IMMUNE MEDIATED BLOOD DYSCRASIAS: CLINICAL PRESENTATION & DIAGNOSIS

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INTRODUCTION
Immune mediated hemolytic anemia (IMHA) and immune mediated thrombocytopenia (IMTP) are both the result of premature immunologic destruction of blood cells tagged by antibody or complement. This immunologic reaction may result from some alteration in the appearance of the blood cell (via infection, drugs, or neoplasia), or by a primary failure of immune regulation. It is always important to “rule out” potential causes of secondary immune destruction of the red cells or platelets, but many cases of IMHA and IMTP in dogs are primary (autoimmune hemolytic anemia [AIHA] and autoimmune thrombocytopenia [AITP]) in nature. In the cat, secondary IMHA and IMTP are more prevalent than primary. Primary AIHA and AITP may occur independently, together (Evan’s syndrome), or as a part of a larger autoimmune disease process (eg, systemic lupus erythematosus; SLE). Many of the autoantigens that trigger binding of complement or autoantibodies in AIHA have been identified. For AIHA, these include membrane glycoproteins, anion exchange molecules, and cytoskeletal components. For AITP, the membrane glycoproteins GPIib and GPIIIa are the predominant autoantigens.

Both AIHA and AITP are the result of type II hypersensitivity reactions in which autoantibody attached to the cell triggers cytotoxicity and subsequent destruction of the cell. There is more variability to the pathophysiology of AIHA than of AITP. In fact, there are five classes of AIHA, each representing a slightly different disease process and each associated with a different prognosis. The most common form of AIHA is class III, wherein antibodies of the IgG type bind to the RBC. As the tagged RBC passes through the organs of the mononuclear-phagocytic system (a.k.a., reticuloendothelial system), the Fc portion of the autoantibody tigers mononuclear phagocytosis. This process results in extravascular hemolysis, most of which takes place in the spleen. The mechanisms for AITP are extremely similar to class III AIHA, although thrombocytopenia rather than hemolysis is the result of the cytotoxic reaction in AITP. Less common but more life-threatening forms of AIHA are described as class I and class II. Autoagglutination is characteristic of class I AIHA, in which both IgG and IgM autoantibodies play a role. The autoantibodies bind multiple RBCs simultaneously, resulting in agglutination followed by extravascular hemolysis. In class I AIHA autoantibodies typically of the IgM isotype activate complement on the RBC, resulting in holes forming in the RBC membrane and extravascular hemolysis. Class IV AIHA is much like class I, with the exception that agglutination occurs at low temperatures. As a result, agglutination occurs only in the periphery of the body, such as the pinnae of the ears or tip of the tail. Because there is little hemolysis, class IV AIHA presents not as an anemia but as dermal necrosis of the extremities resulting from agglutination and occlusion of capillary vessels. Similarly, class V AIHA resembles class II AIHA but only occurs at low temperatures. This disorder is rarely diagnosed clinically, but leads to mild intravascular hemolysis.

AUTOIMMUNE HEMOLYTIC ANEMIA (AIHA)
Initial presentation
Most dogs with primary IMHA are young to middle aged adults. In fact, the diagnosis of hemolytic anemia in either a juvenile or a geriatric animal should prompt a thorough evaluation for non-immunologically mediated causes of hemolysis (parasitism, chemical/toxin damage, fragmentation hemolysis, congenital hemolytic disorders) and for causes of secondary IMHA (infection, drugs, neoplasia). Although any breed of dog may develop AIHA, there are strong breed associations with Cocker spaniels, Springer spaniels, Old English Sheepdogs, German shepherds, and poodles overrepresented. Gender predilections are less pronounced, although females may be at slightly higher risk. Although cats are susceptible to AIHA, secondary IMHA is more common, and most of the following discussion will center on dogs rather than cats. Typically the history of illness is brief, from days to weeks. Clinical signs are largely non-specific, with owners most commonly reporting lethargy, poor appetite, and weakness. Some astute owners may notice pallor, jaundice, or tachypnea. When associated with intravascular hemolysis, hemoglobinuria can be a dramatic presenting complaint. Historical inquiries relevant to AIHA include questions about whelping or estrus in the bitch, recent vaccination, and any recent medications or homeopathic remedies. Abnormalities on physical exam are indistinguishable from non-immunologically mediated causes of hemolysis. Pallor with or without jaundice is typical. When PCV falls below 20%, a soft left basal systolic murmur is often heard. Splenomegaly is a common finding in class I and class III AIHA, while hematomegaly and lymphadenomegaly are more common with class II AIHA. Mild fever may be a reflection of the inflammatory nature of the illness. Tachypnea, tachycardia, and bounding pulses reflect tissue hypoxia. Outright dyspnea occurs in animals with AIHA, but usually from the complication of pulmonary thromboembolism (PTE) rather than as a direct result of anemia. Extent of weakness and depression are quite variable, and depend not only on degree of anemia but also the rapidity with which anemia occurred.

Diagnostic testing
A minimum database including CBC, biochemical profile, and urinalysis provides not only information potentially supportive of AIHA, but helps to rule out causes of non-immunologic hemolysis and of secondary IMHA. Degree of anemia may vary from mild to severe, with PCV commonly measured in the teens. Polychromasia, anisocytosis, nucleated RBC, and most importantly reticulocytosis indicate a regenerative anemia in most dogs with AIHA, although regeneration takes ~3 days to develop and therefore will not be seen in an acute presentation. In some dogs, marrow precursor cells as well as peripheral RBC are attacked, and in these animals regeneration will not be apparent. Bone marrow aspiration may be warranted in such cases, and often erythrophagocytosis will be observed. Class III AIHA is usually accompanied by peripheral spherocytosis, while gross and/or microscopic agglutination are characteristic of class I AIHA. Agglutination must be distinguished from benign roluoaex formation via saline wash of the RBC; true agglutination will persist after washing. Because AIHA is an immunologic and inflammatory condition and marrow is stimulated, neutrophilic leukocytosis is common and sometimes marked. Platelet counts may be normal,
increased (response to inflammation), or decreased. About 10% of dogs with AIHA have concurrent AITP, but other reasons for thrombocytopenia might include disseminated intravascular coagulation (DIC) or PTE. Findings of hematologic parasites or infectious inclusion bodies, markers of oxidative damage, marked schistocytosis, or decreased plasma total proteins would suggest causes of anemia other than AIHA. Biochemical findings should merely reflect hemolysis and tissue hypoxia. Visible hemolysis, bilirubinemia, and elevations of ALT are potential abnormalities. Hypoglycemia, azotemia, or electrolyte disturbances would suggest other causes of hemolysis. Urine should be obtained by free catch pending confirmation of normal platelet count. Bilirubinuria may accompany any class of AIHA, but hemoglobinuria (positive dipstick blood in the absence of intact RBC) suggests class II AIHA.

Infectious disease screening is indicated depending on individual circumstances and geographic location. All cats with hemolysis should be tested for retroviral disease as well as by PCR for *Mycoplasma haemofelis* infection (formerly *Haemobartonella*). In most of the USA, dogs should be tested for *Ehrlichia canis*, and in many areas for *Babesia canis* (esp. greyhounds) or *B. gibsoni* (esp. pit bull terriers) as well. Other specific infectious disease testing would depend on individual circumstances (e.g., splenectomized dog tested for *M. haemocanis*). Each of these infections can induce hemolytic anemia that can be mistaken for AIHA and might delay or derail appropriate therapy.

Imaging studies are often indicated to help rule out neoplastic causes of secondary IMHA. Other causes of hemolysis, such as the presence of pennies in the GI tract, may be identified on occasion.

**Disease confirmation**

Specific immunologic testing can be performed to support a tentative clinical diagnosis of AIHA. Such confirmatory testing is not required when persistent autoagglutination is observed since this provides information identical to the routinely employed test. The direct antiglobulin test (Coombs test) is most commonly used to support the diagnosis of AIHA. However, it is vital to understand that all secondary causes of IMHA as well as blood transfusion may result in a positive test, meaning that a positive Coombs result is not specific for AIHA. Additionally, Coombs testing is not perfectly sensitive and may be falsely negative in the face of AIHA for a variety of reasons (e.g., drug therapy, prozone phenomenon). The test is performed by obtaining the patients RBC and washing away the plasma. In animals with IMHA (primary or secondary), antibodies attached to the RBC will remain attached and will not wash away. The washed RBC are then incubated with species-specific antiserum. This antiserum is usually polyvalent, and contains antibodies that react with IgG, IgM, and complement. If the patient’s RBC are coated with autoantibody, then the antiserum antibodies will attach to the autoantibodies resulting in the positive endpoint of RBC agglutination. Modifications of the test include utilizing different dilution of the patients RBC, conducting the test at both 4°C and 36°C, and the use of monovalent antiserum. Unfortunately, due to numerous false positive and false negative results, the Coombs test is considerably less than an ideal test. Newer methods to detect bound antibodies include flow cytometric analysis, and early results suggest these limited availability tests may be more sensitive. Regardless, positive results can only establish the presence of antibody, not causation.

It is important to understand the role of other immunologically based tests in the diagnosis of AIHA. Often, veterinarians order antinuclear antibody tests (ANA) on dogs with suspected AIHA. Although AIHA is on occasion a part of the systemic autoimmune disease SLE, it occurs far more commonly as an independent condition. Because ANA tests only for the presence of nuclear antibodies, and because the autoantibodies in AIHA are not directed against nuclear components, the ANA test is not expected to be positive in dogs with isolated AIHA (or Evans syndrome). Routine ANA testing can be reserved for animals with other evidence of systemic autoimmune, such as dermatopathy or glomerulonephritis. Similarly, there are several other immunologically based tests for autoimmunity that do not deserve a place in the routine diagnostic testing of animals with suspected AIHA. For example, rheumatoid factor testing is specific for the presence of autoantibodies directed at other antibodies, and is not helpful in the diagnosis of AIHA.

**AUTOIMMUNE THROMBOCYTOPENIA (AITP)**

**Initial presentation**

Similar to AIHA, primary AITP is much more common in dogs than cats. Again, it most often affects young adult to middle aged adults. Secondary causes of IMTP include infectious diseases (esp. rickettsial type disease), neoplasia, and drug therapy. Breeds predisposed to AITP include Cocker spaniels, Old English Sheepdogs, and poodles, and there is weak propensity for females. History may include an owner’s observation of bleeding, or the observed consequences of bleeding. Depending on the site and volume of bleeding, animals may be quite bright and active when presented for examination. Perhaps because petechial and ecchymotic hemorrhage are more common than overt bleeding, it has been the author’s experience that presentation is made more often for the non-specific signs of anemia (weakness, lethargy, anorexia) than for observed bleeding. Overt bleeding (epistaxis, hematuria, melena or hematochezia) are occasionally the presenting signs. An observant owner may notice ocular or gingival petechial lesions, but ecchymotic bruises would be more likely to catch the attention of an owner, particularly on the non-haired ventrum. Hyphema or neurologic dysfunction due to central nervous system bleeding are less common presenting complaints. Physical examination is typically characterized by petechial hemorrhages (often noted on the sclera, retina, or mucus membranes) with or without ecchymosis. Membrane color may be pale if sufficient blood has been lost, but unless part of Evan’s syndrome, AITP does not result in jaundice. Splenomegaly and mild fever are frequent findings, as they are for class III AIHA. Epistaxis and evidence of GI bleeding on rectal exam are occasional findings, but cavitary bleeding should suggest alternate causes of coagulopathy such as rodenticide intoxication.

**Diagnostic testing**

Routine CBC, biochemical profile, and UA may demonstrate no abnormalities other than thrombocytopenia. The thrombocytopenia associated with AITP is often profound, with platelet counts nearly always < 50,000 x 10^3/μL, and very often <5,000. In fact, a very severe thrombocytopenia in the absence of other obvious illness is strongly suggestive of AITP. Bleeding will eventually lead to anemia, which is expected to become regenerative given sufficient time (~3 days for dogs). Red cell morphology reflects the regenerative response (polychromasia,
anisocytosis), but spherocytosis is absent. Neutrophilic leukocytosis may again reflect generalized marrow stimulation and an inflammatory disease process, but leukocytosis tends to be less pronounced in AITP than in AIHA. Plasma total proteins decrease with blood loss. As with IMHA, peripheral blood smears should be carefully examined for the presence of blood parasites or infectious inclusion bodies that would suggest causes of thrombocytopenia other than AITP. There are no specific findings on chemistry profiles, but albumin and globulin will be lost with bleeding, and tissue hypoxia as a result of blood loss anemia may cause elevation of liver enzymes. Urine, which should be obtained only by free catch, may have evidence of microscopic hematuria (gross hematuria is rare). Assessment of coagulation times is not routinely indicated unless it is performed to rule out DIC as a cause of thrombocytopenia. This is usually only necessary in dogs with evidence of illness other than simple thrombocytopenia. In dogs with AITP, both aPTT and OSPT should be within reference range. However, if ACT is used as a rough substitute for aPTT, mild prolongation is expected when platelet count is less than 10,000 x 10³/μL.

Unfortunately, peripheral regeneration of platelets is not evident by routine hematology. Thus, whereas reticulocytosis proves that an anemia is regenerative and rules out problems with marrow proliferation as the cause of anemia, there is no equivalent of reticulocytosis to be used in cases with thrombocytopenia. The presence of large platelets in the peripheral blood as reflected in the mean platelet volume has been suggested to provide such information but is a non-specific finding. For this reason, bone marrow aspiration is often indicated to confirm the presence of megakaryocytosis expected in AITP and to rule out decreased production as a cause of thrombocytopenia. Marrow aspiration is a simple technique that requires little special equipment, and for the purpose of identifying megakaryocytes is simple to interpret. Although abnormal bleeding is expected in animals with severe thrombocytopenia, this is not problematic since bleeding occurs into the marrow allowing the blood to stay within the vascular pool.

A number of infectious diseases may result in IMTP and/or destruction of platelets via non-immunologic means. Chief among these are the rickettsial-type infections. Often, serologic tests for *E. canis* are indicated in thrombocytopenic dogs, as may be tests for *Anaplasma phagocytophilum* (formerly *E. equi*), *R. rickettsia*, and even Babesiosis in certain circumstances. *A. platys* (formerly *E. platys*) causes cyclic thrombocytopenia that is most often asymptomatic, but testing may be indicated in some cases. Cats with thrombocytopenia should be tested for retroviral infection and perhaps also for Ehrlich type pathogens (via PCR and/or serology). Diagnostic imaging studies may be appropriate to look for neoplastic disease that might result in IMTP or other causes of thrombocytopenia.

**Disease confirmation**

There are no reliable, readily available immunologic tests for confirmation of AITP. In the past, a positive platelet factor 3 test was used as supportive evidence. The test is basically a modification of a thrombin time test in which antiplatelet antibodies from the patient result in platelet membrane damage and therefore cause a more rapid coagulation response. This test is neither sensitive nor specific, and cannot be recommended. An alternative test is commercially available for antimegakaryocytic antibodies, but this test requires submission of slides made from marrow aspirates. Flow cytometric tests for antiplatelet antibody have also been developed, but availability is limited (Kansas State; 765-532-4617). As with AIHA, immunologic tests designed for recognition of phenomenon not directly related to the disease in question cannot be used to support a presumptive clinical diagnosis. The Coombs test, designed to test for anti-RBC antibodies, has no bearing on the diagnosis of AITP unless the animal is suspected of having Evans syndrome. When anemia is due to bleeding rather than hemolysis, Coombs testing of thrombocytopenic animals is not indicated. Similarly, serum ANA testing is indicated only when there is a suspicion that AITP is a part of a systemic autoimmune disease such as SLE.

The diagnosis of AITP remains a diagnosis of exclusion. It should be strongly suspected in young adult dogs presented with evidence of primary hemostatic defects in the absence of other illness, particularly when the thrombocytopenia is profound. Appropriate specific testing to rule out infectious causes of thrombocytopenia should be performed. Marrow aspirates typically reveal megakaryocytosis, and if performed, assays of secondary hemostasis will be normal. Finally, response to therapy is often used a supportive evidence of the diagnosis.

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**REFERENCES**