Immune-mediated thrombocytopenia (IMT) is a relatively common cause of bleeding in small animals, particularly the dog. Many differing disease processes may initiate IMT. Despite heterogenous etiologies, most cases of IMT share common pathophysiological features: high levels of platelet-associated antibody, enhanced platelet destruction by the mononuclear phagocytic system (MPS), and markedly decreased circulating platelet life-span. Thrombocytopenia develops when platelet destruction exceeds compensatory platelet production by marrow megakaryocytes.

**Pathophysiology**

Platelet production (thrombopoiesis) by megakaryocytes maintains circulating platelet numbers that far exceed needs. Spontaneous hemorrhage in dogs (assuming normal platelet function) is extremely uncommon at platelet counts above 50,000/µl, well below the canine reference range of 200,000 to 500,000 platelets/µl. The normal circulating life span of a canine platelet is a little over one week. Senescent (aged) platelets are removed from the circulation and phagocytosed by the MPS, particularly within the spleen.

In IMT, platelet-associated antibody levels are usually increased. Increased antibody binding to platelet membranes enhances destruction of platelets by the MPS, a process mediated by macrophage Fc receptor binding of antibody-coated platelets. The spleen is usually the major organ of immune-mediated platelet destruction, and is also a major source of anti-platelet antibodies. Splenic platelet destruction rates are often markedly increased, up to ten times the rate of normal senescent platelet consumption. The marrow responds to increased platelet consumption by increasing megakaryocyte number and volume: thrombopoiesis can expand up to five times normal in states of excessive platelet destruction.
Platelet life span is inversely correlated to platelet-associated antibody levels. Platelet life span in IMT patients is often less than one day, and patients with extremely high platelet-associated antibody levels often have a platelet life span of less than one hour. Surviving circulating platelets in IMT patients typically have normal or increased hemostatic function, presumably because of an expanded population of megathrombocytes (young, large platelets).

Immune-mediated thrombocytopenia typically stimulates vigorous thrombopoiesis. Some IMT patients, however, actually have sub-maximal thrombopoiesis, perhaps because anti-platelet antibodies often cross-react with megakaryocytes. Profound megakaryocytic hypoplasia is an uncommon finding in canine IMT patients, and is associated with high mortality rates. As well as affecting platelet numbers, anti-platelet antibodies can also cause platelet dysfunction (thrombopathia). Clinically, the importance of antibody-mediated platelet dysfunction in small animal IMT patients is uncertain. Variations in the degree of thrombocytopenia necessary to induce spontaneous hemorrhage in IMT patients may reflect a balance between the enhanced function of megathrombocytes and the diminished function of antibody-coated platelets.

**Pathogenesis of IMT**

As with immune-mediated hemolytic anemia (IMHA), IMT may be primary or secondary. Primary IMT is a typical spontaneous autoimmune disease, whereas secondary IMT may be initiated by a diverse array of different disease processes that are probably very similar to those processes known to trigger IMHA (see table in *Immune-Mediated Hemolytic Anemia* lecture notes). Most of the investigations into the pathogenesis of naturally occurring primary IMT have been done in people. Presumably, similar pathogenic processes occur in small animal patients.

Human chronic primary IMT, also called idiopathic or immune-mediated thrombocytopenic purpura (ITP), is a typical autoimmune disease that is clinically very similar to canine IMT. Platelet-associated IgG levels are increased in most patients, and often inversely correlate with platelet count, whereas no consistent correlation has been detected between platelet numbers and platelet-associated IgM, IgA or complement levels. Most primary IMT patients have antibodies directed against platelet membrane glycoproteins such as GP IIb/IIIa and GP Ib/IX. Since these glycoproteins are essential for normal platelet function, the presence of anti-glycoprotein antibodies may explain the platelet dysfunction seen in some patients. Predisposition to develop IMT is thought to be inherited in people, and a genetic predisposition may also explain particular canine breed predilections (including poodle, old English sheepdog and cocker spaniel) for IMT. Primary IMT in cats has been very rarely documented. In the vast majority of instances, IMT in cats is secondary to an underlying disease process.

The pathogenesis of secondary IMT is probably very similar to that discussed in the *Immune-Mediated Hemolytic Anemia* lecture notes.

**Clinical Signs**

Primary IMT most commonly affects middle-aged female dogs, with an average age of onset of six years. Since IMT is usually secondary in cats, it can occur in cats of any age or sex. Canine IMT typically presents as spontaneous hemorrhage in dogs that previously appeared healthy.
Careful questioning, however, may uncover a history of recurrent minor bleeding. Minor trauma or routine surgery may precipitate unexpectedly severe bleeding. Subclinical thrombocytopenia may also be discovered during routine hematology, particularly in cats, since cats seem to be very resistant to significant bleeding despite very low platelet counts. In cases without signs of bleeding, however, it is important to rule out artifact as a cause of a low platelet count. Erroneously low platelet numbers (pseudothrombocytopenia) are very common artifacts seen with hematology analyzer platelet counts, especially in cats.

The hallmark primary lesion in patients with IMT is the petechial (pin-point) hemorrhage. Cutaneous and mucosal petechiae often merge into ecchymotic bruising. Cutaneous bruising typically occurs at sites of either capillary trauma (pressure points) or increased hydrostatic pressure (ventral trunk). Petechiae commonly involve oral, nasal, conjunctival, and urogenital mucosae. Mucosal hemorrhage causes gingival and vulval bleeding, epistaxis, hematemesis, melena, hematochezia and hematuria.

Patients with are often remarkably stable despite marked thrombocytopenia. Cats, in particular, can remain subclinical despite profoundly low platelet counts. Severe thrombocytopenia, however, should always be regarded as a potentially life-threatening disorder. Severe gastrointestinal hemorrhage is the predominant cause of death in canine IMT patients. Less commonly, the loss of even small volumes of blood into a sensitive site such as the eye, brain or spinal cord can cause dramatic clinical signs such as blindness, seizure or paralysis. Nonspecific signs frequently associated with IMT include lethargy, weakness, anorexia, pyrexia, and pale mucous membranes. Splenomegaly is uncommon.

**Diagnosis of IMT**

Routine hematology is the first diagnostic step in patients with suspected IMT. The number of circulating platelets will be reduced, often dramatically (platelet count less than <10,000/µl). Examination of a blood smear may reveal megathrombocytes, indicative of marrow regeneration. Reticulated platelets, immature platelets that are increased in the circulation in conditions causing heightened thrombopoiesis, may also be measured via flow cytometry (when available). Marrow analysis is indicated if megathrombocyte or reticulated platelet numbers are low, since megakaryocytes may be reduced in number. Anemia (due to hemorrhage or concurrent IMHA) and neutrophilia may be present in IMT patients. Assessment of secondary hemostasis (prothrombin time and activated partial thromboplastin time) will generally reveal no abnormalities.

Primary IMT should be suspected in patients with an isolated severe thrombocytopenia in the absence of any detectable underlying causative disease such as disseminated intravascular coagulation, babesiosis or rickettsial infection. The unequivocal confirmation of suspected IMT then requires the demonstration of anti-platelet antibodies. Reliable tests for anti-platelet antibody, however, are often not readily available, although a sensitive flow cytometric assay is currently offered through Kansas State University. The diagnosis of canine IMT in practice often remains a diagnosis of exclusion. In most circumstances, practitioners should feel comfortable with a diagnosis of IMT in patients with isolated moderate or severe thrombocytopenia, reliable indications of increased thrombopoiesis, and no detectable evidence
of either multiple hemostatic abnormalities (suggesting DIC) or non-immunologic platelet sequestration, consumption or destruction. Treatment should not be withheld pending measurement of anti-platelet antibody levels.

Microthrombocytosis (presence of small platelet fragments) has previously been reported as a sensitive indicator of the presence of IMT. The technique, however, has not attained common usage.

**Detection of Anti-Platelet Antibody**

Numerous techniques have been developed in people to measure serum levels of anti-platelet antibody. Several of these methods have been modified for application in dogs and cats. The traditional method of measuring serum anti-platelet antibody is the platelet factor-3 (PF-3) immunoinjury technique. Other indirect methods for measuring serum anti-platelet antibody using various radioactive, enzymatic or fluorescent immunoglobulin labels have also been described. Measurement of antibody in serum is convenient for practitioners, because serum may be frozen for storage or transport, and very small volumes are adequate for testing. Unfortunately, the diagnostic utility of testing serum anti-platelet antibody is limited. Published test sensitivities vary widely depending on the test utilized and the criteria used to define IMT. Many patients with IMT have low serum levels of anti-platelet antibody which do not correlate well with platelet counts. Avid platelet-antibody binding in severely affected animals may effectively remove free antibody from the circulation. Despite low levels of serum anti-platelet antibody, such animals may have profound thrombocytopenia due to high levels of platelet-associated antibody.

The magnitude of antibody binding to platelets or marrow platelet precursors can also be measured. Platelet-associated antibody levels (particularly IgG) appear to consistently inversely correlate with platelet counts. Several techniques for measuring platelet-associated antibody levels in dogs and cats using immunoglobulin labels have been described. Flow cytometric techniques, in particular, hold promise as a means of detecting anti-platelet antibody, even in animals with very few platelets available for measurement because of severe thrombocytopenia. Kansas State University also currently offers flow cytometric measurement of platelet-bound antibodies in suspected IMT cases. Currently available techniques require relatively fresh platelets, necessitating rapid sample handling and transportation. Methods for measuring platelet-associated antibody have not been thoroughly evaluated, and test accuracies are not well determined.

Detection of megakaryocyte-associated antibodies can also provide indirect evidence of concurrent platelet-associated antibodies. High levels of megakaryocyte-associated immunoglobulin have been demonstrated by fluorescent labeling of marrow aspirates from canine IMT patients. Feline primary IMT has also been documented by immunoperoxidase labeling of megakaryocytes in formalin-fixed marrow biopsies. Marrow immunolabeling techniques have, however, not yet been clinically evaluated in large numbers of IMT patients. Immunolabeling will not be possible in those uncommon patients in which megakaryocytic hypoplasia precludes megakaryocyte collection. Adjunct immunodiagnostic testing may sometimes be indicated: patients with SLE may have positive serum ANA, and if IMHA is
suspected, a Coombs test should be performed.

No current test for anti-platelet antibody has indisputable diagnostic accuracy and clinical utility. Results of anti-platelet antibody tests should therefore not be the sole basis for clinical decision making. Confirmation of anti-platelet antibody usually does not assist clinical differentiation between primary and secondary IMT. Additionally, many disorders causing thrombocytopenia, although not usually classified as IMT, do have an immune-mediated component, and may therefore cause positive anti-platelet antibody tests. Positive tests may be detected, for example, in dogs with rickettsial infections, and in cats with thrombocytopenia associated with feline leukemia virus or antithyroid medications.

Identification of Underlying Disease

As with IMHA, primary IMT can only be diagnosed with certainty after underlying causes have been investigated. Screening tests for underlying disease which ideally should be performed in all animals with IMT include hematology, serum biochemistry, urinalysis, thoracic/abdominal radiography and, in cats, testing for retroviruses. Serologic or PCR testing for rickettsial infection is also indicated in endemic areas, as is a treatment trial with doxycycline, and testing for babesiosis is indicated in at-risk breeds such as greyhounds and pit bulls. Tests that may be considered in older animals in which IMT with underlying neoplasia is a possibility include abdominal ultrasonography, lymph node aspiration, and marrow analysis.