Immune-Mediated Hemolytic Anemia and Immune-Mediated Thrombocytopenia in Dogs

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In the last decade there have been insights into the hemostatic and inflammatory changes associated with immune-mediated hemolytic anemia (IMHA), but despite some modifications of therapy, the mortality rate remains unacceptably high. The average mortality rate reported during first hospitalization or immediate follow-up period from referral hospitals had been > 50% for more than two decades. In 2005, a large retrospective study reported a significantly lower mortality rate (< 30%) with a protocol based on azathioprine and low-dose acetylsalicylic acid (ASA), in addition to standard glucocorticoid therapy. This protocol was rapidly widely adopted, although subsequent reports have not always had the same success. Current common standard-of-care for acute/fulminant IMHA includes immunosuppressive and anti-inflammatory therapy with glucocorticoids ± azathioprine ± cyclosporine; ± thromboprophylaxis with ASA, clopidogrel, or heparin; transfusion to correct severe anemia; gastroprotection; and supportive care. Standard-of-care for immune-mediated thrombocytopenia (ITP) includes similar immunosuppression, transfusion, and gastroprotection ± treatment with vincristine. The mortality rate for ITP is lower than with IMHA (<10% in two recent studies), but cases refractory to standard-of-care persist. Standard-of-care management of IMHA and ITP will be reviewed, with a recognition that some of the trend to intensify therapy is born out of frustration and a need to do more, but is not of proven benefit. Other potentially beneficial treatments exist, but many are potentially prohibitively expensive and/or of limited availability.

Etiology and pathophysiology
IMHA results from antibodies binding to red cells. Hemolysis is most often extravascular, where red cells are destroyed by the mononuclear phagocyte system, in particular in the spleen. Red cell autoantibodies may also cause complement-mediated intravascular hemolysis. The period of acute hemolysis is associated with a systemic inflammatory response. IMHA may be primary, or secondary to another disorder or treatment. When a cause cannot be found, IMHA is assumed to be primary, which is the most frequent diagnosis. There are two broad forms of IMHA: the subacute to chronic form, where there is a history of slowly progressive inappetance and exercise intolerance, and initial treatment is on an out-patient basis using prednisone; and the acute-to-fulminant form, which is the focus of this review.

The cause of death in IMHA may be euthanasia due to cost of therapy, refractory anemia, organ dysfunction presumed secondary to thromboembolism, or hemorrhage secondary to disseminated intravascular coagulation (DIC). Natural death is presumed due to thromboembolism. The pathogenesis of thromboembolism is multifactorial. Thrombocytopenia may occur from concurrent ITP (Evan’s syndrome) or DIC.

ITP similarly results from antibodies binding to platelets, is either primary or secondary, and has a spectrum from acute to chronic. It is assumed that most platelet destruction is extravascular. Overall dogs with ITP are not as sick as dogs with IMHA, perhaps because there is less of an inflammatory response. The causes of death in ITP include euthanasia due to cost of therapy, refractory thrombocytopenia, or severe hemorrhage. Natural death is due to hemorrhage, and, if not present, then likely arrhythmias or thromboembolism. Severe hemorrhage is usually gastrointestinal, but central nervous system bleeding may also occur. There is evidence that humans and dogs with ITP are prothrombotic, but the risk for thromboembolism is lower, presumably because of thrombocytopenia itself and possibly because ITP may be a less inflammatory state than IMHA.

Diagnostic plan
Signalment
Middle-aged spayed female dogs are at increased risk, although IMHA and ITP may occur in young and old dogs of either sex. Various breeds have been overrepresented in different studies, but American Cocker Spaniels have emerged as a breed at risk for both disorders.

History
Dogs with IMHA have: 1) acute onset of depression, inappetence, weakness, and exercise intolerance due to acute anemia and acute phase response; and 2) variable vomiting and diarrhea, presumed due to gastrointestinal ischemia and/or pancreatitis. Additionally, 3) owners may report discoloured urine, due to bilirubinuria or hemoglobinuria. Underlying/concurrent disorders may cause additional signs. Dogs with ITP have: 1) variable depression, etc, due to anemia; and 2) variable vomiting and diarrhea. 3) Owners may report/present dogs for hematuria, melena, petechiae and ecchymoses, epistaxis, or other bleeding.

Physical examination
For dogs with IMHA; 1) pale mucous membranes; 2) icterus is common; 3) tachycardia and prominent pulses; a heart murmur due to anemia may be present; 4) polyphagia and, occasionally, dyspnea; 5) variable abdominal pain and fever; 6) hepatosplenomegaly; 7) occasional lymphadenopathy; 8) petechiae and ecchymoses may be evident if there is concurrent ITP. For dogs with ITP: Cutaneous
and mucosal petechiae and ecchymoses are the hallmarks. Ocular hemorrhages may also be present. Physical exam findings of with ITP are otherwise similar to those of IMHA except for icterus.

**Laboratory evaluation/diagnostic imaging**
The goals of the work-up of the dog with suspected IMHA and/or ITP are to: 1) confirm the diagnosis; 2) identify any underlying causes of autoimmunity; 3) identify concurrent disorders of autoimmunity or disorders which may impact therapy; and 4) identify initial prognostic factors. These goals justify a routine CBC, serum chemistry profile and urinalysis. Blood samples of dogs with IMHA should be handled gently because of increased red cell fragility.

Confirming the diagnosis of IMHA - The main disorders that may present with a similar clinical picture as acute IMHA are zinc and onion poisoning, and red cell infections (hemotropic Mycoplasma, Babesia). Hemotropic Mycoplasma infection is rare without a history of splenectomy. Other non-immune causes of hemolysis include hypophosphatemia and hereditary red cell enzyme deficiencies (Springer Spaniels, Basenjis and black Miniature Poodles). The diagnosis of IMHA is most commonly made on the basis of a CBC demonstrating a regenerative anemia and spherocytosis and/or agglutination and/or ghost red cells, and absence of hemoparasites. Regeneration should be present after 3-4 days. Occasionally the anemia is non-regenerative because of destruction of immature red cells in the bone marrow.

To detect agglutination, gently mix equal volumes of EDTA blood and saline in a tube to minimize rouleaux formation, and observe for gross agglutination (blood is flocculent). If agglutination is not seen, place several drops of the mixture on a glass slide and gently rock the slide back and forth to observe for agglutination within 1 minute. (After several minutes rouleaux formation will increase on a glass slide as the sample desiccates.) It is impossible to distinguish rouleaux and agglutination macroscopically. In all cases place a coverslip on the slide and examine the wet-mount microscopically, especially if the diagnosis of IMHA is based on the presence of agglutination. Preparing a wet-mount will also facilitate detection of microscopic agglutination. The common practice of simply observing a drop of whole blood on a glass slide for flocculence and dispersion of potential rouleaux by adding saline is discouraged. Rouleaux microscopically appear as “stacks of coins” and agglutination appears as “clusters of grapes”. If antibodies are not present in a sufficiently high titer to cause agglutination, a lower titer is usually detectable with a Coomb’s test or flow cytometry.

Identifying underlying causes of autoimmunity - Review history for drug therapy (presumptive cause). Thoracic radiographs and abdominal radiographs or ultrasound are used to identify underlying foci of inflammation or neoplasia, and problem-based/risk-factor specific testing rather than routine imaging (especially abdominal ultrasound examination) is strongly advocated. Imaging is expensive and financial resources are often better directed towards therapy. The same principle applies for screening for infectious and parasitic diseases. Bone marrow biopsy should be performed to rule-out histiocytic sarcoma in Bernese mountain dogs and flat-coated retrievers.

Identifying concurrent disorders of autoimmunity, unrelated disorders which may impact therapy, and complications of IMHA - Concurrent autoimmune disorders include glomerulonephritis (proteinuria), polyarthritis (lameness, swollen joints), and dermatitis/vasculitis (skin lesions). These are not common but abnormalities would prompt additional work-up. Animals with IMHA may develop subcutaneous edema with or without pleural effusion and ascites during therapy. This is not a sign of vasculitis, but rather reflects water retention triggered by acute anemia, possibly altered endothelial permeability, and overhydration. Older animals are at increased risk for concurrent unrelated disorders. IMHA may cause pancreatitis, which may increase amylase, lipase, and PLI, but pancreatitis has not been identified as a prognostic factor. Azathioprine may also cause pancreatitis; if this is the putative cause, then the drug should be discontinued. Acute dyspnea with unremarkable thoracic radiographs is suggestive of pulmonary thromboembolism.

Identify initial prognostic factors - Negative laboratory prognostic indicators of variable strength include: 1) Degree of leukocytosis – a neutrophilia with or without a left shift is common, due to stress, bone marrow stimulation and tissue necrosis. The higher the neutrophil count the more likely microthrombi are present. 2) Degree of thrombocytopenia, reflecting Evan’s syndrome or DIC; 3) Decreased albumin – perhaps related to acute phase response or water retention; 4) Degree of bilirubinemia, reflecting severity of hemolysis and other factors – this is the negative prognostic factor most consistently (but not invariably) identified; 5) Degree of elevation in ALT, reflecting hypoxic/ischemic necrosis of the liver; 6) Degree of elevation in urea,9 possibly reflect dehydration, gastrointestinal bleeding, or negative nitrogen balance; 7) Intravascular hemolysis; 8) Prolonged clotting times. Increased activated clotting time (ACT), aPTT, and less often PT, are presumed to be prolonged due to DIC. Thrombelastography (TEG®, a whole blood assessment of hemostasis) also reveals that relatively hypocoagulable dogs have a worse prognosis. Severity of anemia at presentation does not appear to be a prognostic factor.

The principles of work-up are similar for ITP. The diagnosis of ITP is ultimately based on exclusion of other causes of thrombocytopenia. Platelet-bound antibody tests are not widely available or specific; a negative test likely rules-out ITP. The diagnosis is usually made in a dog with thrombocytopenic bleeding that does not have evidence of bone marrow failure or of a disorder causing DIC. Bone marrow biopsy is not necessary if shift platelets are present and/or red cell, neutrophil and monocyte production are normal. Bone marrow biopsy classically reveals megakaryocytic hyperplasia (as may DIC), but cases with initial
normal-to-low megakaryocyte counts may occur, and there is evidence of impaired platelet production in humans.12 In the author’s opinion, megakaryocyte hypoplasia does not imply a worse prognosis for recovery, but does indicate that recovery may take several more days. (Similarly non-regenerative forms of IMHA do not carry a worse prognosis if time is not an issue.)

**Treatment**

Therapeutic goals in IMHA and ITP are: 1) reduce hemolysis and/or platelet destruction; 2) reduce autoantibody production; 3) reduce inflammation; 4) prevent/treat thromboembolism and DIC; 5) correct anemia-hypoxia; 6) prevent hemorrhage; 7) prevent/treat gastrointestinal ulceration; 8) provide supportive care. An understandable although unproven prevailing principle guiding clinician behavior is that the more negative the prognostic indicators are, the more aggressive the therapy should be. There are a large number of case series reported with different treatments and conflicting results, but little firm evidence. It is emphasized that the following describes, more than justifies, clinician behavior.

**Immunosuppressive/anti-inflammatory/antiphagocytosis therapy**

Cyclosporine (a non-myelosuppressive calcineurin antagonist) was first used in dogs as an immunosuppressive agent for organ transplantation, but as familiarity increased, and cost decreased somewhat, it ushered its way into therapy for a variety of immunemediated and inflammatory disorders. It has a rapid onset of action but highly variable oral bioavailability. Currently the tendency in the author’s practice is to use cyclosporine immediately in dogs with IMHA with multiple poor prognostic indicators, and it is also given early consideration in cases of ITP with severe bleeding. It is not recommended to use cyclosporine as a “substitute” for azathioprine. Cyclosporine is used in transplantation at an initial dose of 10 mg/kg q12h with a target trough level of 350 - 500 ng/mL. Currently the tendency in the author’s practice is to use cyclosporine immediately in dogs with IMHA with multiple poor prognostic indicators, and it is also given early consideration in cases of ITP with severe bleeding. Then it is emphasized that the following describes, more than justifies, clinician behavior.

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Vincristine has been used to treat ITP for several decades. The use remains somewhat controversial, as does the mechanism of action, but one trial demonstrated benefit in dogs. Given the relatively low cost, ease of administration and minimal side-effects at “ITP doses”, this drug is routinely given within the first 48 hours of admission in the author’s practice, at a dose of 0.02 mg/kg for dogs <15 kg and 0.5 mg/m² for dogs >15 kg. If a mechanism of action is reduction of macrophage reduction, then it may also be beneficial in IMHA, but has not been evaluated for this purpose.

References & suggested reading


