CPD article

Immune-mediated haemolytic anaemia, part 1: pathophysiology and diagnosis

Immune-mediated haemolytic anaemia is a relatively common condition in dogs, and uncommonly encountered in cats. Prompt and appropriate recognition is paramount to optimal management and improved health outcomes. Currently, no single test has been proven to be definitively diagnostic for immune-mediated haemolytic anaemia. Therefore, a logical and thorough approach is key to achieving an accurate diagnosis.

Lara Brunori DVM CertAvP(ECC) MRCVS, **Nora Romero-Fernandez** DVM DipACVIM (SAIM), DipECVIM-CA (Internal Medicine) MRCVS, Vets Now Emergency and Specialty Hospital Glasgow, 123-145 North Street, Glasgow, G3 7DA, UK; noraromerofernandez@gmail.com

Immune-mediated haemolytic anaemia (IMHA) is a frequent cause of anaemia and is one of the most common autoimmune conditions diagnosed in dogs (Goggs et al, 2015). In cats, IMHA is an uncommon cause of anaemia (Swann et al, 2016), with disorders such as infections and neoplasia appearing to be more prevalent causes (Korman et al, 2013). However, IMHA in cats can still result in a severe and life-threatening condition (Swann et al, 2016).

Generally, IMHA has been categorised into primary (idiopathic) or secondary. The American College of Veterinary Internal Medicine (ACVIM) consensus statement on the diagnosis of IMHA in dogs and cats (Garden et al, 2019) proposed the designation of 'non-associative' for 'primary' and 'associative' for 'secondary' IMHA. The rationale behind this suggested terminology is that the term 'primary' would imply all triggers to have been definitively ruled out, while 'secondary' would indicate a proven causality between concurrent pathologies. In practice many cases may be ambiguous, so the term 'associative' describes cases of IMHA in which one or more comorbidities are identified, whereas 'non-associative' implies that no other disease has been identified with the diagnostic tests used.

Non-associative or primary IMHA is the most common form of IMHA in both dogs and cats (Swann et al, 2016). It typically affects young adults to middle aged animals (Piek et al, 2008), and some dog breeds, such as Cocker Spaniels, English Springer Spaniels, Poodles and Old English Sheepdogs, appear to be overrepresented (Miller et al, 2004).

Associative or secondary IMHA occurs when an underlying disease (such as neoplasia or infections), or exposure to exogenous substances (including drugs, toxins and vaccines) accompanies the presence of antibodies targeting epitopes of red blood cells (Piek et al, 2008). In some cases the anaemia is responsible for initial presentation, while in others IMHA can develop when the animal is already being treated for another condition, such as pancreatitis, and this can be responsible for a sudden deterioration.

Regardless of its classification, animals with IMHA usually present with clinical signs including lethargy, inappetence, pigmenturia, tachycardia, pale mucous membranes and fever (Piek et al, 2008). Diagnosis is based on the demonstration of haemolysis and red blood cell-bound immunoglobulins (Piek et al, 2008) in the anaemic animal.

Treatment differs between cases of non-associative (primary) and associative (secondary) IMHA. While the essential therapeutic aim in non-associative IMHA is to control the aberrant immune response, treatment for secondary IMHA should be directed towards the putative underlying cause, and immune modulation may or may not be required.

Currently, IMHA remains a potentially fatal disease, with reported mortality rates for primary/non-associative IMHA ranging from 29–70% in dogs (Klag et al, 1993; Piek, 2011) and between 24–40% in cats (Kohn et al, 2006; Swann et al, 2016).

Pathogenesis

Normally, canine red blood cells have a life span of 100–120 days (McCullough, 2003) while feline red blood cells remain in circulation for approximately 72 days (Eibert and Lewis, 1997). Once senescent, red blood cells are removed from the circulation via the mononuclear phagocytic system in the liver and spleen
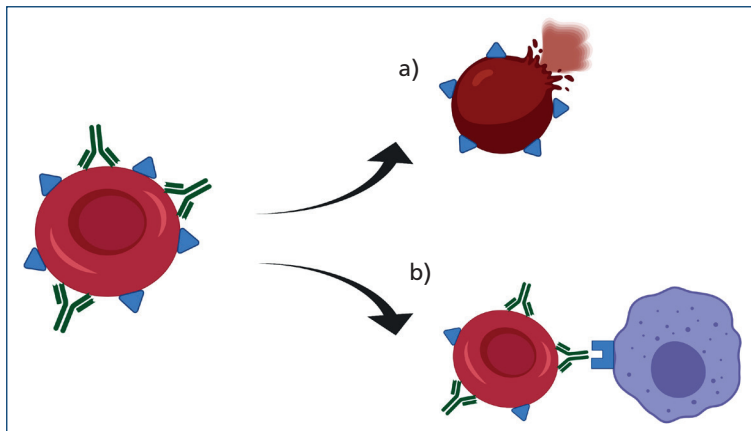
Figure 1. Red blood cells coated with antibodies and complement can a) undergo direct intravascular lysis or b) be uptaken by mononuclear phagocytic system macrophages. Created with BioRender.com
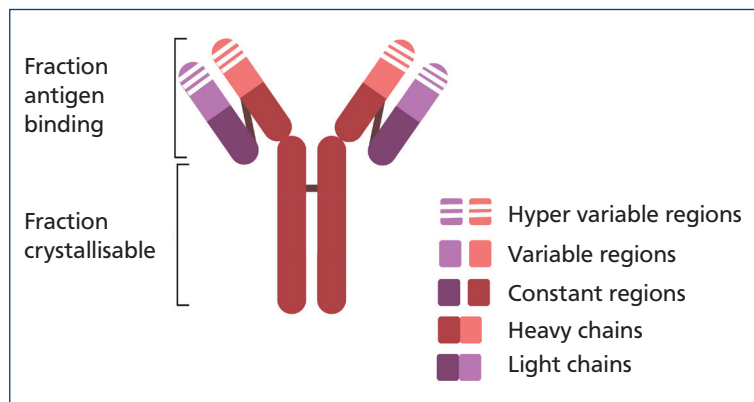

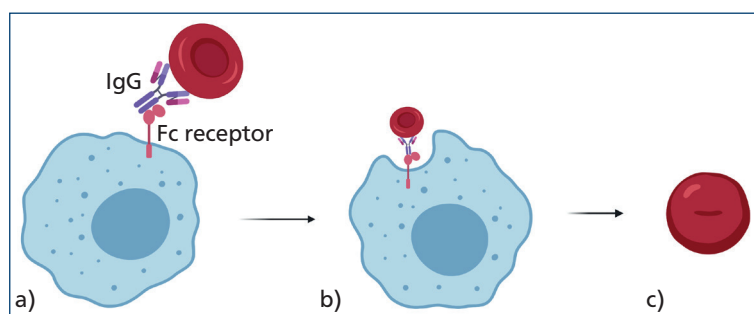*Figure 2. Schematic illustration of the immunoglobulin G structure.*

Figure 3. Interaction between immunoglobulin G and mononuclear phagocytic system macrophage: a) the crystallisable fragment portion of the immunoglobulin G interacts with macrophage's crystallisable fragment-receptor; b) the binding between crystallisable fragment and crystallisable fragment-receptor triggers phagocytosis; c) partial phagocytosis is responsible for the formation of circulating spherocytes.

(McCullough, 2003). IMHA is characterised by premature destruction of red blood cells (McCullough, 2003).

Specifically, IMHA consists of a type II hypersensitivity reaction where red blood cells become coated with antibodies, complement or both. This leads to erythrocyte destruction either by direct lysis (intravascular haemolysis) or by enhanced mononuclear phagocytic system uptake (extravascular) (*Figure 1*).

The degree and site of haemolysis depend on the type and load of immunoglobulins and complement cascade activation. Both immunoglobulin G (IgG) and M (IgM) have been detected on the surface of red blood cells in dogs and cats with IMHA (Morley et al, 2008). IgG-mediated destruction seems to be the most frequent mechanism involved in dogs (Harkin et al, 2012), and IgM have been isolated more frequently in cats (Kohn et al, 2006).

IgG are monomeric immunoglobulins (*Figure 2*) that cause haemagglutination at very high concentrations (Harkin et al, 2012). Thus, autoagglutination is not a common feature for IgG-coated red blood cells.

IgG mainly cause extravascular haemolysis via the mononuclear phagocytic system, which recognises and binds IgG's crystallisable fragment region (McCullough, 2003) as shown in *Figure 3*.

IgM are pentameric immunoglobulins that can easily promote haemagglutination (*Figure 4*) and associated significant intravascular, albeit mainly extravascular haemolysis.

IgM-coated red blood cells can also contribute to complement activation (Harkin et al, 2012). The membrane attack complex causes immediate intravascular lysis, creating holes in the red blood cell membrane and allowing uncontrolled influx of water and electrolytes into the cell, which subsequently swells and ruptures (McCullough, 2003). The C3b-membrane complex (opsonin) can be recognised by splenic macrophages, leading to partial phagocytosis and the production of circulating spherocytes (McCullough, 2003) (*Figure 5*). Spherocytes are ultimately lysed within the spleen or liver because of their increased rigidity.

Signalment

Primary or non-associative IMHA presents in young to middle aged dogs with a median age of 6 years (Piek, 2011). Female dogs appear to be overrepresented (Miller et al, 2004), and the effect of the neuter status remains controversial (Carr et al, 2002; McCullough, 2003; Weinkle et al, 2005; Goggs et al, 2015; Sundburg et al, 2016). Pregnancy-associated IMHA is a well-known occurrence in humans (Piatek et al, 2015) but, to the authors' knowledge, presumed cases have so far been reported only in one pregnant dog (Fernandez et al, 2020) and one pregnant cat (Kopke et al, 2019).

Cocker Spaniels have been consistently recognised as an overrepresented breed; one study reported this breed to be 12.2 times more likely to be diagnosed with IMHA than any other breed (Miller et al, 2004). An Australian study reported Maltese Terrier dogs, Springer Spaniels, Hungarian Vizslas and Airedale Terriers to be overrepresented (McAlees, 2010), while a US-based article reported IMHA to be significantly more prevalent in Poodles, Miniature Schnauzers and Collies (Reimer et al, 1999). These diverging data might suggest that geographical location and exposure to different environments or different breed lines could also play a role in the reported prevalence of IMHA among different dog breeds.

Canine blood types have also been investigated, and dog erythrocyte antigen 7 positivity has been suggested to have a protective effect in Cocker Spaniels in one study (Miller et al, 2004). However, only 10 Cocker Spaniels with IMHA were recruited in that study.

In cats, secondary or associative IMHA was previously thought to be more common than primary IMHA (Scott et al, 1973; Werner and Gorman, 1984). However, a more recent study involving 107 cats reported that a minority of cats with IMHA (32.7%) were diagnosed with a concurrent putative cause (Swann et al, 2016). The same study showed that cats between 2 and 6 years old were twice as likely to develop primary IMHA. Male cats were previously believed to be overrepresented (Kohn et al, 2006), but this was not supported by Swann et al (2016). No feline breed predisposition has been identified so far (Swann et al, 2016).

Clinical presentation

Most animals with IMHA present with clinical signs attributable to anaemia and tissue hypoxia (McCullough, 2003). Common physical exam findings include pale mucous membranes, tachycardia, tachypnoea, hyperdynamic peripheral pulses and systolic murmurs. However, a minority of cases have a much slower disease course, and may have non-specific signs like anorexia, lethargy, a slowly developing exercise intolerance and weakness, which might be accompanied by icterus and discolouration of urine and faeces (Piek, 2011). Fever and concurrent gastrointestinal signs are reported in 46% and 30% of dogs, respectively (Piek et al, 2008).

Icterus in cats with IMHA appears to be uncommon, with an incidence of 10% (Kohn et al, 2006).

A minority of dogs presents with clinical signs secondary to thrombocytopenia (petecchiae, ecchymosis, epistaxis, melaena) (Orcutt et al, 2010), and this is less common in cats (Kohn et al, 2006).

Diagnosis

Demonstration of anaemia

This is more reliably done with a manual packed cell volume, rather than with a calculated haematocrit, especially if agglutination is present (Zandecki et al, 2007). In some cases the reference values vary; neonates (O'Brien et al, 2014) and pregnant dogs (Allard et al, 1989; Kimura and Kotani, 2018) normally have lower packed cell volume; while some sighthounds (Zaldívar-López et al, 2011) and Dachshunds (Torres et al, 2014) have higher red blood cells counts.

Assessment of regeneration

Reticulocytes are anucleated immature red blood cells which still contain ribonucleic acid (RNA). They can be identified in a blood smear with new methylene blue stains (which precipitate the RNA, highlighting it as cytoplasmic blue clumps). Reticulocytosis is an increase in the number of circulating reticulocytes above the reference interval, which reflects a physiological response of the bone marrow to persistent renal cortical hypoxia (Nitsche, 2004), and subsequent elevated erythropoietin concentration (Cowgill et al, 2003), leading to increased proliferation and differentiation of erythroid precursors.

Reticulocyte count is the most accurate means of quantifying the degree of regeneration (Church, 2017). A reticulocyte percentage above the reference interval is interpreted as regeneration. However, reticulocyte percentage may not be
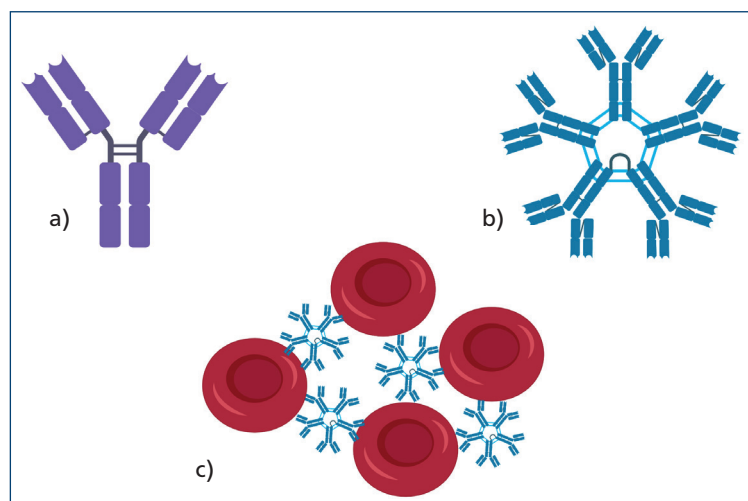

Figure 4. a) Monomeric immunogloblin G. b) Pentameric immunogloblin M. c) Immunogloblin M-mediated agglutination. Immunogloblin M provide multiple antigen-binding domains which can link to multiple red blood cells at the same time and easily result in haemagglutination.
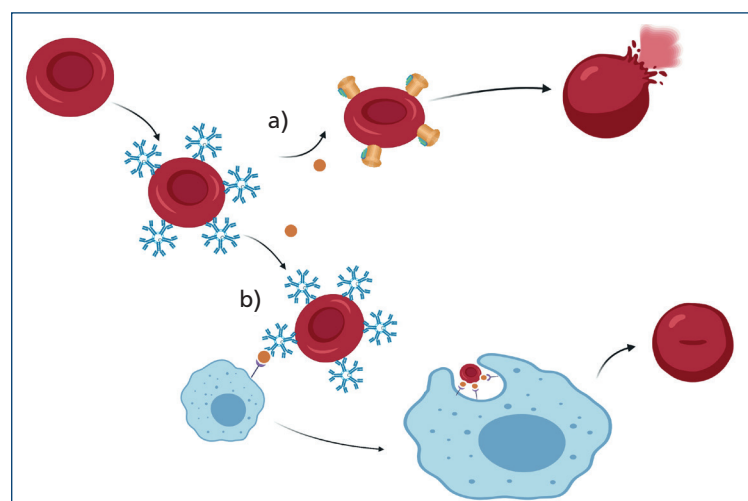

Figure 5. The complement cascade can be activated by immunogloblin M-coated red blood cells. Activated complement components can a) form the C5b6789 or lytic/membrane attack complex and cause intravascular haemolysis or b) be recognised by mononuclear phagocytic system macrophages and contribute to extravascular haemolysis with spherocytes formation.

useful to assess if the degree of regeneration is adequate as it does not consider the severity of anaemia. Means of assessing the adequacy of the degree of regeneration include the absolute reticulocyte count, which is provided by many haematology analysers or may be calculated. This can be done by calculating reticulocyte % × red blood cell count (millions/μl)×10, or the corrected reticulocyte percentage: reticulocyte % × animal's haematocrit or packed cell volume ÷ 'normal' haematocrit or packed cell volume (Cowgill et al, 2003).

In cats there are two types of reticulocytes. Aggregate reticulocytes are reliable indicators of recent regeneration (appearing in circulation after approximately 48 hours) and are the only type that should be included in the reticulocyte count.
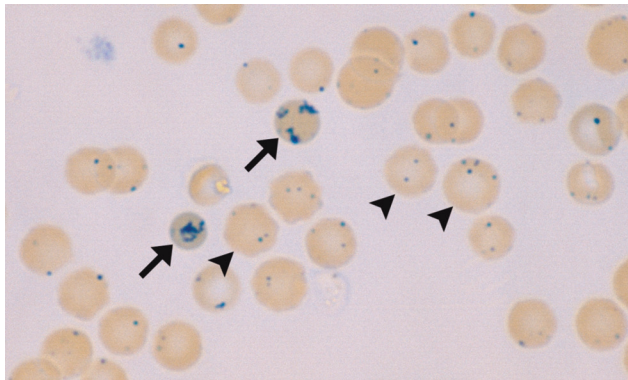
Figure 6. Blood smear from a feline patient with immune-mediated haemolytic anaemia. New-methylene blue stain. Aggregate reticulocytes present with large dark blue intracellular clumps of ribonucleic (arrows) while punctate reticulocytes have small amounts of ribonucleic acid that show as little blue intracellular dots (arrowheads).

Figure 7. Blood smear from a dog with immune-mediated haemolytic anaemia. Romanowsky-type stain. Nucleated red blood cells (arrow) and Howell-Jolly bodies (arrowheads).

Aggregate reticulocytes develop into punctate reticulocytes with time, and can remain in circulation for 14–21 days, so they should not be considered as a sign of recent regeneration (Paltrinieri et al, 2018). Feline aggregate and punctate reticulocytes are shown in *Figure 6*.

Reticulocytes can also be observed as polychromatophils on films stained with Romanowsky-type stains (such as Diff-Quik), which appear more intensely blue stained and larger than mature red blood cells. In one study, polychromasia was deemed the most reliable regenerative sign in dogs, when reticulocyte count was not available (Hodges and Christopher, 2011).

Other indicators of regeneration on smear include macrocytosis (Hodges and Christopher, 2011), Howell–Jolly bodies and nucleated red blood cells (Cowgill et al, 2003), as shown in *Figure 7*.

One third of dogs, and even more cats, with IMHA may lack signs of regeneration on presentation (McCullough, 2003; Piek, 2011; Swann et al, 2016). This may be the result of a pre-regenerative state, as it can take 2–5 days for the bone marrow to mount a regenerative response (Grimes and Fry, 2015). Alternatively, in cases in which the regenerative response appears poor after a few days, consideration should be given to bone marrow affection, including precursor-mediated IMHA (non-regenerative IMHA or precursor immune-mediated anaemia), marrow damage secondary to hypoxaemia, or coexisting bone marrow disease. In these animals, investigation of concurrent bone marrow pathologies should be considered. Weiss (2008) described bone marrow involvement associated with non-regenerative IMHA and concluded that multiple mechanisms may contribute to the lack of erythroid regeneration. Some cases of non-regenerative IMHA are caused by the immune-mediated destruction of bone marrow erythroid precursor cells (pure red cell aplasia), while others may show a variety of intrinsic bone marrow changes including dysmyelopoiesis, myelonecrosis and myelofibrosis. The latter population frequently presents with concurrent neutropaenia and thrombocytopenia, and the 60-day survival rate appears to be lower (Swann and Skelly, 2016).
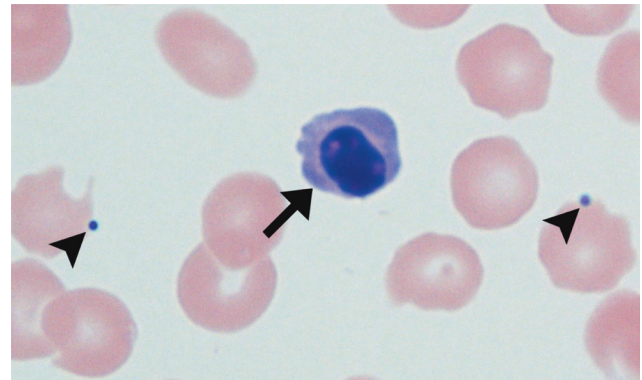
It is fair to conclude that immune-mediated anaemias are a spectrum of related disorders, ranging from pure red cell aplasia, in which the immune system targets early erythroid precursors, to regenerative IMHA in which the target is mature circulating erythrocytes. However, these may occur concurrently in the same animal (Assenmacher et al, 2019).

If a regenerative response has been documented, blood loss should be excluded. *Figure 8* illustrates a suggested approach to the animal with regenerative anaemia. It must be noted that while in the majority of cases only one pathophysiological mechanism may be involved, there are some exceptions where features of haemorrhage overlap with haemolysis, such as in cases of ruptured haemangiosarcomas. It is also worth noting that animals with chronic blood loss frequently present with poor (if any) signs of regeneration.

Signs of haemolysis

Once blood loss has been excluded, signs of haemolysis should be determined to further support management of IMHA.

Spherocytes (*Figure 9*) can be observed on blood smears as small, round, intensively stained red blood cells lacking the typical central pallor (Balch and Mackin, 2007). Spherocytes are considered to be evidence of extravascular immune-mediated erythrolysis, as they are the result of partial phagocytosis.

Five or more spherocytes per high power field showed a sensitivity of 63% and a specificity of 95% for the diagnosis of IMHA, compared to a lower cut-off value of ≥3 spherocytes per high power field (sensitivity of 74%, specificity of 81%) (Paes et al, 2013).

Although typical, spherocytes are not pathognomonic for IMHA as they can also be found in conditions like snake envenomation (Lenske et al, 2018), bee stings (Nair et al, 2019) and inherited red blood cell enzymatic deficiencies (Slappendel et al, 2005).

Spherocytes are not reliably identified in feline smears because normal red blood cells are smaller and lack a well-defined central pallor (McCullough, 2003). As a result, the osmotic fragility test has been proposed as an additional tool to diagnose IMHA in cats (Kohn et al, 2006). An increased osmotic fragility is not
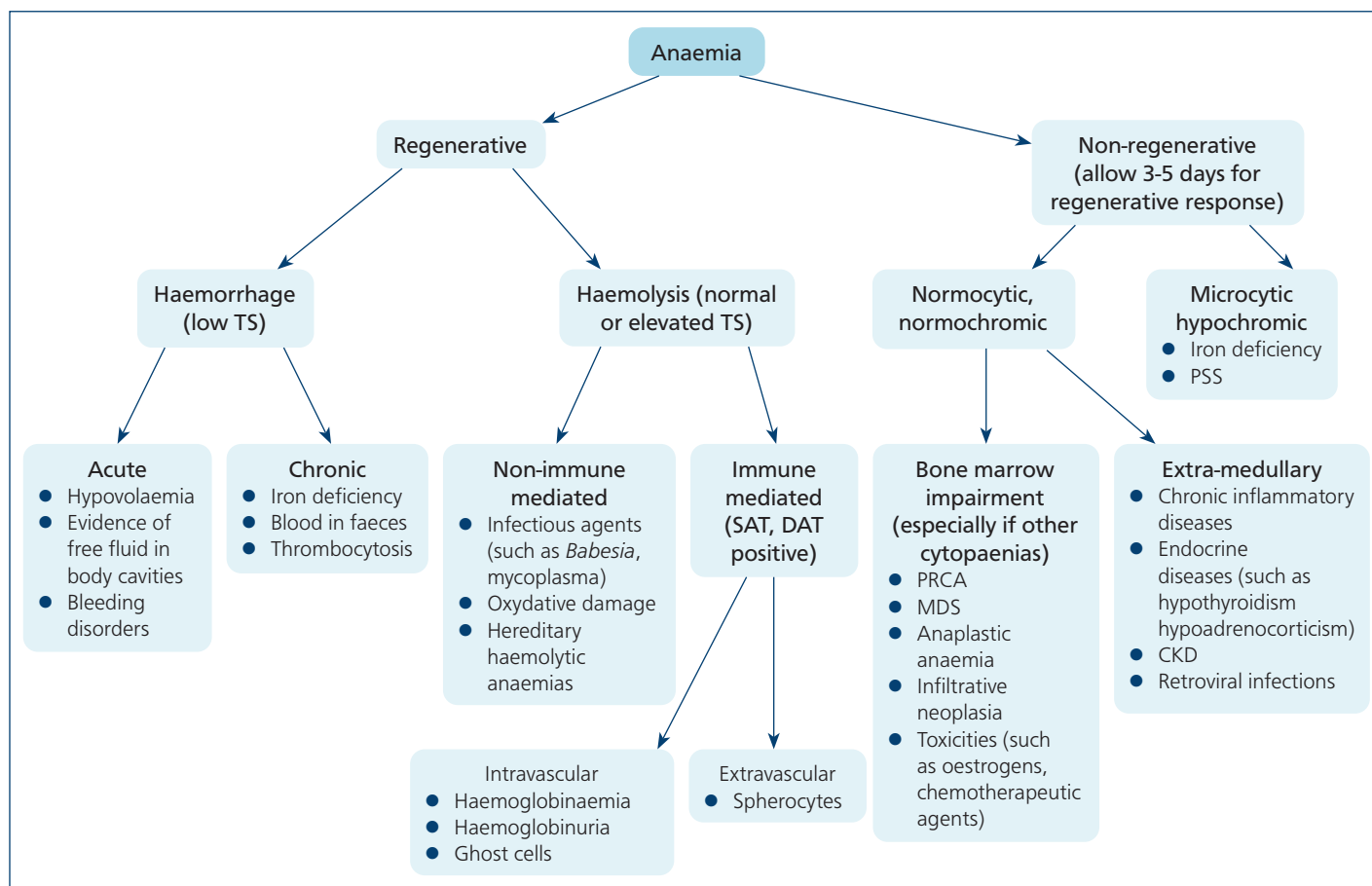
Figure 8. Systematic approach to the patient presented with anaemia. CKD chronic kidney disease, DAT direct antiglobulin test, MDS myelodysplastic syndrome, SAT saline agglutination test; TS total solids; PRCA pure red cell aplasia; PSS porto-systemic shunt.

pathognomonic of IMHA, but may support it. Osmotic fragility may also be abnormal in pyruvate kinase and spectrin deficiency, azotaemia, zinc intoxication (Slappendel, 1998; MacNeill et al, 2019), hypophosphataemia (Adams et al, 1993), infectious and inflammatory diseases (Ottenjann et al, 2006).

Ghost cells are considered a sign of intravascular haemolysis, as they result from partially lysed erythrocytes that retain their basic cytoskeletal structure (Swann et al, 2016), but these may represent an in-vitro artefact in samples not promptly examined (MacNeill et al, 2019).

Urinalysis may show bilirubinuria and/or haemoglobinuria. In dogs, mild bilirubinuria can be a normal finding, especially in concentrated samples from male dogs (Piech and Wycislo, 2019).

Haemoglobinuria occurs when there is excess free haemoglobin in plasma after intravascular haemolysis (Piech and Wycislo, 2019).

With extravascular haemolysis, erythrocytes are degraded within macrophages and free haemoglobin is not released into the circulation. Hence, haemoglobinaemia or haemoglobinuria do not occur, unless concurrent intravascular haemolysis is present (Tasker, 2012).

In some cases, haemoglobin oxidises, becoming methaemoglobin and leading to brown discoloured urine (Piech and Wycislo, 2019).

Demonstration of an immune-mediated component

Although most cases of haemolysis in dogs and cats are immune-mediated, there are instances in which it is not (Fleishman, 2012). Enzymatic abnormalities, like phosphofructokinase or pyruvate kinase deficiencies, that compromise the integrity and resilience of red blood cells have been reported in both dogs and cats (Owen and Harvey, 2012), as well as severe oxidative damage to red blood cells, secondary to zinc (Volmer et al, 2004; Blundell and Adam, 2013) or onion (Tang et al, 2008) toxicity. Additionally, mechanic microangiopathic trauma to red blood cells can occur in cases of haemangiosarcoma (Hammer et al, 1991).

The saline agglutination test is a readily available test that can be performed in-house (*Figure 10*). If false positives are suspected (such as high total proteins or fibrinogen serum concentration), repeating the test, after washing erythrocytes three times in a 1:4 ratio with saline, is recommended (Caviezel et al, 2014). The test should be performed at 37°C, as erythrocytes from normal dogs have been reported to spontaneously agglutinate at lower temperatures (Garden et al, 2019).

The Coombs test, or direct antiglobulin test, uses species-specific antibodies to detect immunoglobin and/or complement bound to erythrocytes (MacNeill et al, 2019). Increasing dilutions of polyvalent or monovalent antibodies are incubated with the animal's red blood cells, and the test is considered positive when
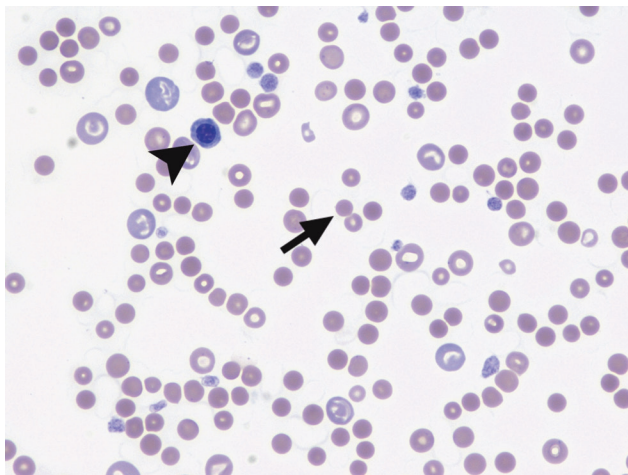
Figure 9. Blood smear from a dog with immune-mediated haemolytic anaemia (Wright Giemsa stain). Spherocytes (arrow) can be distinguished from normal red blood cells as they are smaller, spherical and lack central pallor. A decreased red blood cell density with moderate anisocytosis, polychromasia and a nucleated red blood cell (arrow head) can also be appreciated.
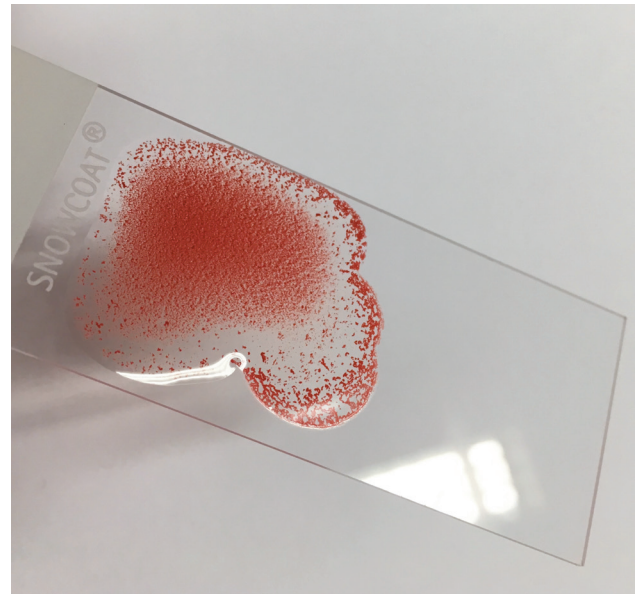

Figure 10. A macroscopically positive in-house saline agglutination test from a dog with immune-mediated haemolytic anaemia.

agglutination occurs (MacNeill et al, 2019) as shown in *Figure 11*. Specific laboratories may consider low positive Coombs titres (1:2) to be of unclear significance, although there is no reliable evidence to support this. Results should be interpreted with caution, and communication with the laboratory is recommended. As for any test result that does not correlate with the clinical picture, repeating the test must be considered to try to reduce the possibility of spurious results.

Reported Coombs test sensitivities are between 37% (Reimer et al, 1999) and 77% (Carr et al, 2002; Weinkle et al, 2005) in dogs and 82% in cats (Tasker et al, 2010), while specificity ranges between 94 and 100% in dogs (Overmann et al, 2007; Paes et al, 2013; Caviezel et al, 2014) and 95 and 100% in cats (Kohn et al, 2006; Tritschler et al, 2016). Coombs test accuracy is thought to be impaired by immunosuppressants, blood transfusions and by
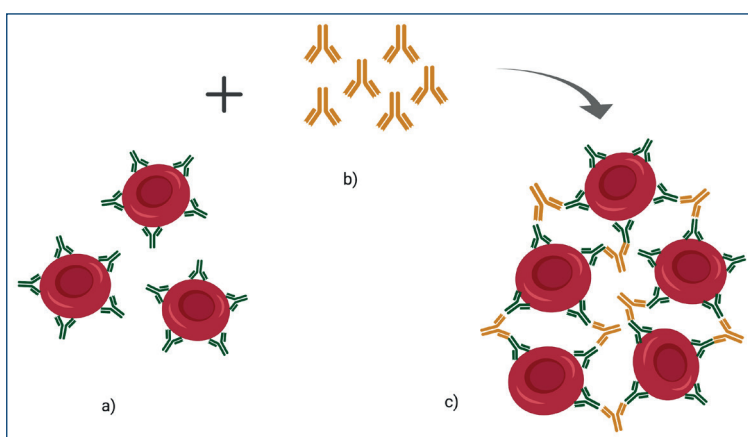

Figure 11. Illustration of a positive direct Coombs' test showing a) animal's antibody-coated red blood cells, b) incubation with species-specific antibodies against immunogloblin G, immunogloblin M or Coomb's reagent, c) agglutination (positive result). Created with BioRender.com.

prolonged blood sample storage. However, one study demonstrated that dogs with IMHA, treated with immunosuppressants, remained positive until clinical remission was achieved; blood transfusions received 1–21 days before testing did not affect direct antiglobulin test status, and preserving ethylenediaminetetraacetic acid samples at 4°C did not affect results (Caviezel et al, 2014).

Flow cytometry can also be applied to detect immunoglobulins bound to red blood cells. In dogs, the test was reported to be 100% sensitive, 87.5% specific (Wilkerson et al, 2000) and have positive and negative predictive values of 70–100% and 93–100% respectively (Quigley et al, 2001; Morley et al, 2008). One study, although limited by a small population, provided encouraging results for the use of flow cytometry in cats with IMHA (Limlenglert et al, 2011).

Evaluation of concurrent diseases

The most common pathologies associated with IMHA in the literature include:

- Infectious diseases
- Neoplasia
- Inflammatory diseases
- Drugs
- Toxins
- Vaccines.

Infectious diseases

Although recent evidence suggests that any infection can trigger an immune dysregulation and loss of immune tolerance in predisposed individuals (Rivera-Correa and Rodriguez, 2018), specific microorganisms have been proven to trigger anti-erythrocyte immunoglobulin synthesis.

Haemoparasites such as *Babesia gibsoni* in dogs (Máthé et al, 2006), and *Babesia felis* (Schoeman et al, 2001) and *Mycoplasma haemofelis* in cats (Weingart et al, 2016) have been reported as potential causes of IMHA.

Coombs-positive anaemias have also been observed with *Leishmania* spp., *Dirofilaria immitis* and *Bartonella* spp. in dogs and feline leukaemia virus-infected cats, but evidence showing the production of auto-antibodies in these cases is currently lacking (Garden et al, 2019).

Neoplasia

In humans, IMHA is a recognised paraneoplastic syndrome (Ugoeke et al, 2017; Yu et al, 2017), but this remains unclear in dogs and cats. Veterinary literature is limited to single case reports of dogs with IMHA and concurrent intestinal lymphoma (Prihirunkit and Chootesa, 2016) and leiomyosarcoma (Yuki and Naitoh, 2019), thoracic and splenic sarcoma (Mellanby et al, 2004), acute monoblastic leukaemia (Kreangsak et al, 2010) and seminoma (Wakamatsu and Takenaka, 2000). There is also one report of IMHA in a cat with renal adenocarcinoma (Brown et al, 2019).

Inflammatory diseases

There is evidence that systemic inflammation is a common consequence of IMHA, with free red blood cell fragments acting as potent danger-associated molecular patterns driving the release of inflammatory mediators (Griebsch et al, 2009; Jeffery et al, 2015). Some studies suggest a potential association between pancreatitis and IMHA, either as a trigger factor or as a consequence of systemic cytokine release and thromboembolic events (Guadarrama-Olhovich et al, 2013; Zoia and Drigo, 2017). However, the current supporting evidence remains sparse in both dogs and cats.

Drugs and toxins

Drug-induced IMHA is a rare but recognised occurrence in people (Garratty, 2009). Its pathophysiology remains unclear, but may involve multiple mechanisms, including anti-drug IgG attaching to drug-coated red blood cell membranes and subsequent clearance by the mononuclear phagocytic system (Garratty, 2009).

In veterinary medicine, the evidence supporting the occurrence of drug-induced IMHA is again limited to a handful of case reports potentially associating IMHA with administration of cephalosporin (Bloom et al, 1988), anti-lymphocyte globulin (DeLong et al, 1990), paracetamol (Raguvaran et al, 2019) and propylthiouracil in cats (Peterson et al, 1984; Aucoin et al, 1985).

Toxin exposure is also a reported putative trigger for IMHA. Four cases of IMHA were described in dogs presented after snake envenomation (Ong et al, 2015), although these animals had also received anti-venom and fresh frozen plasma. A dog that presented with honey-bee envenomation later developed haemolytic anaemia with spherocytosis, thrombocytopenia, haemoglobinaemia and haemoglobinuria (Nair et al, 2019).

Vaccines

A causal relationship has been previously inferred, when a close temporal association was found between vaccination and onset of IMHA. One study showed that 26% of dogs diagnosed with IMHA had been vaccinated within 30 days before presentation (Duval and Giger, 1996), but the significance of this remains unclear

given the broad practice of vaccination. Another study found no difference in timing of last vaccination and hospital presentation between dogs presenting with IMHA and dogs presenting for other reasons (Carr et al, 2002).

Common concurrent clinicopathological abnormalities

An inflammatory leukogram in the complete blood cell count is a common finding in dogs, with leukocytosis and left shift neutrophilia reported in up to 99% and 80% of animals, respectively (Day, 1996; Reimer et al, 1999; Scott-Moncrieff et al, 2001).

In cats, lymphocytosis has been commonly reported (15–52%) (Husbands et al, 2002; Kohn et al, 2006; Swann et al, 2016).

Several studies also showed 65–70% of dogs with IMHA had concurrent mild thrombocytopenia (<200.000/μl), with 20% having a severely decreased platelet count (< 50.000/μl) (Carr et al, 2002; Orcutt et al, 2010). Similar percentages have been shown for feline patients (Kohn et al, 2006; Swann et al, 2016). The causes of thrombocytopenia in animals with IMHA are likely multifactorial, including immune-mediated destruction, consumption secondary to hypercoagulability and splenic sequestration (Kohn et al, 2006).

According to coagulation testing, 85–100% of dogs are in a hypercoagulable state upon presentation (Sinnott and Otto, 2009; Fenty et al, 2011; Goggs et al, 2012). Interestingly, the reported prevalence of disseminated intravascular coagulation in dogs with IMHA is not as high (28–45%) (Scott-Moncrief et al, 2001; Carr et al, 2002). In cats, one study reported a disseminated intravascular coagulation incidence of 5%; altered prothrombin time and activated partial thromboplastin time in 15% of the study population, with no thromboembolic complications observed. However, only 19 cats were recruited for this study (Kohn et al, 2006).

In measurements of serum biochemistry, 60–100% of animals show hyperbilirubinaemia (Kohn et al, 2006; MacNeill et al, 2019) resulting from a combination of increased delivery of bilirubin to the liver and a decreased hepatic clearance, caused by hypoxic hepatocellular damage, which also explains the prevalence of elevated liver enzymes (McCullough, 2003; Kohn et al, 2006).

Azotaemia may also be explained by hypoxia, but renal damage can also occur with persistent saturation of tubular capacity to reabsorb free-haemoglobin (McCullough, 2003). Immune-mediated renal impairment has also been reported in dogs with IMHA as well as concurrent distal renal tubular acidosis (Shearer et al, 2009).

Conclusions

IMHA is an important cause of haemolysis in both dogs and cats. An appropriate diagnostic approach aids in differentiating associative (secondary) from non-associative (primary or idiopathic) IMHA, tailoring therapy and identifying poor prognostic indicators.

Acknowledgements

KEY POINTS

- Immune-mediated haemolytic anaemia is a common cause of anaemia in dogs and, to a lesser extent, cats.
- Primary or non-associative immune-mediated haemolytic anaemia is more common than its secondary/associative counterpart, in both dogs and cats.
- The presence of other conditions needs to be determined, to achieve an appropriate diagnosis of associative or non-associative immune-mediated haemolytic anaemia, as there are no specific tests to differentiate them.
- Neutrophilia in dogs and lymphocytosis in cats are often present, as well as hyperbilirubinaemia with elevated hepatic and renal markers.
- There is no pathognomonic abnormality for the diagnosis of immune-mediated haemolytic anaemia.
- A subset of animals with immune-mediated haemolytic anaemia will not present with a highly regenerative anaemia. Consideration should be given that red cell precursors in the bone marrow may be targeted as well.

Conflicts of interest
The authors declare no conflicts of interest.

References

Adams LG, Hardy RM, Weiss DJ et al. Hypophosphatemia and hemolytic anemia associated with diabetes mellitus and hepatic lipidosis in cats. J Vet Intern Med. 1993;7(5):266–271. https://doi.org/10.1111/j.1939-1676.1993.tb01018.x

Allard RL, Carlos AD, Faltin EC et al. Canine hematologic changes during gestation and lactation. Companion Animal Pract. 1989;19:3–6

Assenmacher TD, Jutkowitz LA, Koenigshof AM et al. Clinical features of precursor-targeted immune-mediated anemia in dogs: 66 cases. J Am Vet Med Assoc. 2019;255:366–376. https://doi.org/10.2460/javma.255.3.366

Aucoin DP, Peterson ME, Hurvitz AI et al. Propylthiouracil-induced immune-mediated disease in the cat. J Pharmacol Exp Ther. 1985;234(1):13–18

Balch A, Mackin A. Canine immune-mediated hemolytic anemia: treatment and prognosis. Compend Contin Educ Vet. 2007;29(4):230–238

Bloom JC, Thiem PA, Sellers TS et al. Cephalosporin-induced immune cytopenia in the dog: demonstration of erythrocyte-, neutrophil-, and platelet-associated IgG following treatment with cefazedone. Am J Hematol. 1988;28(2):71–78. https://doi.org/10.1002/ajh.2830280202

Blundell R, Adam F. Haemolytic anaemia and acute pancreatitis associated with zinc toxicosis in a dog. Vet Rec. 2013;172(1):17–17. https://doi.org/10.1136/vr.100376

Brown P, Stallwood J, Costa M et al. Renin-producing tumor in the kidney of a cat. J Comp Path. 2019;170:70–73. https://doi.org/10.1016/j.jcpa.2019.05.009

Carr AP, Panciera DL, Kidd L et al. Prognostic factors for mortality and thromboembolism in canine immune-mediated hemolytic anemia: a retrospective study of 72 dogs. J Vet Intern Med. 2002;16(5):504–509. https://doi.org/10.1111/j.1939-1676.2002.tb02378.x

Caviezel LL, Raj K, Giger U et al. Comparison of 4 direct Coombs' test methods with polyclonal antiglobulins in anemic and nonanemic dogs for in-clinic or laboratory use. J Vet Intern Med. 2014;28(2):583–559. https://doi.org/10.1111/jvim.12292

Church DB. 'Anaemia'. Presented at proceedings of the New Zealand Veterinary Nursing Association Annual Conference, Auckland, NZ, 4–5 August 2017. https://www.nzvna.org.nz/site/nzvna/files/Quizzes/Anaemia%20PDF.pdf (accessed 24 February 2021)

Cowgill ES, Neel JA, Grindem CB et al. Clinical application of reticulocyte counts in dogs and cats. Vet Clin Small Anim. 2003;33(6):1223–1244. https://doi.org/10.1016/S0195-5616(03)00099-8

Day MJ. Serial monitoring of clinical, haematological and immunological parameters in canine autoimmune haemolytic anaemia. J Small Animal Pract. 1996;37(11):523–534. -https://doi.org/10.1111/j.1748-5827.1996.tb02313.x

DeLong D, Gunther R, Manning PJ et al. Immune mediated hemolytic anemia associated with antilymphocyte globulin therapy in dogs. Lab Anim Sci. 1990;40(4):415–418

Eibert M, Lewis DC. Evaluation of the feline erythron in health and disease. Compend Contin Educ Vet. 1997;19:335–347

Duval D, Giger U. Vaccine-associated immune-mediated hemolytic anemia in the dog. J Vet Intern Med. 1996;10(5):290–295. https://doi.org/10.1111/j.1939-1676.1996.tb02064.x

Fenty RK, deLaforcade AM, Shaw SE et al. Identification of hypercoagulability in dogs with primary immune-mediated hemolytic anemia by means of

thromboelastography. J Am Vet Med Assoc. 2011;238(4):463–467. https://doi.org/10.2460/javma.238.4.463

Fernandez Y, Sharman MJ, Seth M et al. Pregnancy-associated immune-mediated hemolytic anemia in a dog. J Vet Emerg Crit Care. 2020;30(3):308–311. https://doi.org/10.1111/vec.12951

Fleishman W. Anemia: determining the cause. Compend Contin Educ Vet. 2012;34:E1

Garden OA, Kidd L, Mexas AM et al. ACVIM consensus statement on the diagnosis of immune-mediated hemolytic anemia in dogs and cats. J Vet Intern Med. 2019;33(2):313–334. https://doi.org/10.1111/jvim.15463

Garratty G. Drug-induced immune hemolytic anemia. Hematology Am Soc Hematol Educ Program. 2009;2009(1):73–79. https://doi.org/10.1182/asheducation-2009.1.73

Grimes CN, Fry MM. Nonregenerative anemia: mechanisms of decreased or ineffective erythropoiesis. Vet Pathol. 2015;52(2):298–311. https://doi.org/10.1177/0300985814529315

Goggs R, Wiinberg B, Kjelgaard-Hansen M et al. Serial assessment of the coagulation status of dogs with immune-mediated haemolytic anaemia using thromboelastography. Vet J. 2012;191(3):347–353. https://doi.org/10.1016/j.tvjl.2011.03.015

Goggs R, Dennis SG, Di Bella A et al. Predicting outcome in dogs with primary immune-mediated hemolytic anemia: results of a multicenter case registry. J Vet Intern Med. 2015;29(6):1603–1610. https://doi.org/10.1111/jvim.13642

Griebsch C, Arndt G, Raila J et al. C-reactive protein concentration in dogs with primary immune-mediated hemolytic anemia. Vet Clin Path. 2009;38(4):421–425. https://doi.org/10.1111/j.1939-165X.2009.00146.x

Guadarrama-Olhovich M, Garcia Ortuno LE, Ruiz Remolina JA et al. Acute pancreatitis, azotaemia, cholestasis and haemolytic anaemia in a dog: a case report. Veterinarni Medicina. 2013;58(No. 1):44–49. https://doi.org/10.17221/6657-VETMED

Hammer AS, Couto CG, Swardson C et al. Hemostatic abnormalities in dogs with hemangiosarcoma. J Vet Intern Med. 1991;5(1):11–14. https://doi.org/10.1111/j.1939-1676.1991.tb00923.x

Harkin KR, Hicks JA, Wilkerson MJ et al. Erythrocyte-bound immunoglobulin isotypes in dogs with immune-mediated hemolytic anemia: 54 cases (2001–2010). J Am Vet Med Assoc. 2012;241(2):227–232. '. https://doi.org/10.2460/javma.241.2.227

Hodges J, Christopher MM. Diagnostic accuracy of using erythrocyte indices and polychromasia to identify regenerative anemia in dogs. J Am Vet Med Assoc. 2011;238(11):1452–1458. https://doi.org/10.2460/javma.238.11.1452

Husbands BD. 'Idiopathic immune-mediated hemolytic anemia (IMHA) in 25 cats'. Presented at proceedings of the 20th Annual American College of Veterinary Internal Medicine Forum, Dallas, TX, May 29–June 1 2002.

Jeffery U, Kimura K, Gray R et al. Dogs cast NETs too: canine neutrophil extracellular traps in health and immune-mediated hemolytic anemia. Vet Immunol Immunopathol. 2015;168(3-4):262–268. https://doi.org/10.1016/j.vetimm.2015.10.014

Kimura T, Kotani K. Perinatal veterinary medicine-related evaluation in hematological and serum biochemical profiles of experimental beagles throughout pregnancy and parturition. Anim Models Exp Med. 2018;1(4):282–294. https://doi.org/10.1002/ame2.12043

Klag A, Giger U, Shofer FS. Idiopathic immune-mediated hemolytic anemia in dogs: 42 cases (1986–1990). J Am Vet Med Assoc. 1993;202(5):783–787

Kohn B, Weingart C, Eckmann V et al. Primary immune-mediated hemolytic anemia in 19 cats: diagnosis, therapy, and outcome (1998–2004). J Vet Intern Med. 2006;20:159–166. https://doi.org/10.1111/j.1939-1676.2006.tb02836.x

Kopke MA, Pemberton S, Ruaux CG et al. Presumed immune-mediated haemolytic anaemia associated with pregnancy in a cat. JFMS Open Rep. 2019;5(1):205511691984168. https://doi.org/10.1177/2055116919841689

Korman RM, Hetzel N, Knowles TG et al. A retrospective study of 180 anaemic cats: features, aetiologies and survival data. J Feline Med Surg. 2013;15(2):81–90. https://doi.org/10.1177/1098612X12461008

Kreangsak P, Apassara C, Chaleow S et al. Immune-mediated hemolytic anemia in a dog with acute monoblastic leukemia. Comp Clin Path. 2010;19:631–634 https://doi.org/10.1007/s00580-010-1092-4

Lenske E, Padula AM, Leister E et al. Severe haemolysis and spherocytosis in a dog envenomed by a Red-Bellied Black Snake (Pseudechis Porphyriacus) and successful treatment with a bivalent whole equine IgG antivenom and blood transfusion. Toxicon. 2018;151:79–83. https://doi.org/10.1016/j.toxicon.2018.07.005

Limlenglert P, Rungsipipat A, Pusoonthornthum R. Application of flow cytometry for early diagnosis and monitoring in cats with immune-mediated hemolytic anemia. Thai J Vet Med. 2011;41:185–191

MacNeill AL, Dandrieux J, Lubas G, Members of the Veterinary and Comparative Clinical Immunology Society Diagnostic Task Force et al. The utility of diagnostic tests for immune-mediated hemolytic anemia. Vet Clin Pathol. 2019;48(S1):7–16. https://doi.org/10.1111/vcp.12771

Máthé A, Vörös K, Papp L et al. Clinical manifestations of canine babesiosis in Hungary (63 cases). Acta Veterinaria Hungarica. 2006;54:367–385 https://doi.org10.1556/AVet.54.2006.3.7

McAlees TJ. Immune-mediated Haemolytic Anaemia in 110 Dogs in Victoria, Australia. Aust Vet J. 2010;88(1-2):25–28. https://doi.org/10.1111/j.1751-0813.2009.00537.x

McCullough S. Immune mediated hemolytic anemia: understanding the nemesis. Vet Clin Small Anim. 2003;33(6):1295–1315. https://doi.org/10.1016/j.cvsm.2003.08.003

Mellanby RJ, Holloway A, Chantrey J et al. Immune-mediated haemolytic anaemia associated with a sarcoma in a Flat-Coated Retriever. J Small Anim Pract. 2004;45(1):21–24. https://doi.org/10.1111/j.1748-5827.2004.tb00190.x

Miller SA, Hohenhaus AE, Hale AS et al. Case-control study of blood type, breed, sex and bacteremia in dogs with immune-mediated hemolytic anemia. J Am Vet Med Assoc. 2004;224(2):232–235. https://doi.org/10.2460/javma.2004.224.232

Morley P, Mathes M, Guth A et al. Anti-erythrocyte antibodies and disease associations in anemic and nonanemic dogs. J Vet Intern Med. 2008;22(4):886–892. https://doi.org/10.1111/j.1939-1676.2008.0112.x

Nair R, Riddle EA, Thrall MA et al. Hemolytic anemia, spherocytosis, and thrombocytopenia associated with honey bee envenomation in a dog. Vet Clin Pathol. 2019;48(4):620–623. https://doi.org/10.1111/vcp.12747

Nitsche EK. Erythrocytosis in dogs and cats: Diagnosis and Management. Compend Contin Educ Vet. 2004;26:104–118

O'Brien MA, McMichael MA, Le Boedec K et al. Reference intervals and age-related changes for venous biochemical, hematological, electrolytic, and blood gas variables using a point of care analyzer in 68 puppies. J Vet Emerg Crit Care. 2014;24:291–301. https://doi.org/10.1111/vec.12162

Ong HM, Witham A, Kelers K et al. Presumed secondary immune-mediated haemolytic anaemia following elapid snake envenomation and its treatment in four dogs. Aust Vet J. 2015;93(9):319–326. https://doi.org/10.1111/avj.12359

Orcutt ES, Lee JA, Bianco D et al. Immune-mediated hemolytic anemia and severe thrombocytopenia in dogs: 12 cases (2001-2008). J Vet Emerg Crit Care. 2010;20(3):338–345. https://doi.org/10.1111/j.1476-4431.2010.00540.x

Ottenjann M, Weingart C, Arndt G et al. Characterization of the anemia of inflammatory Disease in cats with abscesses, pyothorax, or fat necrosis. J Vet Intern Med. 2006;20(5):1143–1150. https://doi.org/10.1111/j.1939-1676.2006.tb00713.x

Overmann JA, Sharkey LC, Weiss DJ et al. Performance of 2 microtiter canine Coombs' tests. Vet Clin Pathol. 2007;36(2):179–183. https://doi.org/10.1111/j.1939-165X.2007.tb00205.x

Owen JL, Harvey JW Hemolytic anemia in dogs and cats due to erythrocyte enzyme deficiencies. Vet Clin North Am Small Anim Pract. 2012;42(1):73–84. https://doi.org/10.1016/j.cvsm.2011.09.006

Paes G, Paepe D, Meyer E et al. The use of the rapid osmotic fragility test as an additional test to diagnose canine immune-mediated haemolytic anaemia. Acta Vet Scand. 2013;55(1):74. https://doi.org/10.1186/1751-0147-55-74

Paltrinieri S, Fossati M, Menaballi V et al. Diagnostic performances of manual and automated reticulocyte parameters in anaemic cats. J Feline Med Surg. 2018;20(2):122–127. https://doi.org/10.1177/1098612X17699067

Peterson ME, Hurvitz AI, Leib MS et al. Propylthiouracil-associated hemolytic-anemia, thrombocytopenia, and antinuclear antibodies in cats with hyperthyroidism. J Am Vet Med Assoc. 1984;184(7):806–808

Piatek CI, El-Hemaidi I, Feinstein DI et al. Management of immune-mediated cytopenias in pregnancy. Autoimmun Rev. 2015;14(9):806–811. https://doi.org/10.1016/j.autrev.2015.05.003

Piech TL, Wycislo KL The importance of urinalysis. Vet Clin North Am Small Anim Pract. 2019;49(2):233–245. https://doi.org/10.1016/j.cvsm.2018.10.005

Piek CJ, Junius G, Dekker A et al. Idiopathic immune-mediated hemolytic anemia: treatment, outcome and prognostic factors inn 149 dogs. J Vet Intern Med. 2008;22(2):366–373. https://doi.org/10.1111/j.1939-1676.2008.0060.x

Piek CJ. Canine idiopathic immune-mediated haemolytic anaemia: a review with recommendations for future research. Vet Q. 2011;31(3):129–141. https://doi.org/10.1080/01652176.2011.604979

Prihirunkit K, Chootesa A. Immune-mediated hemolytic anemia in a hypereosinophilic dog with intestinal T-cell lymphoma. Comp Clin Pathol. 2016;25(5):1091–1095. https://doi.org/10.1007/s00580-016-2304-3

Quigley KA, Chelack BJ, Haines DM et al. Application of a direct flow cytometric erythrocyte immunofluorescence assay in dogs with immune-mediated hemolytic anemia and comparison to the direct antiglobulin test. J Vet Diagn Invest. 2001;13(4):297–300. https://doi.org/10.1177/104063870101300403

Raguvaran R et al. Management of immune mediated hemolytic anemia (IMHA) secondary to paracetamol toxicity in a dog. Intas Polivet. 2019;20:180–181

Reimer ME, Troy GC, Warnick LD et al. Immune-mediated hemolytic anemia: 70 cases (1988-1996). J Am Anim Hosp Assoc. 1999;35(5):384–391. https://doi.org/10.5326/15473317-35-5-384

Rivera-Correa J, Rodriguez A. Divergent roles of antiself antibodies during infection. Trends Immunol. 2018;39(7):515–522. https://doi.org/10.1016/j.it.2018.04.003

Schoeman T, Lobetti RG, Jacobson LS et al. Feline babesiosis: signalment, clinical pathology and concurrent infections. J S Afr Vet Assoc. 2001;72(1):4–11. https://doi.org/10.4102/jsava.v72i1.601

Scott DW et al. Autoimmune hemolytic anemia in the cat. J Am Anim Hosp Assoc. 1973;9:530–539

Scott-Moncrieff J, Treadwell J, McCullough SM, Brooks MB. Hemostatic abnormalities in dogs with primary immune-mediated hemolytic anemia. J Am Anim Hosp Assoc. 2001;37:220–227 https://doi.org/10.5326/15473317-37-3-220

Shearer LR, Boudreau AE, Holowaychuk MK et al. Distal renal tubular acidosis and immune-mediated hemolytic anemia in 3 dogs. J Vet Int Med. 2009;23(6):1284–1288. https://doi.org/10.1111/j.1939-1676.2009.0406.x

Sinnott VB, Otto CM. Use of thromboelastography in dogs with immune-mediated hemolytic anemia: 39 cases (2000–2008): retrospective study. J Vet Emerg Crit Care. 2009;19(5):484–488. https://doi.org/10.1111/j.1476-4431.2009.00455.x

Slappendel RJ. Abnormal osmotic fragility of erythrocytes in dogs and cats. Vet Q. 1998;20S1:38–39. https://doi.org/10.1080/01652176.1998.10807399

Slappendel RJ, Zwieten R, Leeuwen M et al. Hereditary spectrin deficiency in Golden Retriever dogs. J Vet Intern Med. 2005;19(2):187–192. https://doi.org/10.1111/j.1939-1676.2005.tb02680.x

Sundburg CR, Belanger JM, Bannasch DL et al. Gonadectomy effects on the risk of immune disorders in the dog: a retrospective study. BMC Vet Res. 2016;12(1):278. https://doi.org/10.1186/s12917-016-0911-5

Swann JW, Szladovits B, Glanemann B et al. Demographic characteristics, survival and prognostic factors for mortality in cats with primary immune-mediated hemolytic anemia. J Vet Intern Med. 2016;30(1):147–156. https://doi.org/10.1111/jvim.13658

Swann JW, Skelly BJ. Canine autoimmune hemolytic anemia: management challenges. Vet Med (Auckl). 2016;7:101–112. https://doi.org/10.2147/VMRR.S81869

Tang X, Xia Z, Yu J et al. An experimental study of hemolysis induced by onion (Allium Cepa) Poisoning in Dogs. J Vet Pharmacol Ther. 2008;31(2):143–149. https://doi.org/10.1111/j.1365-2885.2007.00930.x

Tasker S, Murray JK, Knowles TG et al. Coombs', haemoplasma and retrovirus testing in feline anaemia. J Small Anim Pract. 2010;51(4):192–199. https://doi.org/10.1111/j.1748-5827.2009.00869.x

Tasker S. Diagnostic approach to anaemia in cats. In Pract. 2012;34(7):370–381. https://doi.org/10.1136/inp.e4889

Torres AR, Cassle SE, Haymore M et al. Hematologic differences between Dachshunds and mixedbreed dogs. Vet Clin Pathol. 2014;43(4):519–524. https://doi.org/10.1111/vcp.12178

Tritschler C, Mizukami K, Raj K et al. Increased erythrocytic osmotic fragility in anemic domestic shorthair and purebred cats. J Feline Med Surg. 2016;18(6):462–470. https://doi.org/10.1177/1098612X15587574

Ugoeke N, Onweni C, Treece J et al. Inflammatory breast cancer and warm antibody autoimmune hemolytic anemia: a rare paraneoplastic syndrome. J Investig Med High Impact Case Rep. 2017;5(4):232470961774090. https://doi.org/10.1177/2324709617740905

Volmer PA, Roberts J, Meerdink GL. Anuric renal failure associated with zinc toxicosis in a dog. Vet Hum Toxicol. 2004;46:276–278

Wakamatsu I, Takenaka M. Seminoma associated with immune-mediated hemolytic anemia after surgery. J Jpn Vet Med Assoc. 2000;53(7):469–471. https://doi.org/10.12935/jvma1951.53.469

Weingart C, Tasker S, Kohn B et al. Infection with haemoplasma species in 22 cats with anaemia. J Feline Med Surg. 2016;18(2):129–136. https://doi.org/10.1177/1098612X15573562

Weinkle TK, Center SA, Randolph JF et al. Evaluation of prognostic factors, survival rates, and treatment protocols for immune-mediated hemolytic anemia in dogs: 151 cases (1993-2002). J Am Vet Med Assoc. 2005;226(11):1869–1880. https://doi.org/10.2460/javma.2005.226.1869

Weiss D. Bone marrow pathology in dogs and cats with non-regenerative immune-mediated haemolytic anaemia and pure red cell aplasia. J Comp Pathol. 2008;138(1):46–53. https://doi.org/ 10.1016/j.jcpa.2007.10.001

Werner LL, Gorman NT. Immune-mediated disorders of cats. Vet Clin North Am Small Anim Pract. 1984;14(5):1039–1064. https://doi.org/10.1016/S0195-5616(84)50106-5

Wilkerson MJ, Davis E, Shuman W et al. Isotype-specific antibodies in horses and dogs with immune-mediated hemolytic anemia. J Vet Intern Med. 2000;14(2):190–196. https://doi.org/10.1111/j.1939-1676.2000.tb02235.x

Yu H, Fu R, Wang H et al. Paraneoplastic Evans syndrome in a patient with adenocarcinoma of the lung: a case report. Thorac Cancer. 2017;8(1):57–60. https://doi.org/10.1111/1759-7714.12398

Yuki M, Naitoh E. Complete remission of associative immune-mediated hemolytic anemia in a dog following surgical resection of intestinal leiomyosarcoma. Vet Sci. 2019;6(2):55. https://doi.org/10.3390/vetsci6020055

Zaldívar-López, Marín LM, Lazbik MC et al. Clinical pathology of Greyhounds and other sighthounds. Vet Clin Pathol. 2011;40:414–425. https://doi.org/10.1111/j.1939-165X.2011.00360.x

Zandecki M, Genevieve F, Gerard J et al. Spurious counts and spurious results on haematology analysers: a review. Part II: white blood cells, red blood cells, haemoglobin, red cell indices and reticulocytes. Int J Lab Hematol. 2007;29(1):21–41. https://doi.org/10.1111/j.1365-2257.2006.00871.x

Zoia A, Drigo M. Association between pancreatitis and immune- mediated haemolytic anaemia in cats: a cross-sectional study. J Comp Pathol. 2017;156(4):384–388. https://doi.org/10.1016/j.jcpa.2017.02.003