/µL [7,75] and as many as 24% of dogs having severe thrombocytopenia (<50,000µL) [4,7,15,67].

The most common changes in CBC's are summarized in Table 2.

Table 2 - Most Common Changes in CBC's	
Decreased PCV	Mean value of about 15% (reference interval: 35%-55%) [3,4,7–9,15,54,66,67,70]
Leukocytosis	Mean value of about 32 $\times 10^3/\mu L$ (reference interval: 5.0-14.1$\times 10^3/\mu L$) [3,4,7,9,66]
Increased band and segmented neutrophil counts	[7,13,43,47,55]
Monocytosis	[43,66,67]
Decreased platelet count	Associated with bad prognosis [4,7,15,43,67]

6.2. Blood Smear and Reticulocyte Indexes

After correctly collecting a blood sample from the patient a blood smear can be made, allowing to verify two hallmarks of IMHA, spherocytosis and autoagglutination, among other elements that can help the clinician confirm there is a hemolytic event taking place.

Spherocytes are erythrocytes that have lost their membrane and appear on stained blood smears with a smaller diameter and a lack of central pallor (Figure 1) [66].Spherocytes have been identified in as much as 95% cases of IMHA [5,7,13,43,44]. A value of ≥5 spherocytes/×100 oil immersion is thought to support a diagnosis of IMHA, but 3-4 spherocytes/×100 oil immersion will also be indicative of IMHA if no other cause of spherocytosis is found[19].

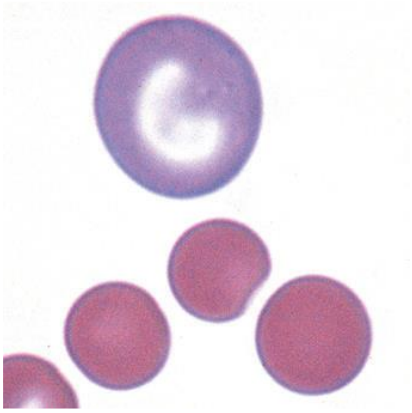


Figure 1: Three spherocytes at the bottom and a polychromatophilic erythrocyte/reticulocyte at the top found in the blood of a dog with IMHA[66].

Apart from the spherocytes there are other morphological characteristic changes that can be found in the blood smears of dogs with hemolytic disease:

- ✓ Polychromasia: presence of bluish-red erythrocytes. Polychromatophilic cells are reticulocytes that stain slightly bluish than erythrocytes due to the presence of both hemoglobin and individual ribosomes and polyribosomes, the latter staining in blue, with Romanowsky-type blood stains. Low numbers of this cells are common findings in the blood of healthy dogs, however, their numbers increase in regenerative anemias[66]. They are present in blood smears of up to 90% of dogs with IMHA[3,13,43].
- ✓ Anisocytosis: variation in erythrocyte diameter. When a significant number of larger-than-normal cells are formed, such as when erythrocytes are created in greater quantities, this can happen. As a result, enhanced anisocytosis is common in regenerative anemias[13,43,66].
- ✓ Erythrocyte "ghosts": indicative of intravascular hemolysis [43,66].
- ✓ Macrocytosis: larger than normal erythrocytes owing to reticulocytes being larger than normal erythrocytes, even though reticulocytes shrink in size as they mature into erythrocytes, larger-than-normal reticulocytes create larger-than-normal erythrocytes. [66].

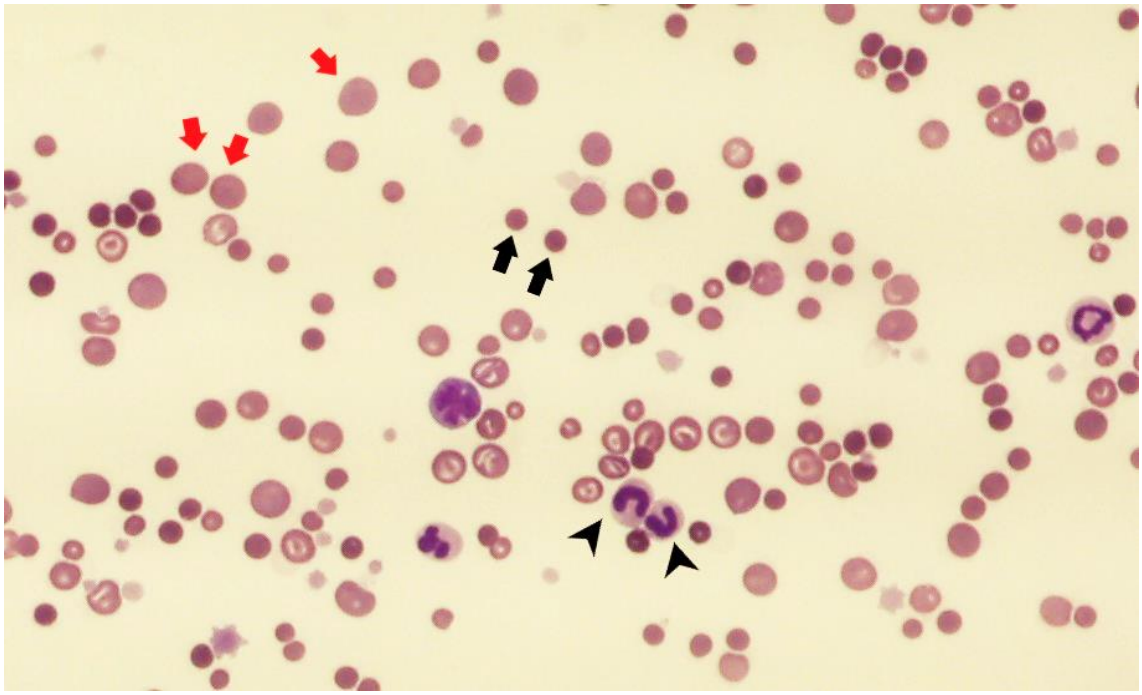


Figure 2: Blood smear from IMHA patient. Several hematological changes can be seen: spherocytes (black arrows); polychromatophilic cells (red arrows) and band neutrophils (arrow heads).

Figure 2 illustrates three common hematological changes in the blood smear of a dog with immune mediated hemolytic anemia.

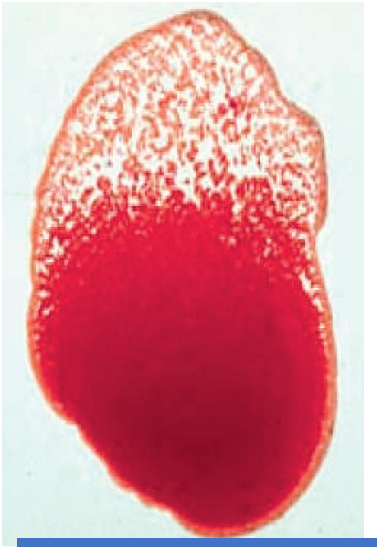


Figure 3: Positive result in SAT [14].

Patients with IMHA frequently have a positive saline agglutination test (SAT) which has been reported in as much as 89% of dogs with the disease[4,5,14,44]. Autoagglutination (aggregation of erythrocytes together in clusters) occurs when RBC are coated with immunoglobulins[66]. One drop of EDTA-blood must be put on a slide and then several drops of isotonic saline must be added, if clumping is present, autoagglutination is confirmed [77]. Although most literature suggests 1-10 drops of saline should be added, a recent study advocates higher concentrations (49:1) result in a higher test specificity[78]. Agglutination can be confirmed macroscopically on a slide, as seen on Figure 3, or by microscopic examination, as seen

on Figure 4. Because repetitive washing may break up erythrocyte aggregates, their absence in the SAT does not rule out IMHA[58].

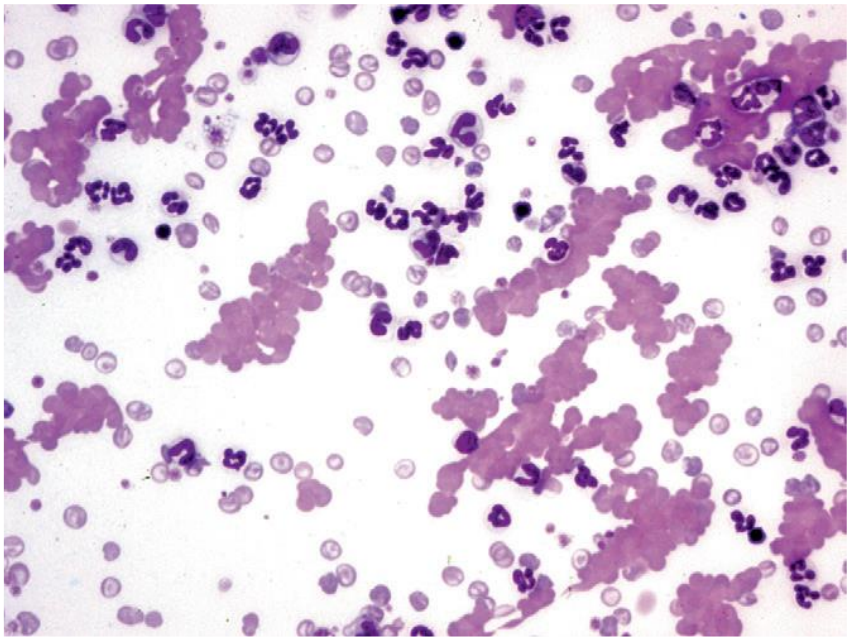


Figure 4: Erythrocyte autoagglutination in blood from a dog with IMHA and severe leukocytosis.[66]

Reticulocyte indexes help us evaluate bone marrow integrity and its role for erythrocyte regeneration, allowing to classify anemias as regenerative and nonregenerative as well as to monitor therapy[79].

Reticulocytes are immature erythrocytes with a reticulum network of RNA, mitochondria, and organelles that can be seen using supravital stains[79]. As a response to blood loss, hemolytic events, or the remission of other types of anemia, their number rises in the peripheral blood which will reflect the bone marrow's erythropoietic activity.

Following the initial hemolytic episode reticulocytes begin to appear in circulation after 2-5 days, which implies that the anemia could appear non-regenerative despite being not. Their numbers peak between 4-7 days returning to normal by day 15 even if the anemia is not entirely resolved[79]. As a result, because the peak in reticulocytes and peripheral regeneration response has already happened, the anemia may appear nonregenerative. When the PCV approaches 30-35%, the bone marrow may simply

react to this mild anemia by releasing more mature RBCs rather than reticulocytes, since the hypoxia is too light at this point to induce more erythropoietin synthesis[79].

Reticulocyte counts can be performed manually, through light microscopic examination of supravitally stained blood smears, or by automated methods like flow cytometry. Automated methods give more precise and reliable numbers than manual counting, nevertheless the quality and features of the blood samples are of vital importance[66,80].

Regardless of the method used to obtain the reticulocyte percentage (manual/automated) we may calculate their absolute count by multiplying the percentage of reticulocytes by the total erythrocyte count in millions in order to obtain the number of reticulocytes in one microliter of blood. In dogs an absolute count of more than 60,000/μL indicates erythrocyte regeneration, however reference intervals for percentage of reticulocytes and reticulocyte number may be higher for automated counting (80,000/μL) [14,79,81].

The bone marrow response should be greater as the hematocrit drops, so correcting the reticulocyte percentage for the PCV is also useful for clinical interpretation. The following formula should be used:

$$\text{Corrected Reticulocyte \% (CRP)} = \text{Reticulocyte \%} \times \frac{Patient\ PCV}{Normal\ PCV}$$

A percentage of 0.5-1% of reticulocytes in the peripheral blood is normal in healthy, nonanemic dogs. In an anemic patient, a corrected reticulocyte percentage of more than 1% is a marker of regeneration[79].

The Reticulocyte Production Index (RPI) can also be calculated to adjust for premature reticulocyte release and extended maturation in peripheral blood[79]. The following formula should be used:

$$\text{Reticulocyte Production Index} = \frac{CRP}{Reticulocyte\ Maturation\ Time}$$

The maturation time used will depend on the PCV, 1, 1.5, 2 or 2.5 days for 45, 35, 25 or 15% PCV respectively.

A RPI of ≤1 is indicative of a nonregenerative anemia, an RPI of 1-2 indicates some regeneration with a functional marrow, an RPI of >2 suggests enhanced erythropoiesis, and an RPI of ≥3 suggests a significant bone marrow response like that observed with hemolysis[79]. Although RPI is corrected for the magnitude of reticulocytosis, according to the severity of anemia, and therefore turns into the best marker of regeneration, the fact that it is calculated based on maturation time of human reticulocytes may somehow limit its accuracy for regeneration in dogs [81].

Interestingly, about a third of all IMHA patients have poorly regenerative anemia at the time of diagnosis, several of these patients have an acute onset and thus require more time to stand an adequate regenerative response, while others have immunoglobulins directed against marrow erythroid precursors or an undetected infectious disorder[5,14,15,71,72] . Verification of nonregenerative anemia requires repeated analysis three to four days after its onset to allow for delayed marrow release of RBC[72].

6.3. Bone Marrow Evaluation

A bone marrow evaluation may also be required, particularly if the patient's anemia is nonregenerative, there is a suspicion that the immune reaction is directed at erythroid precursors, or there is another cytopenia present[2]. In IMHA patients, bone marrow evaluation often demonstrates erythroid hyperplasia, as well as myeloid hyperplasia and megakaryocytic hyperplasia [43,44]. Marrow examination in patients with an immune response directed against marrow erythocyte precursors, on the other hand, may demonstrate reduced erythropoiesis or maturation arrest impacting the erythroid series[14].

6.4. Serum Biochemistry

There are some frequent changes in the biochemistry profile of dogs with IMHA, the most common, occurring in up to 80% of cases reported, is hyperbilirubinemia[4,9,43,44]. This is due to increased bilirubin generation as a result of both hemolysis and decreased hepatic clearance of bilirubin because of compromised hepatic function in

severe anemia. Hyperbilirubinemia is associated with a poor prognosis due to an increased rate of hemolysis and, as a result, the existence of a more severe form of IMHA. Furthermore, higher bilirubin levels may be linked to more severe IgM and complement-related disease, resulting in more fulminant intravascular hemolysis and enhanced intravascular release of procoagulant RBC membranes. This will contribute to the hypercoagulable state associated with the disease and therefore to the risk of thromboembolism [3,7,72]. There is also a report of a dog developing kernicterus due to the hyperbilirubinemia associated with IMHA[82].

Another common finding is the elevation of liver enzymes (ALP; ALT; GGT and AST) especially ALT due to the hypoxic damage the liver suffers with anemia. Elevated ALP levels are associated with an increased chance of developing thromboembolic disease as well as elevated plasma bilirubin levels, and thus a poor prognosis[3,7,55].

Azotemia has also been reported with increases in plasma urea concentration along with creatinine levels, which may be due to renal injury (induced by hypoxia), concomitant thromboembolic renal disease, and prerenal factors[8]. IMHA has been linked to renal injury and distal renal tubular acidosis[83]. The elevation in plasma urea levels may also be related to gastrointestinal bleeding, due to blood coagulation disorders, which will increase protein digestion [11].

Hypoalbuminemia may also occur as a result of the acute phase reaction or due to a decreased production of albumin on account of impaired hepatic function[5,7].

Other changes in the normal biochemistry profile include an elevation in blood lactate values, which may be the result of severe anemia or systemic hypoperfusion leading to decreased oxygenation of the tissues [84] and serum creatine kinase values, that could be a reflection of the combined effects of hypoxia, recumbency, alterations in the permeability of the cell's membranes, and muscle injury resulting from reduced perfusion; thromboembolic complications; recurrent IV catheter placement (thrombosed catheters); and subcutaneous or intramuscular medications and it's related to a worse prognosis [5].

6.5. Coagulation Profile

As previously mentioned, patients with IMHA are often in a hypercoagulable state[55]. Coagulation profiles serve as an early indicator for DIC and thromboembolism, which are related to a worse prognosis[2].

Recently thromboelastography (TEG) has been used to assess global clotting function in dogs, although the availability of TEG machines is currently limited, when available, this methodology yields information on both coagulation and fibrinolysis[55].

Common changes in the coagulation profile of patients with IMHA are summarized in Table 3.

Table 3: Common alterations in the coagulation profile	
Prothrombin Time (PT)	Elevated in up to 46% of patients[4,7,8,43,44].
Activated partial thromboplastin time (aPTT)	Elevated in up to 67% of patients [4,7,8,43,44].
Fibrinogen concentration	Elevated in up to 100% of patients [4,8,44,55]. Reduced in up to 18% of patients [4,8].
Antithrombin activity (AT)	Reduced in up to 50% of patients [44,55].
Fibrin(ogen) degradation products (FDPs)	Elevated in up to 57% of patients [7,44].
D-Dimers concentration	Elevated in up to 91% of patients [44,55].

Abnormal clotting times (PT and aPTT) are a presumed consequence of DIC but can also be altered if anti-coagulation therapy has been initiated [85].

Fibrinogen is an acute-phase inflammatory protein and its plasma concentration is high when systemic inflammation is present, consistent with the inflammatory state in which patients with IMHA are[55,85]. However, during the formation of thrombi, fibrinogen is cleaved into fibrin monomers by thrombin, at which point coagulation is activated[44,55]. This could explain why most patients present high fibrinogen values while some have a decreased plasma concentration. Attention should be paid to

patients with smaller fibrinogen values as they could indicate an end-stage or fulminant DIC[44].

Up to 50% of canine patients have a reduced AT most likely because of excessive consumption in association with DIC but might also occur secondary to urinary loss due to concurrent glomerulopathy[44,86]. AT is an important endogenous anticoagulant and an activity <50% is related to higher risk of thrombosis, besides, along with an increase in D-dimer values, low AT activity is one of the most sensitive indicators of DIC in dogs[85].

FDPs are by-products of clot breakdown and are an indicator of fibrinolysis, however D-dimer assays have mostly supplanted FDP assays, because D-dimers are considered more specific for fibrinolysis[85].

D-dimers are formed when plasmin degrades crosslinked fibrin, a high D-dimer concentration suggests ongoing active coagulation because D-dimers indicates both generation of thrombin (to produce soluble fibrin and activate FXIIIa) and plasmin, thus increased D-dimer concentration is considered a marker of hypercoagulability and DIC[55,85].

The values are of great value when initially evaluating the patient, especially considering how often PTE occurs, and to monitor anticoagulant therapy, thus serial monitoring of these values should be made.

6.6. Demonstration of anti-erythrocyte antibodies

- Direct Antiglobulin Test (DAT)

  The DAT or Coombs's test is employed to identify immunoglobulin, complement or both on erythrocytes membranes (Figure 5). Developed by the veterinarian Robin RA Coombs in 1945 has played an important diagnostic role in both human and veterinary medicine[87]. Although some authors consider that Coombs test is not needed if auto-agglutination prevails after washing [14,19,88].

  While its specificity is up to a 100% its sensitivity varies between 37-89% therefore a negative result does not rule out a IMHA diagnosis[3–5,7,14,15,19,44,58,89].

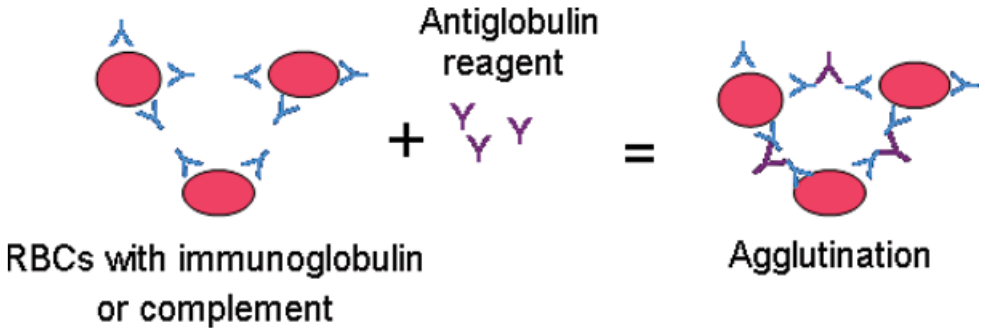


Figure 5: The Direct Antiglobulin Test (DAT). Anti-canine immunoglobulin or complement from rabbit or goat reacts with immunoglobulin or complement-bound RBC causing agglutination of RBCs[87].

Its diagnostic performance depends on:

- Use of polyspecific (containing anti-IgG/IgM/C3) or monospecific reagents instead of both [90]
- A one-dilution tube test as an alternative for multiple plates [87]
- Incubation at 37ºC only opposed to further incubation at 4ºC [58,87,90].

The pattern of antibody reactivity in the DAT might have importance in the diagnostic. A positive anti-IgG DAT is a strong indicator of IMHA, while a positive anti-IgM result is more likely to indicate a concurrent disease[13,58,89,91].

Some authors claim that, while immunosuppression does not immediately result in a negative DAT, there is interindividual variability in the time necessary to become negative DAT following treatment initiation, ranging from days to weeks[70,89]. Blood transfusions are sometimes reported as a cause of false-positive results but must authors agree that this is not an absolute contraindication to perform the test [19,26,89,92]. Prozone effects are also frequently observed. A prozone effect happens when agglutination is found at higher serum dilutions (i.e., high antibody concentrations) but not at lower serum dilutions (i.e., low antibody concentrations)[88,92].

Nonetheless DAT is still a cornerstone in the diagnosis of canine IMHA and new and promising simple in-clinic DAT screening instruments for IMHA are being developed[89].

- Flow Cytometry (FC)

Flow cytometry is a technology for determining the size, internal complexity, and amount of surface fluorescence of cells. Flow cytometry, unlike the DAT, requires a small sample size, employs minimal amounts of antisera, and is a quantitative, objective method considered to be more sensitive and accurate (up to 100% sensitivity) [19,93–96].

In one study, all dogs with IMHA had immunoglobulins bound to erythrocytes membranes detected by flow cytometry, while the antiglobulin test detected just 58% of the cases [97].

IgG is the predominant immunoglobulin detected by this method in patients with IMHA, which corroborates the results using the DAT method[97].

One study demonstrated that dogs with both IgG and IgM on the erythrocyte surface are more prone to have a lower PCV and greater degree of spherocytosis, suggesting that this combination may be more pathogenic than the presence of a single Ig isotype[94].

Flow cytometry allows to determine quantitate levels of surface-bound immunoglobulins which can be used to monitor the effectiveness of immunosuppressive therapy[19]. For example, in one study a rapid decrease in the percentage of Ig on RBC occurred after initiation of immunosuppressive therapy in dogs with IMHA, before changes in hematocrit or reticulocytosis became apparent[96].

- Direct enzyme-linked antiglobulin test (DELAT)

By comparing the absorbance of patient samples to that of healthy control animals, this technology detects immunoglobulins and C3 bound to erythrocytes[88,98].

Despite being a common test in the past century it has fallen in disuse probably because of the improvement and development of better techniques like the Coombs test and flow cytometry[88,99].

Still, the available literature for this test can give us some important information. For example, in one study erythrocytes sensitized with C3 were related to a more severe anemia condition and, the decline of the amount of IgG found on the RBC membrane was associated with the anemia condition improvement, whereas a rise on these levels coincided with relapses [100].

6.7. Additional testing

- Osmotic Fragility Test (OFT)

The OFT is an indirect way to detect spherocytosis seeing that spherocytes hemolyze at higher NaCl concentrations than normal erythrocytes and, possibly due to the oxidative stress IMHA patients are under, the osmotic fragility of these cells is greater as a result of oxidative damage caused by free radicals or reactive oxygen species [8,101,102].

It is a highly sensitive test with positive results up to 89% in IMHA patients and a specificity of 95% for diagnosing IMHA in anemic dogs [8,101]. It is a helpful diagnostic tool because low to moderate spherocyte numbers may not be identified on the blood smear. Despite this, the diagnosis of IMHA cannot be made based on a positive OFT[101].

- Diagnostic Imaging

In terms of radiography, an image of the abdominal cavity allows the clinician to assess the size of the liver and spleen as well as detect mass lesions or zinc bodies, and a radiography of the chest cavity may be effective in distinguishing patients with intrinsic heart disease from those with hemic murmurs induced by anemia, seeing that IMHA patients often have a systolic murmur[14,83]. These tests in combination with abdominal ultrasonography are useful in more old patients if a neoplasia is suspected.

Another useful information the diagnostic imaging can give us is the presence of pulmonary thromboembolism, although difficult to diagnose via thoracic radiographs since there is no pathognomonic finding, some changes can give us a suggestion: pleural effusion; pulmonary artery loss; alveolar infiltrates or an enlarged cardiac silhouette; hyperlucent regions on the lungs and widening of the main pulmonary artery[86,103]. However, PTE cannot be ruled out based on normal chest radiographs.

More modern techniques like magnetic resonance imaging or computed tomography offer potentially significant better diagnostic results, however, since general anesthesia is required, their utility may be somewhat limited[86].

- Urinalysis

Urinalysis is a reliable test to determine how severe the hemolysis is, in account of hyperbilirubinuria, hemoglobinuria (specifically associated with intravascular hemolysis) and increased urobilinogen [19,88].

Besides this, urinary tract infections are not uncommon on these patients and, as previously mentioned there is a chance of developing distal renal tubular acidosis [13,83].

IMHA proposes a diagnostic challenge seeing that not all tests are 100% reliable or even available and some depend on who is performing them. Because of this, recently the American College of Veterinary Internal Medicine proposed a diagram to help with the diagnosis, as seen on Figure 6:

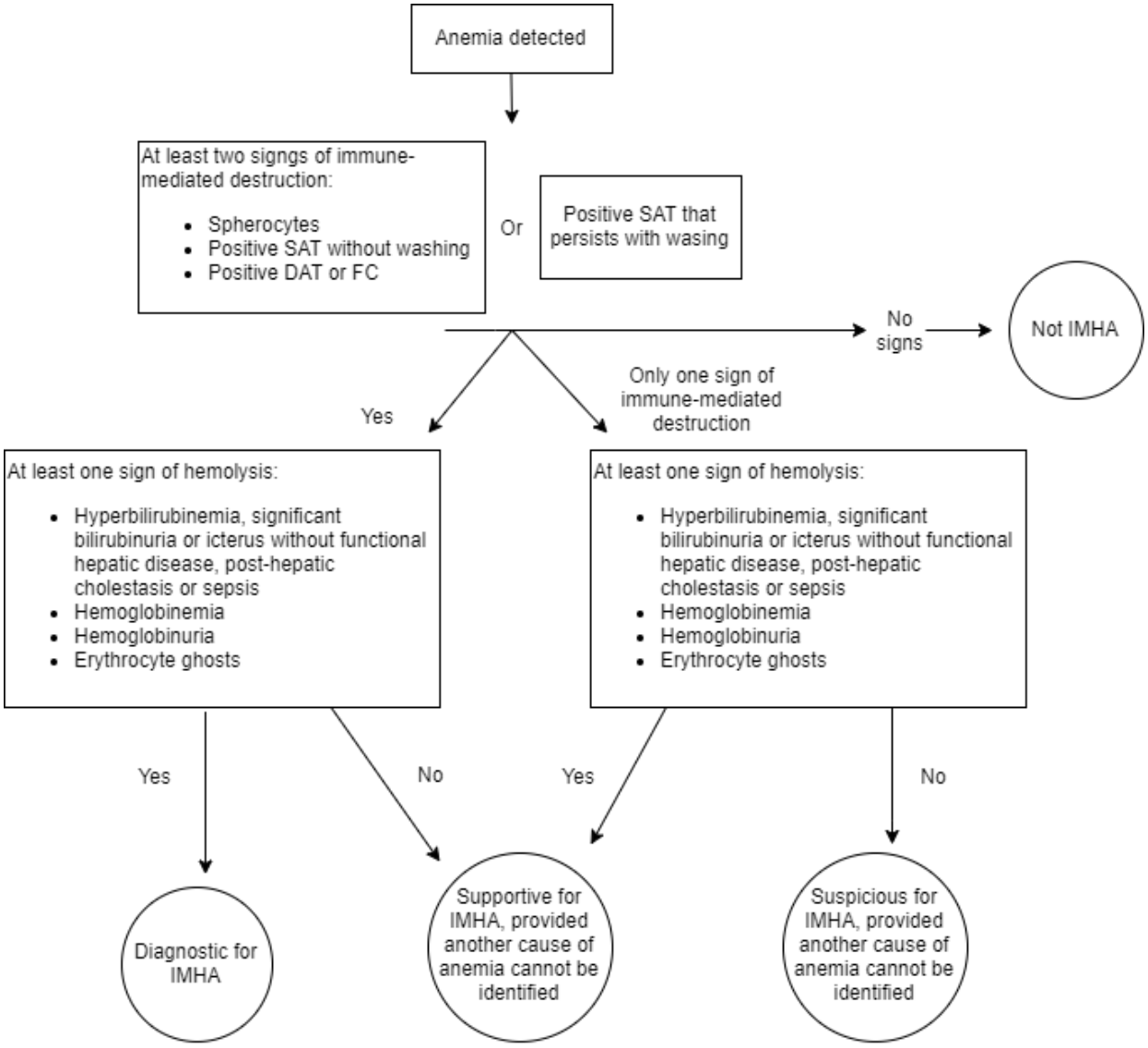


Figure 6: Diagnosis diagram adapted from ACVIM 2019 consensus [19].

7. Treatment

When all other differential diagnostics have been ruled out and a diagnosis of IMHA can be made for a fact treatment should be initiated. Although it is primarily focused on decreasing the erythrophagocytosis and suppressing immunoglobulin production there are other important aspects that must be considered, like restoring tissue oxygenation, preventing thrombus formation and providing supportive care[58]. It is important to mention that treatment protocols to be discussed forward are mainly related to idiopathic cases of

IMHA. When a secondary type of the disease is suspected treatment should be adjusted to cover the secondary pathology.

7.1. Immunosuppression

a. Glucocorticoids

Intravenous or oral glucocorticoids continue to be the basis of treatment for primary immune mediated hemolytic anemia and are effective as single therapeutic agents in many patients. These drugs decrease the Ig affinity for RBC membrane antigens and modify the function or recognition of the constant fragment of the macrophages Ig receptors, and, by doing so, their recognition system for IgG, IgM and C3b is inhibited. They also work by reducing inflammation and suppressing T-cell function and inducing their apoptosis[2,104].

When the patient tolerates oral medication, prednisolone/prednisone can be initiated with a dosage of 2-3mg/kg/day or 50-60mg/m^2/day for dogs ≥25kg, as a single daily dose or separated in two doses, with a recent study claiming that when compared to a fractionated prednisolone regimen, an unfractionated regimen improved side effects faster[105].If the oral drug is not tolerable, IV dexamethasone can be initiated with a dosage of 0.2-0.4mg /kg/day[18,75].

If the prednisone/prednisolone starting dose is more than 2 mg/kg/day, it should be lowered to less or equal than 2 mg/kg/day during the first two weeks, as long as the patient responds favorably to treatment, proved by a stable or increasing PCV[18].

To discontinue glucocorticoid treatment the dosage should first be tampered, decreasing it 25% every 2-3 weeks provided that the PCV remains stable and greater than 30% with an improvement in most measures of IMHA (spherocytosis, agglutination, serum bilirubin concentration and reticulocyte count)[18]. In most cases, prednisone/prednisolone treatment is expected to last 3-6 months[2,18].

Glucocorticoids are undoubtedly efficient at treating IMHA, but they do so with potential side effects due to the iatrogenic hyperadrenocorticism they induce, these effects can range from mild to severe, and in some cases can even be fatal[17]. They can cause restlessness, polydipsia and polyuria, polyphagia, panting, gastrointestinal ulceration, muscle weakness and wasting, thromboembolic events and

proteinuria[17,106]. The higher the dose and the more the treatment prolongs itself the more deleterious side effects may be, chronic usage of glucocorticoids can lead to alopecia or muscle atrophy, among others, worsen a pre-existing condition (i.e., exacerbating a congestive heart failure) or predisposing the animal to other pathologies (i.e., diabetes mellitus and pancreatitis)[13,17,18]. Subclinical bacteriuria is rather common in dogs receiving long-term glucocorticoid therapy and so periodic urinalysis are recommended especially if accompanied by clinical sings[18].

The dosage of glucocorticoid may be further reduced (25-33% every three weeks/25% every two weeks) by introducing a second immunosuppressive drug[18,106], in particular if:

- the patient displays clinical signs associated with severe or imminent life-threatening disease at the time of presentation, seen that many dogs may have an inadequate response to a single drug agent;
- during the first 7 days of treatment the patient's PCV does not remain stable, with an absolute decrease of ≥5% within 24 hours;
- after 7 days of glucocorticoid treatment, the patient requires blood transfusions, or
- the patient experiences or is likely to experience serious side effects as a result of using glucocorticoid drugs.

Available drugs commonly associated with glucocorticoids in IMHA treatment include:

b. Azathioprine

Azathioprine is a purine analogue that diminishes the number of lymphocytes and immunoglobulin synthesis dependent on T-cells through interference of the purine synthesis necessary for DNA and RNA replication[104,106].

The recommended dose is of 2 mg/kg or 50 mg/m^2 PO every 24 hours. After 2-3 weeks, the dose interval can be increased to every other day until treatment is discontinued[18].

Azathioprine administration seems to be related to a better prognosis as reported by several studies, however these results may be conditioned by incomplete information,

a small sample size, and variations in illness severity between groups[3–5,43,107]. On the other hand, a retrospective study suggested that azathioprine had no beneficial effect on the treatment of IMHA patients, however this study too has its own limitations[108]. Therefore, concrete scientific evidence is lacking on the beneficial effects of using azathioprine in combination to a glucocorticoid on the treatment of this disease, nonetheless it continues to be a strong option when aiming to reduce more rapidly the dose of glucocorticoids used[109].

Side effects include gastrointestinal distress, pancreatitis, severe hepatotoxicity (ALT values should be checked every two weeks through the initial two months of treatment) and marked myelosuppression (occurs months into treatment and normally reverses if azathioprine is discontinued)[18,104,110,111].

In human medicine thiopurine methyltransferase (TPMT), an essential enzyme in the azathioprine's metabolism, is used to determine the risk of myelosuppression in patients receiving this drug. If TPMT activity is low, the risk of developing life-threatening and treatment-limiting toxicities is higher, on the other hand, higher TPMT activity is associated with poor drug efficacy[109,110,112]. Some studies tried to determine the distribution of TPMT in canine populations, and while some claim that markedly TPMT-deficient dogs are a rarity [110], others found a significant breed-related variation in the enzyme activity with lower levels in giant schnauzers and greater levels in Alaskan malamutes [112].

c. Cyclosporine

Cyclosporine inhibites the transcription of genes required for T cell activation preventing their proliferation[104].

The recommended dose is 5 mg/kg PO every 12 hours [18].

Evidence on the usage of cyclosporine is scarce and typically of poor quality, one retrospective study suggested that it may be linked to a worse outcome than prednisolone alone or prednisolone in combination with azathioprine [107] and in another study the use of cyclosporine did not significantly alter the probability of survival to discharge[6]. This may be a result of the difficulty to maintain the required

plasma concentration because of incomplete and erratic oral absorption in dogs and substantial intersubject variability[113]. Even though cyclosporine may be more helpful on managing the acute phase of the disease due to its rapid onset of action[75].

In dogs, cyclosporine is normally well tolerated, but it can cause a variety of adverse effects, the most common of which are gastrointestinal symptoms that are either temporary or disappear as its administration is stopped (administering the drug refrigerated or with food may help reduce gastrointestinal adverse effects although it carries a risk of changing the drug absorption profiles)[18,107,109]. Gingival hyperplasia and hypertrichosis have also been reported and, although is not myelosuppressive it can make the patient susceptible to opportunistic infections and subclinical bacteriuria[18].

Therapeutic drug monitoring may be required especially if the patient experiences a poor therapeutic response, relapses, has drug-specific adverse effects, or develops secondary infections [18,106].

d. Mycophenolate mofetil

Mycophenolate mofetil or MMF was developed as a safer alternative to azathioprine due to its lower myelotoxicity and hepatotoxicity, and works by inhibiting the proliferation of T and B cells, as well as the differentiation of Tc cells and antibody responses[109].

The recommended dose is 8-12 mg/kg PO every 12 hours [18].

MF has been recently used in the treatment of IMHA as it is an attractive option for adjunctive treatment due to its oral and parenteral formulations, its rapid onset of action and reduced side effects when comparing to other therapeutic choices as seen on a recent study[114]. However, in another study it appeared to be related to more severe gastrointestinal toxicity although it had its limitations since it was comprised by a small number of dogs and it had no control group[115].

Side effects are uncommon but may occur and are normally related to the gastrointestinal tract, vomiting and diarrhea are the most common although ulcerative colitis mays develop in which case the drug should be discontinued[18].

e. Leflunomide

Leflunomide works by inhibiting the proliferation of T and B cells and has considerable anti-inflammatory effects[109].

The recommended dose is 2 mg/kg PO every 24 hours[18].

Leflunomide is an emerging drug that although it has still to be more studied on the treatment of IMHA is starting to demonstrate its effectiveness as an add-on therapy on different immune mediated/inflammatory diseases in dogs[116–118].

Lethargy, gastrointestinal discomfort, and moderate bone marrow suppression (leukopenia and thrombocytopenia) have been reported as clinical side effects[104].

f. Cyclophosphamide

Although cyclophosphamide was used in the past in association with glucocorticoids recent reports suggest it should not be administered as it offers no benefit compared to treatment with glucocorticoids alone and may potentially be hazardous in terms of long-term prognosis [6,18,69,107,119].

After discontinuing prednisone/prednisolone treatment in patients that are also receiving a different immunosuppressive drug, this other drug may be continued at the same dosage for 4-8 weeks, then stopped without tapering or decreased by 25-33% every 2/3 weeks, depending on the patient's response to treatment.[18].

7.2. Immunomodulation

In veterinary medicine, adjunctive therapies to immunosuppressive regimes are frequently useful in the acute management of patients with immune-mediated diseases. They usually have a rapid onset of action and a low likelihood of side effects; however, these immunomodulatory drugs are usually ineffective as a single-agent therapy. The most common agents used on the treatment of IMHA are:

a. Human Intravenous Immunoglobulin

Human intravenous immunoglobulin or hIVIG is constituted of extremely purified immunoglobulin G (IgG), residual amounts of IgA, IgM, CD4, CD8, and human leukocyte antigen molecules, acquired from large pools of healthy human plasma. It has a complex mechanism of action, involving modification of Fc receptor expression and function, interference with complement, B and T cell activation as well as a reduction in immunoglobulin production[120].

Although it appears to be an attractive possibility due to its rapid onset of action it has been shown to have no effect on long-term survival when compared to a different immunosuppressive protocol in dogs with IMHA[18,120]. Others suggest that its administration may be related with a quicker recovery to a normal PCV or a diminished requirement for transfusion, however these studies failed to have a control group [6,121].

Nonetheless, in circumstances where previous treatments have failed or when significant hemolysis has occurred, hIVIG may be utilized as a last resort[11]. The recommended dosage is 0.5-1g/kg as a single infusion over 6-12 hours depending on how well the dog is responding to it[18].

Side effects may occur especially acute hypersensitivity; other complications include renal failure, hypotension, aseptic meningitis, fluid overload and thromboembolism as well as promotion of an inflammatory state which will only exacerbate the hypercoagulability state the patients with IMHA are in [122,123].

b. Danazol

Danazol is a synthetic androgen that inhibits the expression of macrophages Fc receptors, reduces immunoglobulin binding to RBCs, stabilizes RBCs membranes and disrupts T cell homeostasis[109].

Although it has been used in human medicine to treat immune mediated diseases, in veterinary medicine its usage is not supported by studies and so is not used routinely[109]. In the few studies it was enrolled no beneficial effect was seen in using

danazol as an immunomodulatory drug or it was linked with the development of neoplasia [6,124].

Further investigation is required to determine its real efficacy in treating IMHA in dogs.

c. Liposome clodronate

Liposome clodronate (LC) is a bisphosphonate encapsulated into spherical lipid membrane vesicles which will be phagocytosed by macrophages and dendritic cells making them undergo apoptosis[109]. Within 24 hours, a systemic injection of LC can cause a very effective reduction of splenic and hepatic macrophages and therefore promoting a cutback in RBC clearance [125].

In a small group of dogs that received intravenous LC the drug was well tolerated, and the patient's survival rates increased. These animals received other immunosuppressive drugs, so it is possible that there was a synergetic effect between the high doses of prednisolone used and the LC[125]. The dosage used in this study was 0.5mg/kg.

As well as danazol, liposome clodronate will need further investigation to be considered a safe drug to administer to IMHA patients.

7.3. Splenectomy

Splenectomy may be utilized in dogs who require ongoing immunosuppressive treatment, or experience repeated relapses, therefore is a rescue therapy and must not be used as the first line of treatment[18].

Due to the underlying etiology of IMHA, in which the antibody-mediated RBC destruction happens in the spleen, removing it will diminish the number of mononuclear phagocytic cells and therefore dimmish the destruction on RBC[17].

Although its benefits are not totally obvious, previous studies indicate that splenectomy lowered post-surgery transfusion requirements, potentially allowing PCV to improve rapidly and consequently improving the outcome in dogs with

IMHA[18,50,58,126]. Nonetheless these studies failed to have a control group and the dogs enrolled on them were receiving medical treatment. Thus, as others treatment protocols for IMHA, further studies are required to correctly evaluate its impact in treating the disease.

Side effects are unusual, but this surgery may induce immune-incompetence and precipitate a secondary infection[126].

7.4. Therapeutic plasma exchange

Therapeutic plasma exchange (TPE) or plasmapheresis is a procedure that uses continuous flow centrifugation or membrane filtration to remove high-molecular-weight substances from the circulation[106,127]. In veterinary medicine membrane filtration is most used, the process implicates blood filtration through a large-pore hollow-fiber plasma separator to hold only cellular blood elements while discarding the plasma, which is then replaced by other fluids such as fresh frozen plasma, synthetic colloids and crystalloids, albumin or a combination of various solutions and then returned to the patient.[106,128]. This process is used to reduce the rate of hemolysis by rapidly removing antibodies, immunocomplexes, and activated complement components contained in the plasma, thus letting the PCV to stabilize, and allowing more time for immunosuppressive treatment to take effect[129]. Still the clinician should keep in mind that the effects are transient seeing that resynthesis of autoantibodies takes place constantly and so their levels will certainly rebound after the procedure which may imply a need for a second or even third TPE[129].

Some reports suggest that plasma exchange may also be helpful in reducing bilirubin levels which is most important in cases where bilirubin encephalopathy develops [127,130].

The technique has begun to be more available in common clinical practice, especially membrane filtration TPE, and has shown promising results, with immunoglobulin levels decreasing has much as 68% in IgG and 75% in IgM levels that could remain lower than pre-TPE levels for as long as 72 hours[127,129,131]. Although, even if results appear promising, these studies have several limitations including small group sizes,

having no control group and using different values for plasma volume exchange (1.0-1.5 is recommended).

In a recent retrospective study, the survival rate of patients receiving TPE in addition to immunosuppressive drugs was compared to a control group treated only medically and the results were 82% and 69% respectively. In conclusion, dogs treated with adjuvant TPE had a similar outcome to dogs treated medically, despite a predisposition toward dogs refractory to initial immunosuppression[128].

Complications may occur, the most common one being hemorrhage from the dialysis catheter site that is commonly moderate and self-limiting[129]. Other complications include filter clotting, hypersensitivity reactions and vomiting due to hypocalcemia which may occur due to the loss of electrolytes when the patients' plasma is discarded[128]. Hypocalcemia may be prevented by implementing a calcium chloride infusion during TPE, which rate may need adjustment during the plasma exchange[128].

Limitations of these treatment include its cost and the availability of the technology[129].

When standard treatments have failed in dogs with refractory IMHA, TPE may be a viable option however more studies are needed to evaluate its efficacy[131].

7.5. Blood transfusion

Transfusion is the preferred method for raising blood oxygen levels in anemic dogs. Because there is no conventional recommendation for when to transfuse, the decision should be determined on individual patient-specific parameters such as disease development speed, current PCV, and the nature and severity of clinical signs[106]. Clinicians seem to agree that a PCV<15% and signs of severe hypoxia (weakness, tachycardia, tachypnoea and pallor) are indicative of the need to perform a blood transfusion[7].

There is no evidence that transfusions are contraindicated. The mortality rate does not differ significantly between dogs who had a blood transfusion and those who did not, which is an unusual finding considering that dogs that receive a transfusion are more likely to be severely affected than those who do not[15,17,58,119].

If the transfusion is the first one the dog is receiving, we should consider it like a transfusion naïve dog that does not have preformed alloantibodies against dog erythrocyte antigen (DEA) 1. Thus, these patients are not normally typed or crossmatched before their first transfusion. However, because individuals with a severe onset are more likely to require multiple transfusions, it is preferred to transfuse DEA1 type-specific blood, hence all patients should be typed before transfusion. However, as this approach is financial and time consuming it could not be viable nor advisable to attempt it. In addition, in IMHA patients, hemolysis, agglutination and erythrocyte fragility can affect the results of both typing and crossmatching[106]. If these techniques are imperative the clinician should seek advice from the manufacturers of test kits[18] .

Literature recommends using packed red blood cells (pRBC) seeing that IMHA patients are typically euvolemic and so plasma would not provide any added benefit and would only increase the volume overload risk as well as the possibility of a transfusion reaction[18,132]. Ideally fresh pRBC should not be older than 7-10 days since they have been linked to an increased risk of complications and mortality[132,133]. If pRBC are not available whole blood may be used and only if the last two products are unavailable bovine hemoglobin solutions (BHS) may be used. This is because BHS scavenges nitric oxide and so potentially activates platelets and causes vasoconstriction, raising the risk of hypertension and, in addition to that, BHS has a higher colloid osmotic (oncotic) pressure than do RBCs, which increases the risk of intravascular volume expansion and hypertension [18]. Still some studies suggest that BHS may exert a positive effect on long term survival [5] while others suggest that it was associated with bad prognosis and a substantial mortality rate [6]. The administration of fresh frozen plasma to dogs with IMHA is not advised due to a lack of evidence of benefit[67].

7.6. Thromboprophylaxis

As previously mentioned, most dogs with IMHA are in a hypercoagulable state and thromboembolic disease appear to be a major factor affecting survival and therefore preventing thrombosis may be as important as controlling hemolysis[59]. Recently it has

been proposed that dogs with platelet counts lesser than 30 000/μL should not receive anti-thrombotic drugs because below this threshold, the risk of spontaneous hemorrhage increases[18].

Thromboprophylaxis should be started as soon as the patient is diagnosed and continued until it is in remission and no longer taking glucocorticoids[106]. IMHA patients appear to be at the highest risk of death during the first two weeks after diagnosis, when the disease is less contoled, and patients are receiving blood products and immunosuppressive treatment that may raise the risk of thrombosis[8].

Dogs with IMHA suffer predominantly from venous thrombus, which are fibrin-rich, and their development is less dependent on platelet number or function. Therefore, a regimen including anticoagulant drugs should be preferred for thromboprophylaxis over antiplatelet drugs[18]. Nonetheless the arterial system may be affected as well so using antiplatelet drugs is not contraindicated[59].

7.6.1. Anticoagulant drugs

a. Heparin

Heparin is a highly sulphated polysaccharide used as a general clinical anticoagulant, its anticoagulant efficacy stems from its ability to block various coagulation cascade components. As a serine protease inhibitor, heparin binds to AT and targets coagulation proteins such as factor Xa and factor IIa (thrombin)[134].

Heparin may be administered intravenously or subcutaneously, however in the latter its bioavailability depends on its molecular weight.

i. Unfractionated heparin

The AT-mediated inhibition of thrombin and factor Xa is possible with unfractionated heparin (UFH). Factors IXa, XIa, VIIa, and XIIa are also disabled by this complex[59]. It has been proven to lower mortality rates among IMHA patients [47,135,136].

Recently the ACVIM proposed initiating treatment at 150-300U/kg SC every six hours and individually adjusting the dose daily on the first days of the disease and

then weekly[18]. For intravenously administration the dose is 100 U/kg bolus and then 900 U/kg every 24 hours.

It is advised that its dose be adjusted individually using an anti-Xa assay, however, if not available, activated partial thromboplastin time (aPTT) or viscoelastic assays might be utilized in order to achieve the target plasma levels and to prevent hemorrhage [18,106,137]. This is based on a small randomized controlled trial where dogs who got individually dose-adjusted UFH therapy showed lower mortality rates and had longer median survival times [18,47]. In this trial, patients required UFH doses between 150 and 566 U/kg every 6 hours to reach the target anti-Xa activities of 0.35–0.7 IU/mL.

Nonetheless the reference values for the anti-Xa are extrapolated from human medicine so more studies will be needed to establish the values for this species and, for the aPTT values clinicians should have in mind that apart from also being extrapolated from human medicine its results may be affected by multiple factors like fibrinogen levels (which are high on IMHA patients)[59].

ii. Low-molecular-weight heparin

In contrast to UFH, low-molecular-weight heparin (LMWH) has a more homogeneous size, a more predictable dose-response relationship, and a longer duration of action[138].

Because the anticoagulant action of LMWH is specifically targeted at activated factor X and has no effect on factor II or other components of the intrinsic coagulation pathway at therapeutic doses, anti-Xa analyses are the primary technique of evaluating the anticoagulant effect of LMWH[138]. The recognized therapeutic range for the anti-Xa assay is 0.5–1U/mL [138], however some suggest that drug monitoring for LMWH may not be needed[139].

While some suggest that because of the positive safety profile of LMWH and the more reliable bioavailability of its products, it may be used instead of UFH[140], others suggest UFH is more effective in preventing thrombus formation [141].

There are two LMWH currently used for thromboprophylaxis:

- Dalteparin: 150-175 U/kg (SC) every 8 hours and

- Enoxaparin: 0.8-1.0 mg/kg (SC) every 6-8 hours

Both have been proven to be safe drugs and to prevent thrombus formation, however more studies will be needed to correctly compare its efficacy to UFH[138,141,142].

b. Rivaroxaban

Rivaroxaban works as an anticoagulant by blocking active factor Xa directly, hence the assay for measuring factor Xa activity can also be used to indirectly measure plasma rivaroxaban levels. Measuring prothrombin time and viscoelastic assays like thromboelastography may also be useful[143].

Rivaroxaban is a new drug that has been recently used in human medicine after major orthopedic surgery, for stroke prevention in patients with atrial fibrillation, and for the treatment of acute coronary syndromes in an effective and safe way [144].

The recommended dosage is 1-2 mg/kg every 24 hours PO[18,136].

There is still insufficient data to compare rivaroxaban to other anticoagulant protocols in IMHA patients, however the drug has proven to be safe and well tolerated in dogs[143–145]. One study compared treatment with dalteparin with rivaroxaban in two dogs with venous thromboembolism secondary to IMHA[146]. Both dogs resolved their clinical symptoms and coagulation profiles after initiation of rivaroxaban but did not under the treatment with dalteparin. This could be because heparin needs AT to inactivate factor Xa, but rivaroxaban does not. Additionally, by inactivating both factor Xa and the factor Xa-prothrombinase-complex, rivaroxaban reduces thrombin production more effectively than heparin[146].

Rivaroxaban has other major advantage as it also has antiplatelet properties which in IMHA patients is important seen that their platelets circulate activated[144].

7.6.2. Antiplatelet drugs

As previously stated, platelet activation is enhanced in IMHA patients, thus antiplatelet medications such as aspirin and clopidogrel are appealing

thromboprophylactic treatments because they are easy to use and do not require intense monitoring[59].

a. Aspirin

Ultra-low doses of aspirin (ULDA) inhibits platelet thromboxane A2 generation (a strong platelet agonist) while retaining COX-1 dependent prostacyclin (an antithrombotic) production from endothelial cells[59]. Aspirin is an effective antithrombotic drug because a single dose can impair platelet response for up to one week (the life span of the platelet)[5].

The recommended dosage is 1-2 mg/kg every 24hours and could be associated with clopidogrel[18].

Aspirin is a safe drug at ultra-low doses, however several studies show it can be deleterious especially to the gastrointestinal tract. While one study suggested that administration of prednisone with ultra-low dose aspirin may only lead to mild, self-limiting diarrhea in some dogs[147] other suggests that aspirin administration can induce gastric ulceration and lesions on the intestine in a dose-dependent manner, as well as increase intestinal permeability and decrease barrier function recovery after ischemic insult, which is especially concerning in IMHA patients due to the local ischemic effects of marked anemia.[148].

Nonetheless aspirin has been proven to improve survival time on IMHA patients with one study even suggesting it may be more effective than heparin in preventing thrombus formation [5]. However, as usual, this study has its limitations.

b. Clopidogrel

Clopidogrel inhibits the platelet P2Y12 ADP receptor in an irreversible manner. ADP is an effective platelet activator[59].

Literature suggests clopidogrel should be used preferably than aspirin as it is more effective in preventing arterial thrombosis, however there is no evidence that clopidogrel is effective in preventing venous thrombosis in dogs[18,136,149].

The recommended dose is 1.1-4.0 mg/kg PO every 24 hours[18].

Clopidogrel has been proven to be a safer drug than aspirin because in contrast to the latter, clopidogrel does not cause gastrointestinal hemorrhage in dogs, and its administration with prednisone does not exacerbate gastric lesions or cause clinicopathologic alterations[150].

In a randomized trial, dogs with IMHA that where given clopidogrel had comparable short-term survival to dogs given ULDA or clopidogrel combined ULDA[151].

7.7. Supportive therapy

Symptomatic and supportive treatment is recommended in some patients.

Fluid therapy is an important part of the supportive treatment especially on patients with evidence of dehydration, and in those with intravascular hemolysis in order to prevent kidney damage due to hemoglobinuria. It is recommended that the smallest peripheral catheter required should be inserted in the patient and that the site be checked for thrombophlebitis daily[2].

Gastroprotectant treatment is recommended in patients that have evidence of ongoing gastrointestinal ulceration/bleeding (i.e., melena) or, in patients with other risk factors like concurrent hepatopathy, inflammatory bowel disease, or pancreatitis[18]. Proton pump inhibitors like pantoprazole or omeprazole are indicated during the risk period or until clinical signs disappear[106]. Given that stomach acidity is required for the formation of the active MMF metabolite, proton pump inhibitors may reduce its efficacy. If proton pump inhibitors are required for a medical reason, injectable MMF can be given during the concurrent usage period[106].

Administration of efficacious antimicrobial drugs may be recommended seeing that there is data of connection between some infectious agents and IMHA[19,33,35]. Clinicians should conduct a patient-specific risk assessment that considers client and patient lifestyle characteristics, geographic region, and travel history[106]. High-risk patients should be treated empirically in the absence of diagnostic test findings, and when test results are available, they should be used to guide longer-term antimicrobial treatment[18].

7.8. Novel and emerging therapies

Novel therapies are emerging in order to better manage IMHA.

In one case report, immunoadsorption (IA) was used to treat a female patient unresponsive to immunosuppressive treatment and who required repeated blood transfusions[152]. Hemolysis in this patient stopped immediately after IA despite some adverse effects occurring during the procedure. However further prospective, controlled studies are needed in order to determine if IA can be safely and effectively used in dogs with IMHA[152].

An interventional trial to test a new inhibitor of complement for the management of canine IMHA (C1 esterase inhibitor - C1-INH) is now underway[153]. Because intravascular destruction of erythrocytes by complement activation is highly inflammatory, prothrombotic, and results in a significant reduction in blood oxygen carrying capacity, and hence a worse prognosis, this new drug could transform how we manage canine IMHA patients[153].

As IMHA still remains a clinical challenge more alternative therapies will continue to emerge in order to properly treat this disease.

Since determining the most adequate treatment proposes a challenge, recently the ACVIM introduced a diagram to help with this matter, as seen on Figure 7:

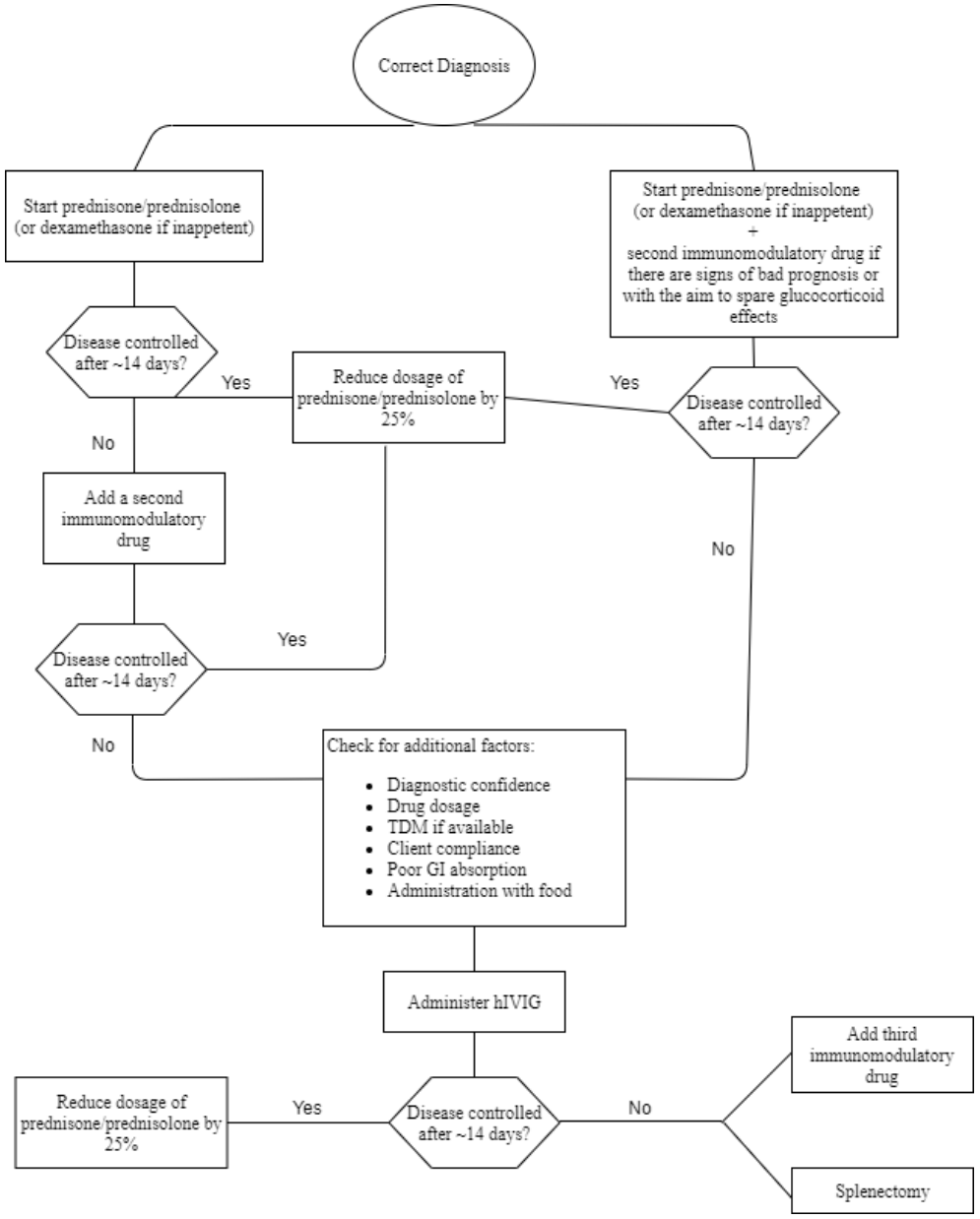


Figure 7: Treatment diagram adapted from ACVIM 2019 consensus [18].

8. Approach to relapse

It is possible that after a patient is discharged and the disease appears controlled a relapse may occur[3].

When a patient returns with a worse condition the first thing the clinician should do is to look for a trigger factor, especially secondary infections that may emerge due to glucocorticoid usage. If there is a trigger factor it should be treated[18].

If the relapse occurred before reducing the dosage of glucocorticoids a second immunosuppressive drug must be added to the treatment protocol, and if the patient is already receiving a second immunosuppressive drugs TDM must be performed, if available[18].

If the relapse occurred when an immunosuppressive drug was being tapered, its dosage should be increased, until remission is established[18]. After that the tapering process may recommence but more gradually.

If recurrent relapses occur, the patient may need lifelong immunosuppressive treatment, attempting to sustain remission with the least amount of immunosuppressive medication possible or, a splenectomy may be considred[18].

9. Prognosis

Several studies have recognized factors for risk of death in patients with IMHA, especially in pIMHA, seeing that in sIMHA treating the underlying condition properly is normally efficient.

Different factors have been associated with a worse prognosis but studies recognize three of them as being the best markers for IMHA severity:

- hyperbilirubinemia (related to intra-vascular hemolysis rate and impaired liver function)[3,5,10,11,15,84,107];
- increased ALP activity [3,7,84] and
- elevated plasma urea concentration (BUN) [8,10,107]

Other markers associated with a bad prognosis are:

i. persistent autoagglutination [5,8,11,15];
ii. severe thrombocytopenia [5,8,11,15];
iii. elevated number of band neutrophils [5,8,11,15];

iv. presence of petechia [5,8,11,15];

v. abnormal coagulation indexes (PT, APTT and FDP) [5,8,11,15];

vi. positive IgM titer[5,8,11,15].

Only two studies relate the degree of anemia to the mortality rate [11,15]. Blood lactate concentration has been proposed as a prognostic factor related to a higher mortality, however it is insufficient for an unequivocal prognosis[84]. Future studies that monitor blood lactate levels during the first 48hours of hospitalization will offer more information for their prognostic values. Likewise, serum IL-17 levels may be a predictive factor, as seen in a recent study where dogs that survived acute hospitalization had a decrease in its plasma concentrations[154]. This implies that blood IL-17 concentrations in dogs with IMHA could be used to predict illness severity and responsiveness to treatment[154].

Some have even evaluated prognostic factors for the development of thromboembolism in IMHA patients with low albumin levels, hyperbilirubinemia and thrombocytopenia being markers of a worse prognosis.

On the other hand, a marked reticulocytosis and the presence of spherocytes are related to a better prognosis[7]. The latter owing to the fact that spherocyte formation occurs in extravascular hemolysis which as a better prognosis in relation to intravascular hemolysis.

A few studies have attempted to establish a scoring system for IMHA. One of them, developed in Tokyo that used sex, seasonality, PCV, total plasma protein and platelet count as a prognostic scoring system [11] seemed inefficient when applied in an Australian population [12] which implies that scoring systems developed for a specific population may be of no utility on other populations. The other one, developed at the British Isles, using a combination of the American Society of Anesthesiologists' clinical grade with the plasma levels of urea, creatinine and bilirubin was more accurate at predicting mortality. Unfortunately, the authors did not provide their own model in the same user-friendly score format[10]. This last scoring system has still not been used to predict the prognosis of a different population than the one it has been developed for.

10.Conclusion

Despite being a common pathology for a long period IMHA remains a clinical challenge and is often associated with a bad prognosis[6–8].

A thorough patient evaluation must be made in order to properly separate secondary etiologies from an idiopathic event[19]. Specificity and sensibility of the several diagnostic tools must be considered[78,87,88,93,101].

The first weeks of treatment are crucial for patient survival and regular monitoring after discharge must be performed in order to detect relapses as soon as possible[18].

Despite having several deleterious side effects glucocorticoids remain the pillar for treating IMHA[5,106]. Their combination with other immunosuppressive and immunomodulatory drugs could reduce mortality rates however reports studying different therapeutic approaches are difficult to compare due to the small number and variety of the populations enrolled, the difference of the adopted treatment protocols and the lack of long-term follow-up of patients among other factors[106,114–116,120,125]. As pulmonary thromboembolism remains the most common cause of death, thromboprophylaxis is of vital importance in the development of the disease[59,135].

Novel pharmaceutical options are being developed which could mean a reduction in mortality rates, nonetheless more studies will be needed to assure their efficacy and security[152,153].

More studies with standardized enrollment criteria and consistent treatment protocols will be necessary for a better understanding of the disease.

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