Immune-mediated hemolytic anemia (IMHA) is a common cause of anemia in dogs and cats. IMHA can be either primary (idiopathic or autoimmune) or secondary. Primary IMHA, a classic autoimmune disorder with no recognised underlying cause, is the most frequent form of IMHA in dogs. The condition typically affects young adult and middle-aged animals, and is most common in cocker spaniels, English springer spaniels, poodles, and old English sheepdogs. IMHA can also occur secondary to a wide range of infectious, inflammatory or neoplastic processes. Important causes of secondary IMHA in small animals include Feline Leukemia Virus (FeLV) or hemobartonellosis (mycoplasmosis) in cats, and recent vaccination or neoplasia (particularly lymphosarcoma) in dogs. Various medications have also been reported to trigger IMHA. Secondary IMHA affects animals of any age or breed, and should be strongly suspected in patients with a signalment atypical for primary IMHA, such as geriatric animals. Unlike the dog, IMHA in the cat is most commonly secondary. Distinction between primary and secondary IMHA is therapeutically important because secondary IMHA will often respond poorly to treatment, or recur, unless the underlying cause is recognized and eliminated.

### Potential Causes of Secondary IMHA

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<thead>
<tr>
<th>Medications</th>
<th>Infectious/Parasitic</th>
<th>Neoplastic</th>
<th>Miscellaneous</th>
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<tr>
<td>Trimethoprim/sulphonamide</td>
<td>Feline leukemia virus infection</td>
<td>Lymphoproliferative disease (esp. lymphosarcoma)</td>
<td>Post-vaccinal</td>
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<td>Penicillins</td>
<td>Hemobartonellosis (mycoplasmosis), esp. in cats</td>
<td>Hemangiosarcoma</td>
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<td>Cephalosporins</td>
<td>Ehrlichiosis</td>
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<td>Levamisole (dogs)</td>
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<td>Propylthiouracil/methimazole (cats)</td>
<td>Dirofilariasis</td>
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<td>Non-steroidal antiinflammatories (phenylbutazone)</td>
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<td>Chlorpromazine</td>
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<td>SLE</td>
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<td>Lymphoproliferative disease (esp. lymphosarcoma)</td>
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<td>Transfusion reactions</td>
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<td>Hemangiosarcoma</td>
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<td>Neonatal isoerythrolysis (esp. cats)</td>
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<td>Antilymphocyte globulin (transplantation patients)</td>
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**Mechanisms of Red Cell Destruction**

The mechanism underlying typical cases of IMHA is antibody-mediated cytotoxic (Type II) destruction of circulating red blood cells (RBCs). Although most cases share this common mechanism, the disease is otherwise very heterogeneous: in primary IMHA, the most studied form of IMHA, both the pattern of immunoglobulin and complement involvement in RBC destruction and the site of antibody attachment to RBC membranes varies widely between patients. Although the most common immunoglobulin type involved in primary IMHA is IgG, less commonly IgM may also be implicated, along with variable involvement of complement. Antibodies have been reported to attach to various components of the RBC membrane, particularly (but not exclusively) glycophorins.

Antibody attachment to cell membranes triggers RBC destruction by a number of different mechanisms. With high levels of antibody attachment and, particularly, complement fixation (with involvement of the membrane attack complex), membranes may be so damaged that extracellular water leaks into the cytoplasm, causing swelling and rupture of the RBC while it is still in the circulation, so-called intravascular hemolysis.

**Mechanism of Intravascular Hemolysis**

![Mechanism of Intravascular Hemolysis Diagram](image)

In the absence of direct RBC lysis, antibody attachment and subsequent cell membrane damage can still lead to an accelerated rate of destruction of affected RBCs by tissue macrophages within the mononuclear phagocytic system (MPS), a process that occurs outside of the circulation (extravascular hemolysis). MPS destruction of RBCs is mediated by Fc receptors on the macrophage surface, receptors which bind the Fc component of the antibodies attached to the RBC membranes. Since the MPS is located throughout the body, extravascular hemolysis can occur in many organs, but typically is most pronounced in the liver and, particularly, the spleen.
In some patients with high levels of anti-RBC antibodies, many individual antibodies can each bind to two different RBCs, a process that causes the cells to clump together (agglutinate). Patients that exhibit significant RBC agglutination at body temperature typically have an increased rate of extravascular hemolysis, since clumping of RBC slows their passage through vessels and facilitates their removal by the MPS.

Typically, IMHA is caused by antibodies directed against circulating, mature RBC, with the marrow mounting a healthy regenerative response to the resultant anemia. However, in some small animal patients (perhaps up to about one third), antibodies may also be directed against marrow RBC precursors at any stage in their development. Hemolytic anemia with an inappropriately poor regenerative response will develop if antibodies are directed against cell membrane components that are present both on mature RBC and their marrow precursors. In contrast, if antibodies are directed against membrane components that are present only on marrow precursors, and not on mature RBC, non-regenerative anemia will develop without peripheral hemolysis. Pure red cell aplasia (PRCA), in which all stages of marrow RBC precursor are dramatically reduced or absent, is the most extreme form of this process.

In primary IMHA, autoantibodies are directed against components of the patient’s own RBC membrane. Although the same process can occur with secondary IMHA, antibodies may alternatively be directed against a foreign antigen (such as a drug or virus) that is attached to the RBC membrane, against normal RBC membrane components that are antigenically similar to non-RBC antigens that are associated with the underlying disease process, or against membrane components that are normally hidden but are exposed by the underlying disease.
Categories of IMHA

Typical IMHA is caused by antibodies that exert their effects at body temperature, so-called warm reactive antibodies. Some animals, however, have anti-RBC antibodies that are only reactive at much lower temperatures. Although such cold reactive antibodies usually cause minimal harmful effects, their presence can potentially cause specific clinical syndromes, and can also lead to a false positive diagnosis of IMHA if tests such as slide agglutination are performed at cold temperatures. Classically, IMHA has been sub-divided into five main categories based on the thermal reactivity of the anti-RBC antibodies, and their major clinical effects at optimal temperature:

1. **Warm Antibody Type, Agglutination:**
   
   High levels of antibody lead to detectable autoagglutination of RBC. Agglutination is often associated with acute severe extravascular hemolysis.

2. **Warm Antibody Type, Intravascular Hemolysis:**
   
   Intravascular hemolysis, usually associated with high levels of antibody and complement fixation, causing severe anemia with detectable hemoglobinemia and hemoglobinuria.

3. **Warm Antibody Type, Incomplete Antibody:**
   
   Anti-RBC antibodies cause extravascular hemolysis, without detectable autoagglutination or hemoglobinemia and hemoglobinuria. Disease onset can be chronic or sub-acute, and resultant anemia varies from mild to severe.

4. **Cold Antibody Type, Agglutination:**
   
   Anti-RBC antibodies are only reactive at cold temperatures, and agglutination does not occur at body temperature. Agglutination can however occur within the vasculature of the extremities, particularly in colder weather. Obstruction of the blood supply to the peripheral vasculature due to agglutination can lead to ischemic necrosis of the ear or tail tips, the end of the nose, and the feet.

5. **Cold Antibody Type, Non-agglutinating Hemolysis:**
   
   Antibodies are again only reactive at cold temperatures, and hemolysis does not occur at body temperature. In cold weather, however, some degree of hemolysis may occur within the extremities, which manifests clinically as transient hemoglobinemia and hemoglobinuria.

Although the above categorisation system is derived by extrapolation from human beings, all five categories of IMHA have been reported in small animals. Agglutinating and (especially) hemolysing cold antibody types of IMHA are however rare in both dogs and cats. Intravascular warm antibody type IMHA is also relatively uncommon.
**Clinical Signs**

Signs typically associated with IMHA reflect the presence of both anemia (lethargy, weakness, pale mucous membranes, and a hemic heart murmur) and compensatory responses caused by tissue hypoxia and stimulation of the sympathetic nervous system (tachypnea, tachycardia, and bounding pulses). Some patients may also show clinical signs of an ongoing immunological or inflammatory process, such as pyrexia, anorexia and, uncommonly, lymphadenopathy. Surprisingly, since the MPS within the spleen and liver is usually the main site of RBC destruction, organomegaly is only variably present in animals with IMHA. Patients with IMHA of acute onset tend to be very severely affected by their anemia, and are often very depressed, weak or even collapsed. Hyperbilirubinemia, bilirubinuria and tissue jaundice are often seen during acute severe episodes of IMHA. Since intravascular hemolysis is uncommon, hemoglobinemia and hemoglobinuria are observed infrequently. Patients with extravascular hemolysis due to sub-acute or chronic IMHA can compensate to some extent for their lack of erythrocytes, and may be remarkably bright despite the presence of severe anemia. In these patients, the liver can often cope with the extra bilirubin released by RBC breakdown, and jaundice does not occur.

Pulmonary thromboembolism (PTE) is a well-recognised complication of IMHA, and is particularly common in those animals with acute severe anemia that are receiving high dose glucocorticoids. Pulmonary thromboembolism should always be suspected in those anemic patients that suddenly develop severe and persistent dyspnea, although other causes of dyspnea such as cardiogenic pulmonary edema or acute bacterial pneumonia should also be considered, especially in dogs already receiving glucocorticoid and immunosuppressive therapy. Disseminated intravascular coagulation (DIC) can also complicate IMHA, but clinically significant DIC is probably uncommon to rare.

**Diagnosis of IMHA**

Hematology in patients with IMHA typically reveals a moderate to severe anemia, which is most commonly regenerative, with anisocytosis, polychromasia, a high corrected reticulocyte count and, sometimes, increased numbers of nucleated RBCs. Reticulocyte counts can however sometimes be inappropriately low, either because antibodies are also directed against RBC precursors, or because anemia is peracute (since it takes about 5 days for the marrow to mount a strong regenerative response). White cell and neutrophil counts are often moderately to markedly increased, probably in response to both non-specific marrow stimulation and the inflammatory process associated with RBC breakdown. Occasionally, white cell counts can be high enough to mimic myelogenous leukemia, a reaction sometimes called a ‘leukemoid response’. Platelet counts are usually normal unless the animal also has immune-mediated thrombocytopenia (IMT) or platelet consumption secondary to PTE or DIC. Concurrent IMHA and IMT, a condition known as Evan’s syndrome, may affect up to approximately 10% of dogs with IMHA, but the frequency of Evan’s may be overestimated if the effects of PTE or DIC on platelet counts are not considered as an alternative diagnosis to IMT.
Hematology can often also reveal clues that suggest a specific etiological diagnosis:

1. **Spherocytosis:**

   Spherocytes are small spherical erythrocytes that, when present in high numbers, strongly suggest a diagnosis of either primary or secondary IMHA. The absence of spherocytes, however, does not absolutely exclude a diagnosis of IMHA. Spherocytes are formed when tissue macrophages remove a piece of RBC membrane without cell destruction or a significant loss of cytoplasm. Since cytoplasm is not lost, RBC volume (as indicated by MCV) remains normal. Spherocytes can be difficult to recognize in cats, because normal feline RBCs tend to be smaller and less discoid than canine RBCs. Experienced veterinary clinical pathologists, however, may be able to recognize the presence of spherocytes in the cat.

2. **Agglutination:**

   Examination of blood smears may reveal microscopic autoagglutination (clumping) of RBCs. Such agglutination can form large rafts of RBC that, when a collection tube containing anticoagulated blood is closely inspected, are visible to the naked eye as multiple red speckles. Similar speckles can however be created by rouleaux formation, a phenomenon that can occur in normal animals, especially cats. Clinicians should therefore perform a saline dilution (one drop of RBCs to one drop of saline in dogs, one drop of RBCs to two drops of saline in cats) slide agglutination test to differentiate rouleaux from genuine autoagglutination. True agglutination can be seen grossly as persistent speckles despite dilution with saline, and microscopically as non-linear clumps of RBCs.
A  Antibody-Mediated Agglutination

Add Saline

Gross Granularity

Persistent Granularity
Positive Slide Agglutination

B  Rouleaux Formation

Add Saline

Gross Granularity

Loss of Granularity
Negative Slide Agglutination
A positive slide agglutination result is highly suggestive of a diagnosis of IMHA, and also suggests that the condition is likely to be acute and severe. A negative slide agglutination does not rule out IMHA, since in fact a negative result has been reported in some studies to be the most common result in small animals with IMHA because most actually have non-agglutinating antibodies. Recent clinical studies of canine IMHA, however, report a much higher incidence of positive slide agglutination, perhaps reflecting a referral bias as a result of practitioners tending to refer only the more severe cases of IMHA. Cell washing techniques using repeated centrifugation and saline washes have been reported to decrease the diagnostic sensitivity and increase the specificity of slide agglutination. Automated hematology analysers sometimes register a clump of agglutinated RBCs as a single cell, often of a size too large to even be recorded as a RBC at all. Resultant erroneous results may include an artificially high MCV or, if clumped cells are not recognised as erythrocytes, lowering of the calculated hematocrit. Since the hemoglobin within all RBCs is still measured by the analyser, this leads to an erroneously high estimation of mean corpuscular hemoglobin concentration (MCHC). When agglutination is suspected to be the cause of a lower than expected hematocrit, packed cell volume (PCV), which is not affected by RBC clumping, should be monitored using microhematocrit tube centrifugation rather than an automated analyser.

3. Other RBC Abnormalities:

Careful examination of RBC morphology may suggest an underlying cause of either immunological or non-immunological hemolysis. Diagnostically useful RBC abnormalities include detection of parasites such as *M. hemofelis* (which may cause secondary IMHA), Heinz bodies (suggesting hemolysis secondary to oxidative damage) and schistocytosis (suggesting a microangiopathic hemolytic process such as DIC).

Serum biochemistry and urinalysis are often normal in dogs with IMHA. Potential abnormalities that may be seen in some patients include mild to moderate elevation of liver enzymes (thought to indicate hepatic hypoxia secondary to severe anemia) and variable hyperglobulinemia. Since serum albumin is usually normal, hypoalbuminemia is an unexpected finding that may suggest that anemia is in fact due to occult blood loss rather than hemolysis, or that the patient also has another illness. Mild to moderate hyperbilirubinemia and bilirubinuria may be seen transiently in animals with acute severe anemia. Since the liver is usually able to cope with all but the transient overwhelming bilirubin loads produced by acute severe hemolysis, severe hyperbilirubinemia or persistence of jaundice for more than 3 to 5 days, even in the markedly anemic animal, usually indicates the presence of concurrent hepatic disease or biliary obstruction. Hemoglobinemia and hemoglobinuria are uncommon, transient events that indicate the presence of severe intravascular hemolysis.

Immunological Testing

Specific immunological testing can be used to support a tentative diagnosis of IMHA. The most widely used test is the direct antiglobulin test (DAT) or Coombs’ test, which detects antibodies and/or complement bound to RBC membranes. A standard DAT as provided by most laboratories typically uses a mix of antibodies directed against IgG, IgM (to a variable extent)
and complement, and is performed at body temperature. Modifications of the routine screening DAT that may increase its diagnostic value include running the test at different temperatures and titers, and using individual antibodies against IgG, IgM, IgA and complement as well as the standard polyvalent antibody/complement mix. Positive DAT results at 4° Celsius, however, are of minimal diagnostic significance unless the patient has clinical signs consistent with cold antibody type agglutination or intravascular hemolysis.

**Mechanism Underlying Coomb’s Test (DAT)**

Strictly interpreted, a positive DAT supports a diagnosis of IMHA, while a negative test suggests a non-immunological cause of hemolysis. Numerous studies, however, have shown that a DAT can often be of only mediocre diagnostic accuracy: although sensitivity and specificity undoubtedly improve with meticulous attention to test methodology, the fact remains that both false positive and false negative results do occur relatively commonly. Veterinarians should therefore be aware that since IMHA can occur in the presence of a negative DAT and, conversely, a positive test does not absolutely prove the presence of IMHA, sometimes a diagnosis must be made based on clinical judgement despite the presence of an apparently discrepant DAT result. Performing a DAT is however still recommended in all patients with suspected IMHA even if criteria such as spherocytosis or a positive slide agglutination already strongly suggest a diagnosis, since a positive DAT will add support to the diagnosis and characterise the disease further by determining the involvement of various immunoglobulin types and complement. Various other immunological tests for detecting anti-RBC antibody have been reported, including an enzyme-linked immunosorbent assay, and a direct enzyme-linked antiglobulin test but, although some of these tests may arguably be more sensitive than the DAT, they have not as yet become commonly available. Regardless of whether a DAT or an alternative test for ant-RBC antibody is used, however, clinicians should be aware that a positive
result merely records the presence of antibody, and does not determine whether IMHA is primary (AIHA) or secondary.

Uncommonly, IMHA (with or without IMT) will be merely one component of systemic lupus erythematosus (SLE), a multisystemic immunological disturbance. Measurement of serum antinuclear antibody (ANA) is therefore indicated in those patients displaying evidence of the concurrent involvement of more than one body system, such as IMT, glomerulonephritis, polyarthritis, polymyositis or immune-mediated skin disease. In contrast, ANA is not indicated (and is usually negative) in those patients suspected to have uncomplicated IMHA.

**Identification of Underlying Disease**

Since IMHA is often secondary, particularly in cats and in dogs with an atypical signalment, confirmation of a diagnosis of IMHA is not necessarily the end of the diagnostic trail. Primary IMHA can only be diagnosed with absolute certainty once potential underlying causes have been thoroughly investigated. Unfortunately, this presents practitioners with a dilemma: although IMHA is unlikely to be treated effectively unless underlying causes have been eliminated, a complete search for such causes can be expensive, time-consuming, invasive and, in the case of primary IMHA, ultimately fruitless. Standard screening tests for underlying disease which ideally should be performed in all animals with IMHA include hematology (including careful examination of a blood smear), serum biochemistry, urinalysis, thoracic and abdominal radiography and, in cats, testing for retroviruses (particularly FeLV). Serologic and/or PCR testing for RBC parasites such as hemobartonellosis, now more correctly termed mycoplasmosis (*Mycoplasma hemofelis* in cats, *Mycoplasma hemocanis* in splenectomized dogs), *Babesia canis* (particularly in greyhounds) or *Babesia gibsoni* (particularly in pit bull terriers) is also often indicated. Since arguably rickettsial diseases may also predispose to secondary IMHA, testing for *Ehrlichia* species may also be indicated in endemic areas. Further tests that might be considered in some patients, particularly in older animals in which underlying occult neoplasia (especially lymphoproliferative disease) is a real possibility, include abdominal ultrasonography, lymph node aspiration cytology, and bone marrow analysis.

**Bone Marrow Analysis**

Bone marrow analysis (aspiration cytology and/or core biopsy histopathology) is also indicated in all patients suspected to have the non-regenerative forms of IMHA. Pure red cell aplasia is characterised by a relative or complete lack of RBC precursors within the marrow, whereas cytological or histopathological evidence of an erythroid 'maturation arrest' (preponderance of immature precursors, with an absence of more mature RBC precursors) suggests that, rather than being directed against very early stem cells, antibodies are directed against a later stage of marrow RBC development. Marrow cytology and/or histopathology may also reveal macrophages phagocytosing erythrocytes or RBC precursors. In such patients, when available, techniques such as immunofluorescent or immunoperoxidase staining of marrow samples may confirm the presence of antibodies directed against RBC precursors.

**IMMUNE-MEDIATED THROMBOCYTOPENIA:**
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.

**Immune-Mediated Thrombocytopenia**

![Diagram of Immune-Mediated Thrombocytopenia](image)

**Reduced Circulating Platelet Survival Time**
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. Non-specific 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.
The most common immune-mediated blood disorders in small animal patients are immune-mediated thrombocytopenia (IMT) and immune-mediated hemolytic anemia (IMHA). Less common disorders that may have an immune-mediated component include pure red cell aplasia (PRCA), aplastic anemia, amegakaryocytic thrombocytopenia and steroid-responsive neutropenia. Immune-mediated blood disorders can be either primary (idiopathic) or secondary. As a general rule, these disorders in dogs are most commonly primary, whereas in cats they are more likely to be secondary. Since treatment of IMHA and IMT has more similarities than differences, most therapeutic approaches apply equally well to both disorders.

Veterinarians have been effectively treating individual patients with IMHA and IMT for many years. Standard therapy is based around transfusion as needed, coupled with immunosuppressive therapy (prednisolone or dexamethasone, with or without concurrent azathioprine, cyclophosphamide or cyclosporine) that is tapered and then discontinued. Unfortunately, however, there is a mounting body of evidence documenting that, with standard therapy, survival rates for IMT and (particularly) IMHA patients are unsatisfactory. One study from Virginia-Maryland, for example, reported that, despite their best therapeutic efforts, one-year survival rate for dogs with IMHA was still only 30%. Most other published studies have long-term survival rates of not much better than 50%. Deaths (naturally occurring or euthanasia) occurred either during initial hospitalization, or at a later date due to disease recurrence or owner intolerance of long-term medication. Undoubtedly, there is a 'referral bias' that will exaggerate the severity of disease in some studies since, with recent advances in in-house diagnostics, better availability of transfusion products, and a greater understanding of immunosuppressive therapy, many general practitioners can now effectively treat the less severe blood disorders without referral. Critical patients with severe or complicated IMHA and IMT are more likely to be referred to specialist centers, and are also more likely to die despite treatment, contributing to the high mortality rates in studies that originate from referral centers. Nevertheless, despite the potential effects of this referral bias, it is still undeniable that mortality rates for the immune-mediated blood disorders are unacceptably high.

Two main priorities can be readily identified from analysis of IMHA and IMT mortality data: firstly, the rate of in-hospital deaths during the initial immune-mediated crisis must be reduced and, secondly, more long-term therapy must be tailored in order to avoid relapses while minimizing expense and drug-induced side effects. This first lecture will therefore concentrate on optimizing the initial emergency management of IMT and IMHA, and the subsequent lectures will focus on long-term management strategies with immunosuppressive therapy.

**Initial Investigation**

Since effective treatment can not proceed without a correct diagnosis, a thorough work-up is
always recommended during the initial management of IMHA and IMT. A standard diagnostic approach has been outlined in the two preceding Immune-Mediated Hemolytic Anemia and Immune-Mediated Thrombocytopenia lectures. Given a working diagnosis of primary immune-mediated blood disease, standard therapy during an initial crisis will typically include immunosuppressive doses of glucocorticoids with or without other immunosuppressive agents, and transfusion as needed. Even if an underlying cause for secondary IMHA or IMT has been identified and removed, immunosuppressive therapy is still usually indicated during the initial treatment phase.

**Emergency Drug Therapy**

Glucocorticoid therapy, although a mainstay of both the initial and the chronic treatment of IMT and IMHA, is outlined in greater detail in the following Immune-Mediated Blood Disorders: Chronic Management lecture. Oral prednisolone (or prednisone) dosage at the commencement of therapy should be 2 mg/kg once or twice daily. Although some clinicians prefer to commence therapy with an initial dose of either intravenous dexamethasone (0.1 to 0.2 mg/kg) or intravenous high dose methylprednisolone (11 mg/kg daily for up to 3 days), there is minimal hard evidence that starting with intravenous steroid therapy hastens recovery. Typically, regardless of route of administration or starting dose, steroids are not immediately effective.

Immunosuppressive therapy with drugs such as azathioprine, cyclophosphamide, cyclosporine or mycophenolate is also is discussed in greater detail in the following Immunosuppressive Therapy lectures. Even in severely affected patients, these drugs are usually given orally at standard starting dose rates. Cyclophosphamide, however, is sometimes also given intravenously (200 mg/m²) in dogs with acute, severe IMT or IMHA. There is little evidence that commencing with a high-dose intravenous bolus of cyclophosphamide hastens recovery. In fact, several retrospective studies have reported high mortality rates in IMT patients that are initially treated with cyclophosphamide, even at standard conservative oral doses. Given the limitations of a retrospective study, however, it is by no means proven that cyclophosphamide actually increases mortality rates, since factors such as case selection bias (for example, clinicians may reserve the use of cyclophosphamide for their sickest patients) may influence apparent survival rates in animals treated with cyclophosphamide. Cyclosporine is also available in a solution for intravenous use (6 mg/kg, given over 4 hours) as is mycophenolate mofetil (same doses as oral doses discussed in later lectures) although, like cyclophosphamide, there is minimal strong evidence that intravenous administration hastens recovery during crises.

Dogs with IMT may respond to a single intravenous bolus of vincristine (0.02 mg/kg). The vinca alkaloid is inexpensive and usually well tolerated, and prospective studies have reported that a single initial dose of vincristine hastens recovery of platelet numbers in some canine patients. The vinca alkaloids have both mild immunosuppressive (impairment of MPS function, and inhibition of cell-mediated and humoral immunity) and thrombocytotic (stimulation of transient megakaryocyte platelet release) properties. Intravenous vinca alkaloids induce transient platelet number increases in many IMT patients: circulating platelet life-span may be prolonged following treatment, suggesting that the increased platelet number is due to decreased destruction as well as enhanced megakaryocyte platelet release. Vinca alkaloids avidly bind to tubulin, a major component of platelet microtubules. The antibody-coated vinca-containing platelets of
IMT patients are subsequently phagocytosed by tissue macrophages. Vinca alkaloids are therefore selectively delivered in cytotoxic doses to the macrophages involved in platelet destruction (so-called ‘poison platelets’).

**Mechanism of Action of Vincristine in IMT Patients**

Vincristine is the vinca alkaloid most commonly used in the dog. Intravenous vincristine markedly increases platelet numbers in some canine IMT patients, often within two to three days. Vincristine (a single intravenous dose) is therefore recommended for the emergency management of canine IMT.

Intravenous vinca alkaloid boluses are cleared from the circulation too rapidly for optimal vinca-platelet binding. Although weekly vinca boluses maintain remission in some human IMT patients, most eventually become refractory. Techniques maximizing vinca-platelet binding have improved remission rates: either constant vinca infusion over four to eight hours, or transfusion with platelets pre-incubated with vinca alkaloid (‘vinca-loaded’ platelets). Although reported, similar techniques have not been thoroughly clinically evaluated in the dog. Such techniques are labor-intensive, and are not commonly used in veterinary medicine.

Vincristine is extremely corrosive if extravasated. Single vincristine doses are otherwise well tolerated. Chronic vincristine therapy has been associated with reversible peripheral neuropathy in humans, and a comparable vincristine-associated neuropathy has recently been reported in the dog. Vincristine inhibits platelet function *in vitro*. However, clinically significant platelet dysfunction of any significant duration which can be unequivocally attributed to vincristine has not been documented *in vivo*.

**Supportive/Ancillary Therapy**

IMT and IMHA patients with severe blood loss or hemolytic anemia will be suffering from generalised tissue hypoxia, and will benefit from reducing oxygen demand by instituting strict
cage rest until anemia responds to therapy. The severely compromised patient can also be supported with oxygen supplementation. Hemoglobin oxygen saturation is however already near maximal, and supplementation with oxygen therefore increases saturation only minimally. Oxygen supplementation is also laborious and expensive. Since patients with IMHA have a normal blood volume, crystalloid or colloid fluid therapy is of little benefit and may contribute to volume overload. Hypovolemic IMT patients, in contrast, may benefit from fluid therapy. An additional benefit of strict cage rest in IMT patients is that rest reduces the chances of traumatic vascular injury, which in turn reduces the chances of life-threatening bleeding in severely thrombocytopenic animals.

Since patients with IMHA are prone to pulmonary thromboembolism (PTE) and, to a lesser extent, disseminated intravascular coagulation (DIC), particularly those with severe anemia and/or a positive slide agglutination, and those requiring transfusion, some clinicians recommend using prophylactic heparin during the hospitalisation of severely affected animals. A safe low dose of heparin that does not cause spontaneous bleeding, and does not require careful monitoring of coagulation parameters, is 75 to 100 U/kg three to four times daily subcutaneously. Much higher doses of heparin (starting at 200-250 U/kg SC four times daily), titrated upwards in order to prolong partial thromboplastin times by at least 1.5 times baseline values, may however be more effective at preventing thromboembolism. Measurement of plasma heparin levels, with subsequent dosage adjustments to attain a therapeutic range, may prove to be another means of maximizing the benefit of heparin therapy. Heparin may also be administered as a constant rate infusion. Plasma heparin assays are available: the Cornell University Hemostasis Laboratory indirectly measures plasma heparin levels via an inhibition of factor Xa assay. The standard form of heparin that is currently used in veterinary medicine is unfractionated heparin. However, the use of low molecular weight forms of heparin such as dalteparin or enoxaparin (which, in people, have a more predictable bioavailability than unfractionated heparin) may allow safer and more effective anticoagulation: we are currently using enoxaparin at a dose of 0.8 mg/kg SC q6hrs, based on Xa inhibition assays. Low dose aspirin and/or clopidogrel (Plavix) can also be considered. Since intravenous catheters, particularly jugular catheters, can predispose to thromboembolism, catheter placements in IMHA patients should be minimized. Unfortunately, however, despite our best intentions the use of escalating doses of heparin and other anticoagulant drugs and the avoidance of unnecessary catheters have not been shown to reliably prevent PTE in IMHA dogs. There is clearly a pressing need for us to develop a more effective means of preventing this common and disastrous complication in our IMHA patients.

Transfusion

Cage rest and standard glucocorticoid and immunosuppressive drug therapy are successful in most small animal patients with non-life-threatening IMHA and IMT. However, initial response to therapy can sometimes be sluggish (a week or more), particularly in those animals with poor marrow responsiveness due to either peracute anemia or immune-mediated damage to bone marrow RBC or platelet precursors. In the meantime, transfusion may be needed to support those patients with life-threatening acute and severe anemia (PCV less than about 15%, or signs of severe compromise, such as collapse, nystagmus or stupor). Transfused red blood cells often have a very short life span (days or even hours) in patients with IMHA, and transfusions may
actually increase the rate of hemolysis (‘add fuel to the fire’). For this reason, transfusions should be avoided when possible in stable patients with IMHA. However, in those IMHA patients that are severely compromised, blood transfusions are life-saving, and should not be withheld. Transfused platelets in IMT patients typically have an extraordinarily short circulating survival time and, in fact, platelet numbers have often not even detectably risen immediately after a platelet transfusion. Transfusion to replace lost platelets is therefore rarely of value in IMT patients, although there is no real contraindication to trying a single test dose of a platelet product. Transfusion of RBC products in order to support hypovolemic or anemic IMT patients, on the other hand, can often be life-saving, even if the transfusion had no impact on platelet numbers.

In normovolemic animals, such as most patients with IMHA, whole blood may be safely transfused at a rate of up to approximately 5-10 ml/kg/hour, usually at a maximum daily volume of 20 ml/kg. Multiple transfusions as often as every day or two may be needed in very severely affected animals. Since IMHA patients are typically normovolemic, volume overload after transfusion can become a significant risk in animals that have already recently received high volumes of blood or other fluids. In these patients, blood transfusions should be given slowly (maximum rate of 4 ml/kg/hour). When available, packed red blood cells are preferable to whole blood. Since cross-matches are often positive in patients with IMHA (because the animal has antibodies against its own RBC, and can even ‘cross-match’ positive against its own blood, as well as donor blood), compatible or universal donors should be used if blood typing is available.

Over the past decade or so, bovine purified polymerized hemoglobin (Oxyglobin®) was used as an effective means of providing temporary (several days) oxygen-carrying support for the severely anemic IMHA patient. Bovine polymerized hemoglobin was a very convenient blood product for use in general practice, in that it was associated with almost no risk of transfusion reaction, could be safely used without blood typing or cross-matching, and could be stored for up to two years at room temperature. Although the product was developed and marketed for use in dogs at doses of 10-30 ml/kg, bovine polymerized hemoglobin was also reported to provide effective temporary support to anemic cats at a dose of 10 ml/kg given over several hours. Since the product was a colloid, it had the potential to cause volume overload if given too fast. One retrospective study reported a very high mortality rate in canine IMHA patients that received bovine polymerized hemoglobin, although these results may potentially have been affected by a pre-treatment case selection bias (that is, the sickest patients got the polymerized hemoglobin). Bovine polymerized hemoglobin was expensive and, unfortunately, has become for all practical purposes unavailable, although it may be re-entering the market soon.

In IMT patients with severe blood loss anemia or hypovolemia, fresh or stored packed red cell or whole blood products can be life saving. In severely hypovolemic IMT patients with ongoing bleeding, blood can be given to effect at rates that can greatly exceed 20 ml/kg/day if needed. Although products such as platelet concentrate, platelet-rich plasma and fresh whole blood can be given in order to provide platelets, the transient survival time of most transfused platelets typically renders such treatments ineffective. The main focus of transfusion therapy in IMT patients therefore should be to provide red cell and volume support in the bleeding patient.
Advanced Emergency Therapy

Unfortunately, some animals with IMHA and IMT, despite appropriate standard therapy and multiple transfusions, succumb to severe anemia or blood loss during the first weeks of treatment. Additional treatment options which may be used in a crisis include gammaglobulin, plasmapheresis and splenectomy.

High intravenous doses of human immunoglobulin (HIVIG), as a 6 to 12 hour infusion at doses ranging from 0.5 to 1.5 g/kg, occasionally cause rapid and sometimes sustained remission of immune-mediated disorders, including IMHA, PRCA and IMT. Human intravenous immunoglobulin is a pooled preparation of IgG obtained from the plasma of multiple healthy blood donors. Although HIVIG were initially produced for treatment of immunodeficiencies, they have also been shown to be beneficial in the treatment of human immune-mediated diseases such as IMT and IMHA. The main proposed mechanism of action of HIVIG is that the 'antibody soup' bathing the MPS binds to and overwhelsms available macrophage Fc receptor sites, leaving no receptors left to bind antibody-coated cells. Alternatively, there may be some antibodies in the HIVIG soup that actually bind to and inactivate circulating anti-platelet or anti-RBC antibodies.

Proposed Mechanisms of Action of HIVIG

The use of HIVIG in dogs is associated with few side effects, although there is some concern that treated animals have a higher incidence of pulmonary thromboembolism. Certainly, a high rate of PTE in HIVIG-treated patients was reported in the human literature, raising concerns about using HIVIG in canine IMHA patients. Pulmonary thromboembolism is less of a concern in IMT patients and, recently, HIVIG has been shown to safely and effectively shorten the duration
of thrombocytopenia in dogs with IMT. Human gammaglobulin has not attained common usage in veterinary medicine, probably because of high cost and occasional limited availability.

Plasmapheresis and splenectomy, although reported to be useful in isolated cases, have also not entered into common use, and are usually considered treatments of last resort. Plasmapheresis, when available, is a very effective method of rapidly removing unbound anti-RBC or anti-platelet antibodies from the circulation, although antibodies that are already bound to cell membranes will persist and may cause ongoing disease.

Splenectomy is potentially a particularly effective treatment for IMT and IMHA because many different splenic elements contribute to the mechanisms reducing circulating blood cell numbers: anti-RBC or anti-platelet antibody production (splenic lymphocytes), antibody-coated platelet or RBC destruction (splenic MPS), and platelet or RBC sequestration (splenic vasculature). Splenectomy is the treatment of choice for most humans with chronic IMT or IMHA, with higher remission rates than medical therapy. In human IMT patients, platelet numbers often rise within several hours of splenectomy, with maximal increases within one to two weeks. Most human IMT and IMHA patients (60% to 80%) subsequently maintain adequate platelet or RBC counts without further medical therapy. Splenectomized patients that do require further treatment frequently demonstrate an improved response to medical therapy. Splenectomy is therefore recommended early in the course of chronic human IMT or IMHA.

Splenectomy has not been thoroughly clinically evaluated in a large group of small animal IMT or IMHA patients, although several case series reporting early splenectomy in small groups of canine IMHA patients certainly showed some promising preliminary results. Other than these relatively recent studies, published post-splenectomy remission rates for canine IMT and IMHA (each study limited to small patient groups) vary from poor to excellent. Since response rates appear to be unpredictable, early splenectomy currently cannot be strongly recommended for canine IMT or IMHA, particularly as medical therapy is often far better tolerated than it is in people. Splenectomy is indicated in canine patients refractory to glucocorticoids, immunosuppressive agents and danazol, particularly if associated drug side effects are unacceptable.

Life-threatening post-splenectomy complications in people (overwhelming infection, DIC) are rare in the dog. The most commonly reported small animal post-splenectomy complication, erythrocyte parasitemia (*Hemobartonella [Mycoplasma], Babesia*), usually responds well to medical therapy.

Persistent IMT or IMHA post-splenectomy indicates ongoing platelet or erythrocyte destruction by the non-splenic MPS (usually hepatic macrophages). Uncommonly, post-splenectomy platelet or RBC destruction can also occur within an 'accessory spleen' (a detached splenic remnant with residual MPS function). Some authors recommend exploratory laparotomy in humans with persistent IMT or IMHA: removal of an accessory spleen may induce complete remission.
Immune-mediated polyarthritis (IMPA) is a common disease process in the dog. The immune-mediated polyarthropathies are divided into two major categories: erosive (or deforming) and non-erosive (or non-deforming).

Pathophysiology

Immune-mediated non-erosive polyarthritis is believed to be driven by a type III hypersensitivity reaction, where immune complexes comprised of antigen bound to antibody accumulate in the joint space. Implicated antigens are typically found in the systemic circulation, but can originate from within the joint space itself. Systemic immune complexes can arise from a variety of chronic antigenic stimuli, including but not limited to viruses such as distemper virus, other microbial agents, neoplasia, drug haptenes or even dietary elements. In addition, antibodies directed against self-antigens, such as heat shock proteins, immunoglobulins (rheumatoid factors) and nuclear elements (anti-nuclear antibodies) can also form complexes that accumulate in the joint space. The presence of immune complexes in the joint space activates complement along the synovial membrane and within the synovial fluid. Complement fixation results in tissue damage and release of cytokines, some of which attract neutrophils. These neutrophils also release cytokines and lysosomal enzymes that cause further tissue damage.

In canine rheumatoid arthritis, a disease characterized by erosive joint damage, antibody directed against type II collagen has been found along the joint surface as well as rheumatoid factors within the joint fluid. In addition, chronic persistent synovitis exists that is characterized by perivascular accumulation of mononuclear cells, indicating a possible type IV hypersensitivity component to this destructive disease. T lymphocytes, macrophages and fibroblasts release matrix-degrading enzymes such as metalloproteinases, which cause cartilage degeneration and further inflammation.

Erosive Polyarthritis

The erosive forms of immune-mediated polyarthritis are very rare compared to the non-erosive forms, and represent only about 1% of all canine polyarthritis cases. These forms are characterized by the presence of radiographic changes consistent with subchondral bone destruction. Radiographic changes may include irregular joint surfaces, a narrowing or widening of the joint space, and punched out lesions along the joint surface. It is important to note that radiographic changes can take up to 6 months to appear. Therefore, dogs with apparently non-erosive forms of polyarthritis in which clinical signs persist should be periodically re-evaluated for erosive changes.

Rheumatoid arthritis is the most notable form of immune-mediated erosive polyarthritis, but Felty’s syndrome and erosive polyarthritis of Greyhounds have also been described. Felty’s syndrome is a disease triad characterized by rheumatoid arthritis, neutropenia and splenomegaly.
described in humans and several dogs. Erosive polyarthritis of greyhounds has been reported most frequently in Australia and England. *Mycoplasma spuman* has been isolated from at least one affected Greyhound.

Rheumatoid arthritis is typically diagnosed in small, middle aged dogs. Diagnostic criteria for rheumatoid arthritis in the dog are adapted from those defined for humans. The presence of 5 criteria is suggestive of rheumatoid arthritis, and the presence of at least 7 out of 10 criteria is considered supportive of a definitive diagnosis of canine rheumatoid arthritis.

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<tr>
<th>Diagnostic Criteria for Rheumatoid Arthritis in the Dog</th>
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<tr>
<td>1. Stiffness</td>
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<td>2. Pain on manipulation of at least one joint</td>
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<tr>
<td>3. Signs of arthritis for at least 3 months</td>
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<td>4. Periarticular soft tissue swelling</td>
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<td>5. Typical radiographic changes such as subchondral bone destruction, indicated by irregularity of articular surface or ‘punched out' erosions and loss of mineralization of epiphysis, calcification of soft tissue around joint, changes in joint space (increased or decreased width) or extensive bone destruction with gross joint deformity</td>
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<tr>
<td>6. Inflammatory synovial fluid</td>
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<td>7. Characteristic, symmetrical deformations of distal joints</td>
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<tr>
<td>8. Detection of rheumatoid factors (anti-globulins) in serum</td>
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<tr>
<td>9. Three of the following histopathologic changes in synovial membrane: marked villous hypertrophy, synovial cell proliferation, fibrin deposits, foci of necrosis and lymphocytic-plasmacytic infiltration</td>
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<tr>
<td>10. Extra-articular symptoms such as lymphadenopathy</td>
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The stifle and carpal joints in dogs with rheumatoid arthritis are commonly affected, and also occasionally the digital joints. Antibodies directed against IgM, IgG and IgA (rheumatoid factors) have been identified in the joint fluid and blood of dogs diagnosed with rheumatoid arthritis. Establishing a definitive diagnosis of rheumatoid arthritis is important since this disease has a more guarded prognosis compared to most of the more common non-erosive polyarthropathies.

**Non-Erosive Polyarthritis**

Non-erosive forms of immune-mediated polyarthritis that lead to neutrophilic inflammation in multiple joints include idiopathic polyarthritis, vaccine- and drug-induced polyarthritis,
polyarthritis/polymyositis syndrome, steroid-responsive meningitis-arteritis, and breed specific polyarthropathies such as juvenile-onset polyarthritis of Akitas and familial Chinese Shar-pei fever. Systemic lupus erythematosus (SLE) also commonly involves the joints but less than 20% of canine cases of polyarthritis can be attributed to SLE. Idiopathic polyarthritis is by far the most common form of non-erosive immune-mediated polyarthritis in the dog.

**Idiopathic Polyarthritis**

Idiopathic polyarthritis refers to all cases of immune-mediated arthropathy that cannot be classified into the other groups previously mentioned. Sporting dogs and large breed dogs are over-represented, and the majority of affected patients are young adults with ages ranging from about 2 to 5 years. Breeds that are commonly diagnosed include the Labrador Retriever, Golden Retriever, German Shepherd, Cocker Spaniels and American Eskimo.

Idiopathic polyarthritis has been categorized into four sub-types: Type I - no underlying disease, Type II - reactive, Type III - enteropathic and Type IV - neoplasia-related. Types II to IV are often grouped together and referred to as the reactive polyarthritides. It should be noted that regardless of subtype, the pathophysiologic change that occurs in the joint space is the same. These subtypes merely point to the presence or absence of a concurrent disease process.

In *Type I* (uncomplicated) polyarthritis, the cause is unknown, and no underlying disease can be detected. This is the most common form of the idiopathic polyarthritides, accounting for approximately 50%-65% of all idiopathic polyarthritis cases.

In *Type II* (reactive) polyarthritis, an infectious or inflammatory disease distant from the joints is the underlying cause of the polyarthritis. These diseases produce antigens that combine with antibodies to form immune complexes that accumulate in the joints, activating complement and leading to inflammation. Type II polyarthritis accounts for approximately 10-25% of all idiopathic polyarthritis cases. The underlying infection can be bacterial, fungal, protozoal or viral, and can be located anywhere in the body including the heart valves, vertebral bodies or disc spaces, uterus, kidneys or lower urinary tract including prostate, respiratory tract including tonsils, oral cavity, skin, or even ears. Non-infectious inflammatory diseases such as pancreatitis have also been reported in association with immune-mediated arthritis.

*Type III* (enteropathic) arthritis is associated with the presence of gastrointestinal or hepatic disease. Only about 5% of all idiopathic polyarthritis cases are in this category. It has been theorized that disease of the gut leads to an increase in intestinal permeability to potential antigens, which then stimulate the production of immune complexes.

*Type IV* arthritis is associated with neoplasia that exists outside the joints. This is an uncommon manifestation of idiopathic polyarthritis, occurring in only about 2% of dogs with polyarthritis. It has been reported to occur in dogs with neoplasms such as pancreatic adenocarcinoma, renal carcinoma, tonsillar carcinoma, squamous cell carcinoma, mammary carcinoma, leiomyosarcoma, and lymphoma. Neoplasia may act as an antigenic stimulus against which antibodies are formed, leading to circulating immune complexes that deposit in the joint spaces.
**Vaccine-Induced Polyarthritis**

Vaccine-induced polyarthritis can occur after a first vaccination or after a booster vaccine. Clinical signs are evident within 30 days of receiving the vaccine. Vaccine-induced polyarthritis is usually transient, resolving within several days. However, some breeds such as the Akita appear to be predisposed to this condition, and suffer a longer course of vaccine-associated polyarthritis. Reports in Akitas describe profound joint pain and cyclic fevers lasting 24-48 hours, with initial signs occurring 3 to 30 days following vaccination. Prognosis is guarded in affected Akitas, who often do not respond to immunosuppressive therapy.

**Drug-Induced Polyarthritis**

Drug-induced polyarthritis has been reported with multiple different drugs in many different breeds, although the most widely reported manifestation occurs secondary to sulfonamide administration, particularly in Doberman Pinchers. Other drugs that have been implicated include phenobarbital, erythropoietin, penicillins, lincomycin, erythromycin and cephalosporins. Typically, the affected animal has either received the inciting drug in the past or has been on the medication long-term. Clinical signs usually resolve within 2-7 days of discontinuing the drug. Reactions associated with sulfonamide in dogs occur an average of 2 week after drug initiation or 1 hour to 10 days after drug re-exposure.

**Polyarthritis/Polymyositis Syndrome**

Polyarthritis/polymyositis syndrome is characterized by polyarthritis initially accompanied with focal or generalized muscle pain and swelling followed by eventual muscle atrophy and fibrosis. Most recorded cases of polyarthritis/polymyositis syndrome have been identified in spaniels. Animals present with fever and painful joints and muscles. Muscle enzymes including creatine kinase are often elevated, and diagnosis is based on the combination of electromyography, muscle biopsies and joint taps.

**Steroid-Responsive Meningitis-Arteritis**

Steroid responsive meningitis-arteritis (SRMA) is currently the most widely accepted term describing a disease syndrome that causes meningitis in medium and large breed dogs less than two years of age. The syndrome was first recognized in young laboratory Beagles, and was referred to as ‘Beagle pain syndrome’. Canine pain syndrome, canine juvenile polyarteritis syndrome, canine meningeal polyarteritis, aseptic suppurative meningitis, and necrotizing vasculitis are also terms that have been used to describe essentially the same disease condition. Other breeds that are predisposed to SRMA include the Boxer, Bernese Mountain Dog, Akita and German Short Haired Pointer. Typically, affected animals present with an acute onset of neck pain, fever and lethargy.

Cerebrospinal fluid analysis reveals inflammation and an increase in protein. More specifically, a high level of immunoglobulin A is found both systemically and intrathecally. Paired measurements of IgA in the serum and CSF have proven useful for diagnosing SRMA. Histopathology of affected dogs shows migration of inflammatory cells into the meninges and inflammation of the meningeal arteries causing stenosis of these vessels. Wide spread arteritis in dogs with SRMA, however, can affect any organ system. The coronary arteries are often
involved in the Beagle, and affected dogs commonly have a combination of myositis, meningitis and polyarthritis.

The combination of immune-mediated polyarthritis and apparent SMRA is more common than suspected. Up to one in three dogs with IMPA also has spinal pain, which may often be due to SMRA. Spinal pain was most commonly demonstrated in the cervical region when the head was flexed or extended. It is recommended that a CSF tap be performed on all dogs suspected IMPA that have concurrent joint and spinal pain.

**Juvenile-Onset Polyarthritis of Akitas**

Juvenile-onset polyarthritis occurs in affected Akitas between 9 weeks and 9 months of age. Affected dogs experience cycles of fever and severely painful and swollen joints that result in a reluctance to stand or walk. Neck and back pain can also be present, as well as mild to moderate lymphadenopathy. Episodes last about 24-48 hours before spontaneously resolving. Joint fluid analysis reveals neutrophilic inflammation. Occasionally, sterile suppurative meningitis is revealed via CSF analysis. Significant laboratory findings may include a mild to moderate non-regenerative anemia, neutrophilic leukocytosis, mild hypoalbuminemia and mild hyperglobulinemia. Pedigree analysis in affected Akitas suggests that the disease is inherited. Akitas that exhibit characteristic clinical signs should therefore not be breed. Some clinicians believe that the development of this disease and its close association with immunization suggests that juvenile-onset polyarthritis in Akitas may be an immune-mediated response triggered by viral antigens or other components of vaccines. Others believe the apparent association with vaccination is coincidental, and that the disease naturally arises during the age when multiple booster vaccines are required.

**Familial Chinese Shar-Pei Fever**

Familial Chinese Shar-Pei fever is an inherited autoinflammatory disease characterized by unexplained, recurring attacks of inflammation that seem to be triggered by stress. Symptoms consist of waxing and waning 24-36 hour episodes of high fever usually starting before 18 months of age, although adult onset attacks are not uncommon. Of those affected Shar-Peis that experience fever, approximately half have concurrent ‘swollen hock syndrome’. Swollen hock syndrome is characterized by periarticular swelling due to cellulitis with or without inflammation in the joint itself. Although several joints can be affected, most cases involve the tibio-tarsal joint. Occasionally, the muzzles of affected dogs may be warm, swollen and painful. Other physical symptoms may include mild vomiting or diarrhea, abdominal pain and signs of back, joint or pleural pain. Complete blood count and blood chemistry results may reveal leukocytosis with a left shift and an elevation of alkaline phosphatase.

Dogs affected with familial Shar-Pei fever have abnormally high resting levels of interleukin-6. Interleukin-6 is a pro-inflammatory cytokine that stimulates the liver to make acute phase proteins and the hypothalamus to increase the body’s core temperature. Many Shar-Peis that have intermittent fever episodes eventually develop amyloidosis, although amyloid accumulation can occur without clinical evidence of fever, and fever can occur without the development of clinically significant amyloidosis. Dogs that develop amyloidosis often die of protein-losing nephropathy or renal failure at a mean age of 4 years. Since it has been demonstrated that
familial Shar-Pei fever is compatible with autosomal recessive inheritance, Shar-Peis with cyclic fever should not be breed.

Persistent overwhelming levels of acute phase proteins or an inability to metabolize these proteins leads to accumulation of amyloid in several body organs. In the Shar-Pei, the specific protein that accumulates is amyloid AA, a breakdown product of serum amyloid A. Amyloid AA accumulates primarily in the kidneys, but other organs can be affected, most notably the liver. Diagnosis of amyloidosis is usually based on renal biopsy, although fine needle aspirates of the liver containing amyloid have also been described. Microscopic examination of renal parenchyma tissue stained with Congo Red reveals accumulation of beta pleated sheets of amyloid AA, mostly in the renal medulla. Detecting early renal disease in affected Shar-Peis by monitoring both specific gravity and urine protein concentration is therefore imperative.

Systemic Lupus Erythematosus

Systemic lupus erythematosus is a multisystemic immune-mediated disease reported infrequently in the dog. Predisposition to SLE is thought to be inherited. Mixed breed dogs as well as German Shepherds, Shetland Sheepdogs, Beagles, Afghan Hounds, Irish Setters, Old English Sheepdogs, Cocker Spaniels, Collies and Poodles are over represented. Onset of disease typically occurs between 2 to 4 years of age, although older dogs can be affected. Multiple concurrent immunologic reactions can be present in SLE patients, including type III (antigen-antibody complex mediated), type II (antibody directed against cellular self antigens including nuclear material, red blood cells, white blood cells and platelets) and, to a lesser degree, type IV (cell mediated activity against self antigen) hypersensitivities.

Clinical findings can be separated in two main categories (major signs and minor signs) based on their importance in contributing to the diagnosis of SLE. Major signs include polyarthritis, glomerulonephritis, hemolytic anemia, leukopenia, thrombocytopenia, characteristic skin lesions and polymyositis. Minor signs include fever, central nervous signs, oral ulcerations, lymphadenopathy, pericarditis and pleuritis. Non-erosive polyarthritis is the most frequent primary sign of SLE in dogs, and about 80% of canine SLE patients have polyarthritis. Consequently, the resultant shifting leg lameness is the most common finding on physical examination of dogs diagnosed with SLE.

Major Presenting Complaints and Clinical Signs

Immune-mediated polyarthritis can cause non-specific systemic signs such as weight loss, inappetance, lethargy and reluctance to move. Owners may or may not report more specific clinical signs such as swollen joints, altered gait or lameness. Vomiting and diarrhea may also be mentioned in the recent history. Various retrospective studies of dogs with IMPA have found a range of clinical signs, although most reported fever, lethargy, weakness, reluctance to walk, a stiff or stilted gait, lameness, swelling of multiple joints often in a bilaterally symmetrical pattern, and pain on palpation of these joints. Neck and back pain may also be present if vertebral articular facets are involved or if meningitis is present. Clinicians should be aware that dogs with IMPA commonly present with no obvious joint swelling or localizable pain. IMPA has been reported as one of the most common causes of fever of unknown origin in dogs.
Diagnostics and Differential Diagnoses

Ultimately, immune-mediated polyarthritis is diagnosed by arthrocentesis and synovial fluid analysis. Further diagnostic testing is necessary, however, to determine if an underlying disease is causing a reactive polyarthritis. In addition, other causes of joint disease, including septic arthritis, degenerative joint disease, neoplastic arthropathy, trauma and hemophilic arthropathy, should be ruled out. Most of these, however, are more likely to affect a single joint versus the multiple joints typically affected in dogs with IMPA.

When evaluating a dog with suspected polyarthritis, a recommended minimum database should include a complete blood count (CBC), serum chemistry panel, urinalysis and urine culture. Common CBC and blood chemistry findings noted in dogs with IMPA include leukocytosis, mild non-regenerative anemia and mild hypoalbuminemia. A mild to moderate elevation in serum alkaline phosphatase of as yet undetermined cause is also common. A positive urine culture can help to identify a urinary or blood-borne bacterial infection.

A thorough search for evidence of underlying infection, inflammatory disease, or neoplasia should be undertaken, including thoracic and abdominal radiographs and abdominal ultrasonography. If back or neck pain is detected, radiographs of the spine are indicated. In addition, radiographs of multiple joints should be considered to look for evidence of synovial effusion, soft tissue swelling and signs of joint erosion and destruction, or to rule out other possible causes of joint disease. The hocks, carpi and stifles are the most commonly affected joints in dogs with IMPA, while more proximal and larger joints are more likely to be involved in patients with infectious arthritis.

Rheumatoid factor is an autoantibody with specificity for the constant region (Fc) portion of an immunoglobulin molecule. Many infectious and inflammatory diseases can be associated with the presence of rheumatoid factor in the serum, including infectious arthritis and osteoarthritis. Testing for rheumatoid factor is indicated if bilateral symmetrical, erosive changes are present in joint radiographs, especially if distal joints are affected. A positive test would support a diagnosis of rheumatoid arthritis in a dog in which erosion of articular cartilage is present but extra-articular manifestations of immune disease are absent, especially if joint cultures are negative.

Systemic infectious diseases can lead to reactive immune-mediated polyarthritis. If the patient has lived in or visited areas in which such diseases are endemic, appropriate testing should be performed. Such diseases would include Lyme disease, bartonellosis, ehrlichiosis, anaplasmosis and Rocky Mountain spotted fever. Paired antibody titers, polymerase chain reaction testing and specific Bartonella culture techniques are some of the tests that may be considered.

Septic arthritis should also be ruled out before considering treatment with immunosuppressive drugs. Culturing the blood, urine and synovial fluid may help identify a possible local or systemic bacterial infection. Many cases of bacterial infective arthritis with no history of a surgical procedure or penetrating wound at the affected joint have evidence of pre-existing osteoarthritis. Most bacteria cultured from infected joints are skin commensals, including Staphylococcus and Streptococcus species. The elbow is the most common joint affected, followed by the hip, stifle and hock joints. Other bacteria that have been reported to cause septic
arthritis and may require special culture techniques include *Mycoplasma* species and L-form bacteria. Evaluation of the heart valves via echocardiography may also be necessary to screen for endocarditis, particularly if a new or progressive murmur is auscultated. Endocarditis can lead to either a sterile reactive immune polyarthritis or a true infective arthritis via hematologic spread of organisms to one or more joints. Culturing bacteria from blood can be insensitive. Further testing for organisms such as *Bartonella*, *Aspergillus* and *Mycobacterium* should be considered if infective endocarditis is suspected despite negative bacterial blood cultures. Uncommonly, cytology of synovial fluid may also help confirm septic arthritis by revealing the presence of infectious agents such as *Mycoplasma* species, *Borrelia burgdorferi* spirochetes, *Ehrlichia ewingii* and *Anaplasma phagocytophilum* morulae in neutrophils, *Leishmania* amastigotes within macrophages or fungal hyphae.

Non-immune joint diseases diagnosed by synovial fluid analysis include synovial neoplasia and hemophilic arthropathies. However, further confirmatory testing will often be needed, such as a surgical biopsy if neoplasia is suspected or a complete coagulation profile if hemophilic arthropathy is suspected. Synovial cell sarcoma is one of the more common joint neoplasms and is most likely to affect a single joint in a large breed dog, particularly the stifle or elbow joint.

Further diagnostic testing depends on history, clinical signs and suspected underlying diseases. Cerebrospinal fluid analysis, including IgA levels, should be considered if neck pain is noted and steroid responsive meningitis-arteritis (SRMA) is suspected. If muscle pain is present or creatine kinase is elevated and polyarthritis/polymyositis is suspected, muscle biopsies should be obtained. Lastly, if immune-mediated disease is demonstrated in multiple organs, screening for systemic lupus erythematosus (SLE) should be considered. A definitive diagnosis of SLE can be made if 2 major signs and a positive anti-nuclear antibody (ANA) titer are identified, or if 2 minor signs and 1 major sign are identified along with a positive ANA titer. A probable diagnosis of SLE can be made if 2 major signs are present with a negative ANA titer. Positive ANA titers represent detection of serum antibodies directed against nuclear material such as DNA, RNA, nucleoproteins and histone proteins. More than 90% of SLE cases have a positive ANA titer. Positive ANA titers can also be detected with infectious, inflammatory or neoplastic disorders. Therefore, clinicians should only run ANA titers when a multi-systemic immune disorder is strongly suspected. The lupus erythematosus (LE) cell preparation test is much less sensitive than the ANA test, but is more specific for SLE. This test identifies opsonized nuclear material within neutrophils and macrophages. Documenting the presence of other major signs besides polyarthritis may lead to looking for anti-platelet antibodies if thrombocytopenia is present, performing a urine protein-creatinine ratio if glomerulonephritis is suspected, performing a slide agglutination or Coombs test if anemia is present or performing a skin biopsy if characteristic lesions such as erythema, scaling, crusting, depigmentation or alopecia are found on the skin, at mucocutaneous junctions or within the oral cavity.

**Arthrocentesis and Synovial Fluid Analysis**

The diagnosis of IMPA is confirmed by demonstrating neutrophilic inflammation in the synovial fluid of multiple joints. Immune-mediated polyarthritis is classically considered to be a ‘polyarthropathy’, suggesting that 5 or more joints are involved. However, many cases of canine IMPA may involve fewer than 5 joints, and could therefore be more strictly defined as
‘oligoarthritis’ (involvement of 2-4 joints). Rarely, immune-mediated monoarthritis involving only one joint has been reported. If attention is paid to aseptic technique, collection of joint fluid is associated with little risk of introducing infection or causing significant trauma to the joint.

The carpal and hock joints are the most common joints affected by immune-mediated inflammatory joint disease in the dog and should be aspirated when attempting to confirm a diagnosis of IMPA. Aspiration of at least one larger and more proximal joint, such as the stifle joint, is also recommended. Shaving hair over or taking radiographs of joints in question can help identify more subtle expansion of the joint capsule if the presence of joint effusion is unclear.

In most dogs, arthrocentesis can be performed using standard sedative protocols without the need for general anesthesia. In preparation for collecting joint fluid, the skin over each joint should be shaved and cleansed using a sterile technique. A 25 or 22 gauge needle of sufficient length to enter the joint cavity should be used: a needle as short as 1/2 inch will suffice for smaller joints such as the carpus or hock, a 1 to 1½ inch needle is typically sufficient for the stifle, elbow and shoulder, and a needle as long as 2 inches may be needed to access the hip joint in big dogs. A 3 cc syringe will provide adequate negative pressure to draw out the joint fluid. The needle is attached to the syringe before attempting arthrocentesis. Most joints are in a more open configuration when in moderate flexion. While in the ideal position, palpation helps to further identify the best route to take to enter the joint capsule. The clinician should minimize blood contamination by avoiding superficial vessels. Once the joint space is entered, the syringe plunger should be gently drawn back and the needle hub carefully observed. Since viscous joint fluid can take time to enter the syringe, patient observation of the hub of the needle is required before making the decision to redirect and try again if no fluid appears. In the smaller joints of normal dogs, less than 0.25 mls of joint fluid is obtained during arthrocentesis: if greater than 0.5 mls is collected, the joint is considered likely to be diseased.

The color of the joint fluid that initially enters the needle hub can help determine whether blood contamination is iatrogenic. If the fluid is initially clear but later appears reddish, iatrogenic contamination is likely, whereas if the sample is initially red-tinged, joint inflammation most likely led to pathologic hemorrhage. Regardless, samples with significant blood contamination should be placed in an EDTA tube to avoid clot formation and submitted with a current CBC. Comparing peripheral blood and joint fluid cell counts may enable the clinical pathologist to distinguish nucleated cells present due to inflammation from those introduced by blood contamination.

Hyaluronic acid is responsible for maintaining the viscosity of joint fluid and coating the synovium. The presence of inflammatory cells or bacteria in the joint can lead to degradation of hyaluronic acid by leukocyte proteases or bacterial hyaluronidases. Hyaluronic acid can also be diluted by an influx of plasma if the joint vasculature is more permeable due to inflammation. To evaluate the viscosity of joint fluid, clinicians can use the subjective ‘string test’ or the laboratory mucin clot test. For the string test, a drop of fluid is placed between two gloved fingers. The string of normal synovial fluid should only break when the fingers are parted by more than approximately 1 inch. Alternatively, a drop of synovial fluid can be allowed to fall from the end of the aspiration needle, and should produce a string of similar length before
breaking. Although a decrease in fluid viscosity is typically expected in inflamed or infected joint fluid, only about one half of all joint samples taken from dogs diagnosed with IMPA has a gross reduction in viscosity.

Ideally, a sufficient amount of joint fluid will be collected to make several direct smears with enough volume left over to place in a standard purple top tube for fluid analysis, including protein concentration, total nucleated cell count and differential cell count. In many cases, only a small quantity of fluid is obtained. If so, one 1 drop should be placed on a glass slide, smeared using standard methods and allowed to air dry. If blood contamination of the fluid is suspected, a CBC should also be submitted.

Normal canine synovial fluid contains less than 3,000 cells/ml. Greater than 90% of these cells are mononuclear and less than 5% are mature, non-degenerate neutrophils. The majority of mononuclear cells are non-reactive macrophages with few vacuoles. Small lymphocytes are also present in lower numbers and, on occasion, a synoviocyte may seen. In canine immune-mediated polyarthritis, synovial fluid volume is often increased. Fluid obtained may be turbid and/or discolored. It typically has a decreased viscosity, and the protein and nucleated cell content are usually increased, with cell counts often greater than 5000/µl. Fluid from abnormal joints is actually more likely to clot. The percentage of neutrophils is usually increased to 10-95% of total nucleated cells, and they are typically non-degenerate. Although the results of synovial fluid analysis in dogs with IMPA almost always support the diagnosis, on rare occasions a synovial membrane biopsy is required.

Joint sepsis and immune-mediated arthritis can produce very similar changes in total joint fluid nucleated and differential cell counts. Although degenerate neutrophils are suggestive of bacterial or possibly a fungal joint infection, neutrophils from infected joints are often not significantly degenerative. Consequently, further testing of joint fluid may be needed to distinguish infected from non-infected joints. If only small amounts of joint fluid are obtained, a drop of fluid can be added to culture medium. Alternatively, traces of synovial fluid can be washed from the syringe and needle by aspirating sterile saline or enrichment broth. Direct culture of joint fluid on blood agar, however, produces false negative results in 50-70% of patients with septic arthritis. One canine study demonstrated that inoculating a pediatric blood culture bottle with synovial fluid, incubating for at least 24 hours and then streaking the blood culture medium on appropriate plate media significantly reduced false negative culture results. This approach was also more successful than synovial membrane biopsy cultures. More recently, real-time broad-based PCR assays have been described that rapidly identify bacteria in synovial fluid, and these methodologies may eventually replace joint fluid culture as a means of identifying joint infection in dogs.

Infectious agents are occasionally identified by direct cytologic examination of joint fluid. For example, rickettsial morulae have been found in neutrophils in the synovial fluid of up to 1% of affected patients. Other organisms that may be seen include bacteria within neutrophils, *Borrelia burgdorferi* spirochetes, *Mycoplasma* species, *Leishmania* amastigotes within macrophages, and fungal hyphae or yeast bodies.
Treatment

Treatment for polyarthritis should target the underlying cause if applicable, and concurrently address pain and inflammation. The overall goal of therapy is to achieve long term remission with the lowest possible dose of medication, and to prevent recurrence of joint inflammation.

Non-steroidal anti-inflammatory drugs (NSAIDs) can be administered to treat pain and inflammation, and may be the only medication needed for mild transient cases of polyarthritis, such as those induced by vaccination. NSAIDs should be used with extreme caution in dogs with impaired renal or hepatic function, and in dogs that are dehydrated. If steroids are indicated for further treatment, NSAIDS should be discontinued for at least 48 hours before glucocorticoids are administered. A longer washout period may be needed depending on the NSAID, or in older or more debilitated dogs. The prostaglandin analogue misoprostol can be added to help avoid or treat gastric ulceration induced by NSAIDs alone or by overlapping NSAIDs and glucocorticoids. Misoprostol should not be handled by pregnant women. Although less effective than misoprostol for NSAID-associated gastric ulceration, the proton pump inhibitor omeprazole can also be used as a substitute.

In areas where tick borne disorders such as Lyme disease and rickettsial infection are prevalent, initial therapy for polyarthritis should be limited to analgesics and empirical treatment with doxycycline. A recent American College of Veterinary Internal Medicine consensus statement on Lyme disease in dogs recommended treating with doxycycline at 10 mg/kg every 24 hours for 1 month. The same doxycycline dose should be effective for most common tick borne diseases. Dogs with polyarthritis induced by tick borne disease typically demonstrate clinical improvement within the first 7 days of doxycycline administration.

Immunosuppressive therapy, primarily with glucocorticoids, is the cornerstone of therapy in most dogs with IMPA. It can, however, be dangerous in patients with bacterial arthritis, especially if this is related to a systemic bacterial infection. Since IMPA is almost never immediately life threatening, it is preferable to withhold immunosuppressive therapy until bacterial arthritis has been reasonably excluded. In patients where bacterial arthritis is suspected, treatment should be initiated with a broad spectrum, beta-lactamase-resistant bactericidal antibiotic until culture and sensitivity results return or until diagnostic test results suggest that a bacterial infection is not present.

Specific treatment protocols have been described for the various manifestations of polyarthritis described previously. Treatment for drug induced polyarthritis, for example, requires discontinuation of the inciting drug and possibly a short tapering course of glucocorticoids. Dogs diagnosed with polyarthritis/polymyositis have been treated with a combination of cyclophosphamide and a tapering dose of prednisone with varying success. Dogs diagnosed with SRMA have been successfully treated with prednisone alone or a combination of prednisone and azathioprine. Juvenile onset polyarthritis in Akita dogs has also been treated with multi-drug protocols including a combination of prednisone and azathioprine, but with a less reliable positive outcome. In dogs with Type II to Type IV idiopathic polyarthritis, joint inflammation will usually resolve when the underlying disease process is successfully identified and treated or removed. Occasionally, however, additional treatment with analgesics and possibly
glucocorticoids will be required. In many cases of Type II (reactive) polyarthritis triggered by infection, immunosuppressive doses of steroids will be contraindicated. Tick borne diseases, however, may benefit from concurrent treatment with doxycycline and a tapering dose of glucocorticoids. Sulfasalazine has been used to treat cases of Type III (enteropathic) arthritis and rheumatoid arthritis associated with gastrointestinal disease, and provides a combination of antibacterial, anti-inflammatory and immunosuppressive properties.

Once no underlying cause of polyarthritis has been identified, infection has been reasonably excluded, and a provisional diagnoses of Type I (uncomplicated) idiopathic polyarthritis has been established, a tapering dose of glucocorticoids is indicated if symptoms persist after more conservative treatment protocols have failed. Immunosuppressive doses of prednisone, prednisolone or methylprednisone (2-3 mg/kg/day once daily or divided) can be started initially. This dose is administered until there is no cytologic evidence of joint inflammation or the clinical signs of arthritis are no longer present. The dose can then be tapered every 2-3 weeks by 25-30% until the approximate physiologic dose of 0.2-0.3mg/kg/day is reached. Tapering glucocorticoids too quickly can lead to relapses that may be less responsive to treatment than the original manifestation of the disease. The addition of supplementary immunosuppressive agents should be considered if remission is not attained or if relapse occurs during treatment with steroids alone. Immunosuppressive drugs that have been used in dogs with IMPA include azathioprine, cyclosporine, mycophenolate mofetil and leflunomide. If side effects with glucocorticoids or other immunosuppressive agents are intolerable, monotherapy with leflunomide can also be considered.

When treating with a combination protocol, once remission of clinic signs is achieved, the drug causing the most concerning side effects (or, if no major side effects are seen, the most expensive drug) is typically tapered first. There are no controlled studies in dogs that show that one immunosuppressive agent is better than another for treatment of IMPA, and efficacy seems to depend on the individual patient. Therefore, if one immunosuppressive agent does not appear to be working, it can be replaced by another. In some patients, all drug therapy can gradually be tapered then discontinued without relapse. In other patients, an ongoing low dose of glucocorticoid is required in conjunction with an immunosuppressive agent. In these cases, the lowest possible doses that maintain clinical remission should be administered. Controlling pain is also an important part of restoring quality of life and allowing for tapering of immunosuppressive medications. Oral analgesics that may be combined with either NSAIDs or corticosteroids to control pain include tramadol, gabapentin, amantadine and acetaminophen.

In cases of canine SLE, immunosuppressive doses of glucocorticoids are the cornerstone of treatment with additional cytotoxic agents added if needed: azathioprine, cyclosporine, cyclophosphamide, and chlorambucil have all been reportedly used by clinicians in refractory SLE patients. The goal of treatment is to achieve complete remission with resolution of clinical signs and a negative ANA titer. After complete remission is attained, medications are tapered gradually over at least 6 months. In addition to more traditional immunosuppressive agents, levamisole may be considered in refractory SLE patients. Other more specific therapies depend on the organ system being attacked, and may include avoiding exposure to ultraviolet light in dogs with skin lesions or treating glomerulonephritis with low-dose aspirin, omega 3 fatty acids, a low protein renal diet and ACE inhibitors.
Treatment for dogs with Shar-Pei fever focuses on prevention of amyloidosis, decreasing inflammation and relieving episodic clinical signs of fever. The mainstay of prevention of amyloidosis is colchicine. Colchicine impairs the release of serum amyloid A from the liver by binding to hepatocyte microtubules and preventing amyloid secretion. Although colchicine is unable to reverse renal disease that has already occurred, the drug has been shown to decrease proteinuria in dogs with renal amyloidosis, slow disease progression and decrease the number or fever episodes. Colchicine should be started in any Shar-Pei with cyclic fevers since up to 30% of these dogs may develop life threatening renal amyloidosis. A wide range of antioxidant, dietary supplements and anti-inflammatory medications have been suggested for use in Shar-Peis with clinical fevers including omega 3 fatty acids, methylsulfonylmethane (MSM), vitamin C, vitamin K2, vitamin E and selenium, cobalamin, curcumin with bioperine, alpha-lipoic acid, boswellia, resveratrol and lecithin. In addition to these supplements, a high quality, balanced, commercial dog food that contains an adequate level of Vitamin D3 that is low in simple carbohydrates and grains is recommended. Monitoring magnesium and cobalamin levels as well as careful attention to gastrointestinal, skin and thyroid health is also paramount. The use of NSAIDs such as dipyrone and meloxicam has been described to control fever in dogs with temperatures over 105°. Protein-losing nephropathy in dogs with familial Shar-Pei fever is treated with angiotensin-converting enzyme (ACE) inhibitors, a renal diet and low-dose aspirin to help prevent thromboembolic disease.

Erosive arthritis in dogs is more refractory to treatment and may require life long therapy. Since *Mycoplasma* has been found in the joints of some Greyhounds with erosive arthritis, a trial therapy using an antibiotic effective against this organism (such as tylosin) should be administered before considering immunosuppressive therapy. There is little published data available regarding the treatment of rheumatoid arthritis in dogs. Treatment recommendations are therefore usually extrapolated from the human literature. In human patients with only rheumatoid arthritis, glucocorticoids are used as the first line of defense, and are frequently combined with additional medications referred to as disease-modifying anti-rheumatic drugs (DMARDs). Depending on the response of the individual patient to therapy, more than one DMARD may be used at the same time. DMARDs that are used in human medicine include hydroxychloroquine, sulfasalazine, methotrexate, D-penicillamine, gold salts, azathioprine, cyclophosphamide, cyclosporine, and leflunomide. Chrysotherapy using gold salts has also been recommended for refractory cases of canine rheumatoid arthritis. In addition to immunosuppressive drug therapy, splenectomy is often the last resort treatment of Felty’s syndrome (characterized by rheumatoid arthritis, neutropenia and splenomegaly). Splenectomy is based on the presumption that the spleen is the site of immune-mediated neutrophil destruction.

Patients on immunosuppressive therapy should be monitored closely for infections. Urinary tract infections, often with minimal clinical signs and detectable only via culture, are commonly associated with chronic immunosuppressive therapy. Pneumonia is another possible infection that may go undiagnosed due to vague clinical signs such as lethargy and inappetence. Fungal infections are also more likely in animals that are immunosuppressed.

Some dogs with Type I idiopathic polyarthritis, and most dogs with rheumatoid arthritis, will require long term or life-long management. Although cage rest is recommended during acute
episodes when affected dogs are still significantly painful, a gradual increase in activity should
be allowed over time since long-term management should encourage lean body condition
through exercise and dietary restriction. Diets or dietary supplementations high in omega 3 fatty
acids should also be considered in an effort to control inflammation.

In dogs with apparently non-erosive IMPA that is refractory to therapy, periodic radiographs of
multiple joints are warranted to look for progression to erosive arthritis. Permanent damage to
joints in dogs with the erosive form of IMPA may cause collapse of the joint space. In these
cases, surgical treatment of badly affected joints may be warranted, particularly if the underlying
polyarthritis is in remission. Arthrodesis of collapsed joints may increase comfort, and
synovectomy may help reduce local inflammation within that joint. Intra-articular injection of
glucocorticoids such as triamcinolone or methylprednisolone has also been suggested as a means
of controlling local inflammation.

Monitoring Response to Therapy

Swelling and pain associated with immune-mediated polyarthritis often respond to
glucocorticoids, or a combination of glucocorticoids and other immunosuppressive agents,
within 7 days of commencing therapy. Since significant joint inflammation may still be present
despite resolution of clinical signs, the gold standard for monitoring response to therapy would
be to repeat arthrocentesis before the first tapering of medication to ensure that the patient is in
tue remission. Documentation of a substantial decrease in total white cell count and neutrophils
in synovial fluid on repeat joint taps is considered to be a good prognostic sign. If no significant
improvement is observed in synovial fluid cytology, continuation of aggressive
immunosuppression or commencement of alternative medications should be considered.

Measuring acute phase protein levels during the course of treatment of IMPA may offer a
practical alternative to either repeat arthrocentesis or simple observation of clinical signs for
monitoring response to therapy. C-reactive protein is an acute phase protein produced by the
liver in response to inflammation that is commonly used in human medicine to monitor several
inflammatory disorders, including rheumatoid arthritis. The rapid production of C-reactive
protein in response to inflammation, and subsequent short circulating half-life, make the protein
a good biologic marker of progression for many inflammatory diseases. Serum C-reactive
protein is significantly elevated prior to treatment in dogs with IMPA, and also when the disease
is active during relapse. The prognosis for long term remission appears to be better in those dogs
with various forms of idiopathic polyarthritis in which C-reactive protein concentrations
normalize rapidly after starting glucocorticoids.

Prognosis

Erosive arthritis in dogs is associated with a poor long-term prognosis. In most cases, multi-drug
protocols are required to keep the disease in remission. Permanent joint destruction often leads to
an inferior quality of life, even if the underlying inflammatory process responds to medical
therapy.

In contrast, the prognosis for most forms of non-erosive polyarthritis in dogs is fair to good. The
prognosis in dogs with idiopathic polyarthritis depends on the sub-type. In dogs with types II to IV, the prognosis is good and relapse is unlikely if the underlying disease can be identified and resolved. Outcomes in dogs with Type I (uncomplicated) idiopathic polyarthritis are also generally favorable. The prognosis for dogs with vaccine- and drug-induced IMPA is also excellent. Recovery times are usually relatively short, and relapse is unlikely. Akitas with vaccine-induced IMPA, however, are reported to typically require more intense initial therapy compared to most other dog breeds and are less likely to respond to treatment.

Polyarthritis/polymyositis can also be challenging to treat. Most dogs with steroid responsive meningitis arteritis respond to immunosuppressive therapy, although relapses are possible. Older SRMA patients with high CSF IgA levels tend to experience more frequent relapses and require a longer duration of therapy, whereas Boxers tend to have an excellent clinical outcome. Akitas that develop juvenile-onset polyarthritis have a guarded prognosis. Break-through symptoms are common, even on multi-drug protocols combining prednisone and azathioprine. However, some Akitas recover after tapering of immunosuppressive drugs, and do not relapse. Prognosis for dogs with familial Shar-Pei fever that have already developed significant amyloidosis is guarded. Many die from kidney or liver failure at a median age of 4 years. However, if amyloidosis is prevented or controlled, particularly with colchicine, dogs with Shar-Pei fever have anecdotally been reported to live for over 10 years. Prognosis in dogs with SLE varies, with 40% of patients being dead within one year and more than 50% achieving long-term survival. Prognosis is favorable in dogs that are treated early in the development of the disease, and in those that respond to glucocorticoid therapy alone. Prognosis is less favorable if renal failure with proteinuria is present, initial response to therapy is less evident or relapses occur frequently.
A number of established immunosuppressive agents have been used in small animal medicine for many decades. Some have justifiably fallen out of favor whereas, for others, new and promising uses have been described in the recent veterinary literature. This lecture will discuss some ‘old favorites’: cyclophosphamide, chlorambucil, azathioprine, danazol and vincristine.

**Cyclophosphamide**

Cyclophosphamide, a cell-cycle nonspecific nitrogen mustard derivative alkylating agent, was one of the first major chemotherapeutic agents approved by the FDA over 50 years ago, and has since become very well-established in human medicine as both an antineoplastic drug and as an immunosuppressive agent. Within a few years of FDA approval in the late 1950s, the use of cyclophosphamide for the prevention of transplant rejection in experimental models and for the treatment of both neoplasia and immune-mediated diseases was described in both dogs and cats. Cyclophosphamide has persisted to this day as one of the core drugs used in many small animal cancer chemotherapeutic protocols. In contrast, after many years as one of the most commonly immunosuppressive drugs utilized to treat immune-mediated diseases in cats and dogs, the use of cyclophosphamide as an immunosuppressive agent in small animal patients has in the past two decades essentially faded away. The reasons for the steadily diminishing popularity of cyclophosphamide as an immunosuppressive agent are myriad, and include a high incidence of unacceptable side effects, the development of other immunosuppressive agents that are generally safer and more convenient to administer, and the publication of a number of papers a decade or so ago that suggested that cyclophosphamide was associated with poor outcomes when used to treat conditions such as immune-mediated hemolytic anemia.

Cyclophosphamide is a prodrug that is metabolized by the hepatic cytochrome P450 enzyme system to eventually form active metabolites such as 4-hydroxycyclophosphamide, 4-hydroperoxycyclophosphamide, and aldophosphamide. These metabolites can enter cell cytoplasm, where they are ultimately metabolized to phosphoramidemustard and acrolein. Phosphoramidemustard is an alkylating agent that replaces a hydrogen atom with an alkyl group on the guanine base of DNA, which interferes with nuclear DNA replication and cytoplasmic RNA transcription by forming crosslinks both within and between nucleotide strands. Cyclophosphamide has long been reported to be a potent immunosuppressive that inhibits humoral and cell-mediated immunity, including inhibition of primary and secondary immune responses, reduction of antigen trapping in lymph nodes, and inhibition of local inflammatory responses, although in a number of experimental studies in dogs, cyclophosphamide often appears to be relatively mildly immunosuppressive compared to other drugs.

Cyclophosphamide shares the toxicity profile of most alkylating agents, with common side effects including gastrointestinal signs, myelosuppression, and hair loss. Gastrointestinal signs
are relatively common, and include nausea, anorexia, vomiting and diarrhea. Dose reduction and antiemetic agents are often enough to control gastrointestinal signs, but occasionally persistent gastrointestinal side effects will prevent ongoing use of the drug, especially in cats. Myelosuppression appears to be dose dependent, and is associated with both the use of high drug doses and the use of lower doses over a sustained period of time. Moderate to severe neutropenia is the most potentially life-threatening feature of cyclophosphamide myelosuppression, but moderate thrombocytopenia and mild anemia may also occur. Neutropenia is typically reversible with drug dose reduction or discontinuance, but can occasionally persist for weeks or even months, particularly after chronic cyclophosphamide therapy. Recombinant granulocyte colony-stimulating factor can be used to hasten recovery in dogs with severe cyclophosphamide-induced neutropenia. Alopecia is most common in susceptible breeds such as poodles and old English sheepdogs. Interestingly, cyclophosphamide has been used in the past to ‘chemically shear’ sheep.

A major side effect of cyclophosphamide that is (to a large extent) unique to this drug is the development sterile hemorrhagic cystitis. Cystitis is mediated by urinary excretion of the cyclophosphamide metabolite acrolein. Cystitis is often severe and debilitating to the patient, and will not resolve until the drug is discontinued. Unfortunately, distressing signs of cystitis can sometimes persist for days or even weeks after drug discontinuation. Cystitis is more likely to develop after long-term therapy with cyclophosphamide, which can present a particular problem for patients with immune-mediated diseases because such diseases are often persistent, and tend to relapse if immunosuppressive therapy is discontinued prematurely. The incidence of cystitis can be reduced significantly by the concurrent administration of furosemide or sodium 2-mercaptoethane sulfonate (mesna), a sulfhydryl donor that binds acrolein, by ensuring ready access to water, and by taking canine patients for regular walks. The chronic local bladder inflammatory effects of cyclophosphamide have also been reported to predispose to the development of irreversible bladder wall fibrosis and transitional cell carcinoma.

Over the years, a number of immune-mediated and inflammatory diseases in dogs and cats have been treated with cyclophosphamide, including immune-mediated hemolytic anemia (IMHA), immune-mediated thrombocytopenia, megakaryocyte hypoplasia; pure red cell aplasia, systemic lupus erythematosus, immune-mediated polyarthritis, inflammatory bowel disease, glomerulonephritis, noninfectious inflammatory meningoencephalitis, immune-mediated vasculitis and pemphigus. For many years, cyclophosphamide was considered a ‘big gun’ to be used in dogs with severe or life-threatening IMHA. However, in the late 1990s and early 2000s, a number of case studies were published that suggested that, at best, cyclophosphamide was not better than glucocorticoids alone for the treatment of IMHA and, at worst, associated with a higher than expected mortality rate. Given the known limitations of retrospective studies, including the associated potential for ‘case selection bias’ (that is, the dogs with the most severe IMHA may have been given cyclophosphamide because it was the drug perceived to be most potent), it is hard to know with any real certainty whether cyclophosphamide actually worsens prognosis in dogs with IMHA. Nevertheless, there is no doubt that, since publication of these papers, the use of cyclophosphamide to treat conditions such as canine IMHA has markedly decreased.

Compared to many immunosuppressive agents, cyclophosphamide is relatively cheap, with a generic 25 mg tablet costing under $2, and a 50 mg tablet costing under $4. One of the major
problems associated with using cyclophosphamide as an immunosuppressive agent is that it is difficult to dose accurately, and even more difficult to taper, especially in smaller patients. Cyclophosphamide is available as 25 or 50 mg tablets that composed of an active inner tablet surrounded by an inert outer flecked tablet. Because of uneven distribution of the drug through the tablet, cyclophosphamide tablets cannot be split or crushed without a risk of major dosing inaccuracies. Without drug compounding, cyclophosphamide doses must therefore be in multiples of 25 or 50. Published immunosuppressive doses for cyclophosphamide in dogs include 50 mg/m$^2$ or 1.5 to 2.5 mg/kg every second day or daily on a ‘4 days on, 3 days off’ weekly protocol. In cats and small dogs, similar total weekly doses can be used, but ‘pulsed’ at infrequent dosing intervals that ensure that the total weekly dose is equivalent to seven times the calculated daily dose. Since myelosuppression can occur at any time during chronic cyclophosphamide therapy, complete blood counts must be performed regularly throughout the course of drug treatment. Cyclophosphamide is available in an intravenous form as well as an oral form, and a recent study in dogs confirmed that equivalent oral and intravenous doses of cyclophosphamide achieved comparable blood levels of the active metabolite 4-hydroxycyclophosphamide. Intravenous cyclophosphamide may therefore be a viable treatment option in vomiting animals that are unable to tolerate oral immunosuppressive agents.

**Chlorambucil**

Chlorambucil is a nitrogen mustard derivative cell-cycle nonspecific alkylating agent that has, for many decades, been used in both human and veterinary medicine predominantly as an antineoplastic agent for the treatment of cancers such as lymphoid leukemia, lymphoma, mast cell tumors, multiple myeloma and polycythemia vera. Antineoplastic cytotoxicity is derived from inappropriate cross-linkage of cellular DNA and RNA by insertion of alkyl radicals on the purine base, guanine. Chlorambucil also has immunosuppressive properties, and has occasionally been used in human medicine to treat immune-mediated and inflammatory conditions such as glomerulonephritis. More than 30 years ago, some veterinary clinicians began suggesting the use of chlorambucil as an immunosuppressive agent for our small animal patients. Since then, the use of chlorambucil for the treatment of a number of feline inflammatory skin conditions, such as pemphigus and eosinophilic granuloma complex, and for treatment of diseases such as immune-mediated thrombocytopenia and refractory inflammatory bowel disease, has become very well established, primarily because of a paucity of viable alternative medications that could be accurately dosed with safety in cats. The use of chlorambucil as immunosuppressive agent in dogs has been slower to evolve, but its use has been described for the treatment of pemphigus, glomerulonephritis and, most recently, inflammatory bowel disease. It is somewhat surprising that chlorambucil has not attained more common usage as an immunosuppressive agent in dogs, since it appears to have much the same mechanism of action as cyclophosphamide with significantly less onerous side effects (specifically, chlorambucil does not cause sterile cystitis), and comes in a more convenient tablet size.

Chlorambucil is metabolized predominantly in the liver, primarily to the active metabolite phenylacetic acid mustard. Compared to other alkylating agents, chlorambucil is relatively well tolerated, especially at immunosuppressive doses, but does occasionally cause gastrointestinal side effects such as vomiting and diarrhea, and/or myelosuppression with neutropenia, thrombocytopenia and non-regenerative anemia (anemia is usually mild). Alopecia and poor hair growth are sometimes reported in susceptible dog breeds, such as poodles. Neurologic side
effects are reported with chronic chlorambucil use in people, and chlorambucil-associated neurologic signs (including myoclonus, twitches and seizures) have been reported in cats.

Chlorambucil is available as a coated 2 mg tablet that cannot feasibly be divided, and dosing recommendations in smaller patients are therefore typically provided in multiples of two, and/or ‘pulsed’ at infrequent dosing intervals (given at an interval that ensures the overall weekly dose is equivalent to seven times the calculated daily dose) in order to avoid overdose. For immunosuppressive therapy, chlorambucil is almost always given in combination with an oral glucocorticoid. In dogs, recommended starting oral immunosuppressive chlorambucil doses (with a glucocorticoid) range from 0.1 to 0.2 mg/kg (or, alternatively, 4 to 6 mg/m²) every one to two days, with dosing individualized based on patient size and disease severity. In cats (and small dogs) with inflammatory or immune-mediated disease, a starting oral chlorambucil dose of 2 mg every second day (with a glucocorticoid), tapered to every 3rd or 4th day, is my preferred dosing regime, although a number of other tapered dosing protocols are also available. Lower daily doses of chlorambucil, comparable to dog dosing regimes, can also be used in cats if the drug is compounded, but the effects of compounding on drug efficacy have not been established. Complete blood counts must be monitored regularly (weekly at first) and, since myelosuppression tends to be dose-dependent rather than idiosyncratic, doses can be tapered ‘to effect’ rather than discontinued completely. Myelosuppression, provided it is detected promptly, is typically reversible.

Compared to many other immunosuppressive agents, chlorambucil has until recently been moderately priced. Unfortunately, the patent on the only available chlorambucil product, Leukeran®, recently expired, leading to a change in ownership of the company responsible for distributing the drug, and the US price of chlorambucil has doubled as a result, to over $10 for a 2 mg tablet. There are currently no other US generic alternatives, apart from compounded products.

Azathioprine

Azathioprine has been used as an immunosuppressive agent in dogs for over 50 years. The drug was initially primarily used in studies that utilized dogs as a model for investigations of organ transplantation and the effects of immunosuppression on various body systems. Within a few years, azathioprine was also being used to treat naturally occurring diseases in dogs. Despite almost half a century of cumulative clinical and research experience on the use of azathioprine in dogs, however, there have been remarkably few studies that actually elucidate the precise effects that azathioprine has on the canine immune system. Most of our understanding of the mechanism of action of azathioprine in dogs is extrapolated from work in other species.

Azathioprine is a prodrug for the active metabolite 6-mercaptopurine, and the primary mechanism of action was long believed to be inhibition of the synthesis of the purines adenine and guanine by blockage of enzymes such as amidophosphoribosyltranserase, with resultant production of nonfunctional nucleic acid strands. Disruption of purine synthesis inhibits DNA and RNA synthesis, thereby inhibiting the proliferation of fast-growing cells such as lymphocytes. In the past few decades, however, multiple other mechanisms of action mediated by various azathioprine metabolites have been proposed, including blockage of T cell activation and stimulation of T cell apoptosis. Azathioprine has long been reported to be more effective against T cell function than B cell function, although strong evidence supporting this is lacking.
One of the key enzymes involved in azathioprine metabolism and inactivation is thiopurine methyltransferase (TPMT). Individual human patients (about one in 300 people) inherit a marked deficiency in the TPMT enzyme that renders them highly susceptible to azathioprine toxicity, particularly life-threatening bone marrow suppression. Interestingly, cats have also been shown to have a marked deficiency in TPMT enzyme activity, which may explain why azathioprine causes marked myelosuppression in cats at standard canine doses. Although the use of azathioprine at a very reduced dose rates has previously been published in cats, given the narrow margin for safety it is probably wisest to recommend that azathioprine never be used in cats at any dose, especially considering the availability of other immunosuppressive agents that appear to be much safer in cats, such as chlorambucil and cyclosporine. Although TPMT expression in dogs is widely variable, severe deficiencies in enzyme activity of the magnitude seen in cats and some people have not been commonly reported, and TPMT deficiency does not appear to be associated with the severe drug toxicities sometimes seen in dogs.

The standard azathioprine starting dose in dogs is 2 mg/kg orally once daily. This dose is usually well-tolerated and, although gastrointestinal side effects such as nausea, anorexia, vomiting and diarrhea are occasionally reported, they are typically mild and self-limiting. Although, in dogs, marked myelosuppression is uncommon, chronic azathioprine usage almost invariably causes a mild to moderate poorly regenerative anemia. Since anemia is an expected outcome in dogs receiving azathioprine, and is typically very well tolerated (that is, sub-clinical), mild to moderate anemia alone should not be mistaken as evidence of either drug overdose or treatment failure. Uncommonly, azathioprine can also cause profound myelosuppression or severe hepatotoxicity in dogs. Marked myelosuppression and hepatotoxicity appear to be idiosyncratic non-dose-dependent drug reactions (Type B reactions), and are typically reversible if the problem is recognized early enough and azathioprine is discontinued. Complete blood counts and serum biochemistry panels (especially ALT) should therefore be monitored regularly during initial azathioprine therapy. Several individual case reports have also reported pancreatitis in dogs receiving azathioprine, but cause and effect has not been established.

Azathioprine has, over the years, become well-established as an ‘add on’ immunosuppressive agent to be considered for the treatment of many different immune-mediated and inflammatory conditions when glucocorticoids alone are ineffective or poorly tolerated, including immune-mediated hemolytic anemia, immune-mediated thrombocytopenia, inflammatory bowel disease, chronic hepatitis, glomerulonephritis, immune-mediated polyarthritis, myasthenia gravis, non-infectious meningoencephalitis, immune-mediated skin diseases, and anal furunculosis. Despite decades of azathioprine usage, evidence supporting immunosuppressive efficacy for many of these common diseases is remarkably limited. Interestingly, because (despite a relative paucity of evidence) azathioprine has commonly been recommended as the standard immunosuppressive drug of choice for many conditions, the efficacy of newer drugs for the treatment of these conditions is sometimes compared to a parallel group receiving azathioprine. One perceived ‘limitation’ of azathioprine compared to other immunosuppressive agents, that it can take many weeks or even months to exert its effects, is based on limited and dated data derived predominantly in humans. In my experience, azathioprine in a clinical setting exerts its immunosuppressive effects in dogs about as rapidly as most other comparable agents.

Compared to most other immunosuppressive agents, azathioprine is relatively inexpensive, which is an important consideration with long-term immunosuppressive therapy, especially in
large dogs. While the proprietary product (Imuran® or Azasan®) typically still costs over $5 per 50 mg tablet, the generic equivalent can be obtained for less than $1 a tablet. The smallest tablet size is 50 mg (although tablet scoring permits a 25 mg dose), which can present dosing problems in small (under 20 lb) dogs.

**Danazol**

Danazol, a synthetic androgen with weak (‘impeded’) androgenic effects, has in the past been suggested for the treatment of canine immune-mediated hemolytic anemia and immune-mediated thrombocytopenia, in combination with glucocorticoids, in order to reduce the dose of steroid that is needed. Danazol is derived from the synthetic steroid ethisterone, a modified progestogen. Danazol’s most important mechanism of action is probably to reduce macrophage Fc receptor/antibody binding affinity. Danazol also competes with glucocorticoids for combination with steroid-binding globulin, consequently increasing the availability of active unbound glucocorticoid. Concurrent danazol therefore enables significant glucocorticoid dose reduction. Danazol may also reduce the degree of binding of antibody and complement to the red blood cell or platelet surface. Side effects are uncommon, and include hepatotoxicity and masculinization of female dogs. However, although some dogs with refractory IMHA and IMT have been reported to benefit from danazol, the drug fell out of favor a few decades ago, probably because it was very expensive at the time, and response to therapy was sluggish and highly unpredictable.

Reported oral danazol doses in dogs with IMHA or IMT, in combination with glucocorticoids, range from 5 to 15 mg/kg daily, either given as a single dose or 2-3 divided doses. Danazol comes in 50 mg, 100 mg, and 200 mg capsules. Danazol currently costs a little less than $2 for a 50 mg capsule.

**Vincristine**

The vinca alkaloids are biologically-active dimeric alkaloids derived from the Madagascar (or rosy) periwinkle plant, Catharanthus roseus. Vincristine, a naturally-occurring vinca alkaloid, were originally characterized phytochemically more than fifty years ago. The diverse biological effects of vincristine have traditionally been attributed to drug-induced disruption of various intracellular microtubules. Microtubules are elongated, tubular cytoplasmic organelles involved in a broad spectrum of cellular processes including chromosomal migration, conduction of cellular secretions, ciliary and flagellar motility, and maintenance of cell shape. Microtubules are composed predominantly of complex helical polymers of the structural protein tubulin. Vincristine binds directly to tubulin, causing both inhibition of microtubule synthesis and disruption of intact microtubules. Microtubular structures susceptible to the effects of vinca-tubulin binding include the mitotic spindle in dividing cells, the neurotubules in neurons, and the cytoskeletal microtubules in platelets. Vincristine may also exert biological effects that are independent of disruption of intracellular microtubules, such as inhibition of RNA, DNA and protein synthesis, and modification of prostaglandin production.

Vincristine is a cell-cycle-specific cytotoxic agent. Vincristine disrupts microtubules within the mitotic spindle of dividing cells, thereby arresting chromosomal separation in metaphase. Vincristine at standard therapeutic doses is minimally myelotoxic, and is therefore commonly used in combination with more myelosuppressive chemotherapeutic agents. Vincristine is
frequently used in veterinary cancer chemotherapy, both as a single agent for the treatment of canine transmissible venereal tumors, and as a component of combination protocols for the treatment of acute leukemia, lymphoreticular neoplasms, mast cell tumors, and various carcinomas and sarcomas.

Vincristine is usually administered intravenously as a sulfate salt, which is chemically more stable than its corresponding free base. Inadvertent subcutaneous or intramuscular administration causes severe local tissue irritation and necrosis. Oral absorption of vincristine is poor. Plasma disappearance of vincristine following intravenous administration is markedly biphasic, with a short initial half-life and a prolonged terminal half-life. The short initial clearance phase reflects extensive extravascular drug redistribution due to a combination of both avid binding to intracellular tubulin and rapid biliary excretion. The prolonged terminal clearance phase is due to the gradual release of vincristine bound to circulating plasma proteins and intracellular tubulin. Platelets demonstrate a remarkable ability to concentrate vincristine from plasma, and are therefore the principal circulating cellular carriers of the drug.

The degree of immunosuppression induced by vincristine at intravenous therapeutic doses is minimal compared to that induced by glucocorticoids, cyclophosphamide or azathioprine, and vincristine therefore is not used as an immunosuppressive agent for the treatment of most immune-mediated or inflammatory diseases in dogs and cats. The one exception is immune-mediated thrombocytopenia (IMT), where vincristine has become a mainstay of treatment.

During early clinical trials in human cancer patients, it was observed that the administration of vincristine was frequently associated significant but transient increases in circulating platelet numbers. A similar phenomenon has since been reported in dogs, both in research animals and in cancer patients. This effect appears to be due to increased megakaryocytopoiesis and thrombopoiesis, although the precise mechanisms of vincristine-associated thrombocytosis are still uncertain. Circulating platelet life-span does not appear to be significantly affected by standard low doses of vincristine in healthy animals.

The serendipitous discovery that vincristine induced thrombocytosis in human cancer patients with normal pre-treatment platelet numbers prompted conjecture that a similar outcome could be obtained in thrombocytopenic patients. Following publication of several anecdotal reports describing prompt, marked increases in circulating platelet numbers after administration of vincristine to people with IMT, vincristine gained favor with some hematologists as the treatment of choice for chronic refractory IMT. Vincristine frequently induces partial or complete remission of thrombocytopenia within one week of commencing therapy, although such remissions are typically transient. Only a relatively small proportion of human chronic refractory IMT patients achieve complete sustained remission with vincristine therapy.

Rapid drug clearance from plasma reduces the therapeutic efficacy of a standard intravenous bolus of vincristine. Several alternate methods of vincristine administration have therefore been used in human IMT patients in order to sustain therapeutic plasma concentrations. Constant intravenous vincristine infusion (over six to eight hours) effectively maintains therapeutic plasma concentrations the drug throughout the period of administration. Alternatively, the ability of platelets to concentrate vinca alkaloids from plasma has been utilized to enhance therapeutic efficacy via transfusion of vincristine-loaded platelets. Incubation of donor platelets in high concentrations of vincristine (vinca loading) prior to transfusion maximizes intracellular vinca-
tubulin binding. Following transfusion, circulating vinca-loaded donor platelets gradually release vincristine into the recipient's plasma, thereby sustaining therapeutic plasma drug concentrations. Both constant rate infusion with vincristine and transfusion with vinca-loaded platelets induce sustained remissions in some human chronic IMT patients previously refractory to single intravenous boluses of the drug.

Vincristine, typically in combination with prednisone, has been reported to similarly facilitate remission of thrombocytopenia in many canine patients with IMT. Original case reports demonstrating an apparent rapid response to vincristine in dogs with IMT have been supported, decades later, by evidence obtained from prospective studies. Circulating platelet numbers increase markedly within three to five days of vincristine administration in responsive dogs, and the addition of vincristine to standard immunosuppressive therapy in dogs with IMT appears to shorten hospitalization time by several days. Most authors currently recommend an intravenous vincristine bolus dose of 0.02 mg/kg for the treatment of canine IMT. Vincristine boluses may subsequently be repeated weekly if thrombocytopenia recurs. Apparent rapid clinical response to vincristine-loaded platelets has been reported in one dog with refractory IMT. Vincristine has been used in cats with IMT, although evidence of clinical efficacy is lacking. One significant advantage of vincristine compared to other therapeutic options for IMT (such as human intravenous globulin) is that vincristine is inexpensive (a 1 ml vial of 1mg/ml vincristine sulfate costs around $20).

The pathogenesis of vincristine-induced remission of thrombocytopenia in IMT patients is uncertain. Clinicians initially assumed that remissions were due to increased megakaryocyte production and release of platelets, the principal mechanism assumed to underlie the vinca-induced thrombocytosis seen in healthy animals and cancer patients. However, since IMT patients typically already have high levels of circulating thrombopoietic factors and maximal thrombopoiesis, platelet precursors may be refractory to further stimulation by vincristine. Furthermore, studies in people suggest that the main therapeutic effect of vincristine in IMT patients is not increased thrombopoiesis. Post-treatment average platelet life-spans are significantly prolonged in human IMT patients that respond to vincristine, suggesting that remission is due to reduced platelet destruction rather than increased platelet production. Since platelets are the major circulating cellular carriers of vincristine, researchers have speculated that antibody-coated platelets selectively deliver vincristine to those phagocytes within the mononuclear phagocytic system that are actively involved in platelet destruction. This proposed mechanism explains why, despite being an ineffective immunosuppressive agent for the treatment of most conditions, vincristine can still be very effective for the treatment of IMT.

During electron microscopic studies of platelet ultrastructure, it was discovered that prolonged incubation of platelets in vincristine solutions caused marked disruption of cytoskeletal microtubules. Laboratory investigations have since demonstrated that as well as disrupting platelet structure, exposure to high concentrations of vincristine also significantly impairs platelet function. Based on the in vitro evidence that exposure to vincristine impairs platelet function, hematologists expressed concern that using the drug in patients with IMT could similarly induce platelet dysfunction. Subsequent studies revealed that vincristine affected platelet function (aggregation) in dogs with lymphoma, but not in healthy dogs. Since several recent prospective studies showed no significant increase in bleeding in IMT dogs receiving
vincristine, the effect of vincristine on platelet function, if it occurs, does not appear to be severe enough to be clinically significant.

Neurotoxicity, although uncommon, is the most frequent significant side-effect associated with therapeutic doses of vincristine in dogs and cats. Reversible vincristine-induced neurotoxicity in the dog has been reported with chronic cancer chemotherapy, but is not likely to be an issue with the single doses used to treat IMT. Other side-effects such as gastrointestinal disorders (including megaesophagus and gastric hypomotility) and alopecia, occur less frequently and are typically mild and temporary. Vincristine at doses used for IMT typically causes minimal myelosuppression in dogs, although dogs with the ABCB1-1Δ (MDR1) gene mutation and some Border Collies have been reported to be more susceptible than other dog breeds to myelosuppression at antineoplastic vincristine doses. In affected Border Collies, this effect appears to sometimes be independent of the MDR1 gene mutation reported in this breed. Temporary erythrodysplasia of erythroid precursors in the bone marrow and peripheral blood smears, featuring bizarre mitotic figures, abnormal nuclear configurations, and Howell-Jolly bodies, can be observed after administration of vincristine in dogs, but is of little clinical significance. An unusual transient pulmonary toxicity has been reported in a cat receiving chemotherapeutic doses of vincristine. Vincristine has no known mutagenic or carcinogenic potential.

Given the high price of many human immunosuppressive agents, and also given the dosing difficulties associated with giving human tablet and capsule sizes to our smaller patients, veterinarians are often tempted to instead use compounded equivalents of these drugs. Compounded versions of many of these drugs can be found at on-line pharmacies that cater to the veterinary market at attractive prices and convenient dosing sizes. However, the bioavailability and clinical efficacy of most of these products in our patients is not established and, with the few drugs where the compounded version has been evaluated (cyclosporine, for example), drug bioavailability was markedly variable and often led to subtherapeutic blood concentrations. Using these products in our patients, especially in those animals with life-threatening disease, therefore represents a major gamble.

In animals that are difficult to give pills to, it is tempting to use a liquid compounded formulation. However, many of the common immunosuppressive agents are potentially mutagenic, carcinogenic and teratogenic. In fractious animals that end up with more medication on their whiskers and fur than in their mouth, given liquid suspensions has the potential to significantly increase the level of owner exposure to these potentially dangerous drugs.
Several potent immunosuppressive drugs developed over the past few decades in human medicine have recently made the leap to our small animal patients, and our use of them is growing. This lecture will discuss cyclosporine, leflunomide and mycophenolate.

**Cyclosporine**

Cyclosporine is a potent immunosuppressive drug indicated for the treatment of inflammatory and immune-mediated diseases, and for organ transplantation. Cyclosporins are cyclic polypeptide macrolides originally derived from the soil fungus *Beauveria nivea* (*Tolypocladium inflatum*), but are also produced by other fungal organisms. Cyclosporine A is the molecule developed for commercial use as an immunosuppressive agent. Discovered in the 1970s, the use of cyclosporine as an immunosuppressive agent was first described in humans to prevent rejection of renal allografts. Within a decade, cyclosporine had become the cornerstone of immunosuppression for organ transplantation. In veterinary medicine, oral cyclosporine capsules received FDA approval in 2003 for the treatment of canine atopy, and were more recently also approved for allergic skin disease in cats. Cyclosporine has been used in an extra-label fashion for many years for renal transplantation in dogs and cats, and for the treatment of a variety of inflammatory and immune-mediated conditions.

Cyclosporine’s primary immunosuppressive mechanism of action is inhibition of T lymphocyte function. Cyclosporine acts to inhibit calcineurin, an intracellular protein phosphatase that activates gene transcription factors through dephosphorylation. In the untreated patient, activation of T cells results in activation of calcineurin, which dephosphorylates inactive nuclear factor (NFAT). NFAT translocates into the nucleus, where it upregulates transcription of genes coding for several important cytokines, including IL-2, IL-4, TNF-α, and INF-γ. Production of IL-2 in particular plays a key role in the activation and proliferation of T cells. Calcineurin inhibitors, including cyclosporine, act by binding to intracellular cyclophilins, which are proteins that facilitate protein folding. Binding of cyclosporine to cyclophilin A creates a complex with high affinity for calcineurin. Through inhibition of calcineurin, cyclosporine specifically inhibits T cell function and thus, cell-mediated immunity, but has little immediate impact on humoral immunity. Decreased IL-2 expression in CD4+ Th1 cells associated with cyclosporine therapy leads to inhibition of proliferation and activation of both T-helper and T-cytotoxic lymphocytes, and blunting of the immune response. Cyclosporine has also been shown to have many other local anti-inflammatory and immunosuppressive effects, especially in the skin.

Cyclosporine is a large lipophilic molecule which must be solubilized prior to intestinal absorption. Commercial cyclosporine is available as two very different types of oral formulations. Cyclosporine was initially approved in humans as a vegetable-oil based preparation (Sandimmune®), but variability in oral bioavailability caused marked variability in blood drug concentrations. A more recent formulation, an ultramicronized preparation approved
in 1996 (Neoral®), forms a microemulsion upon contact with aqueous fluids, resulting in more consistent and predictable absorption. Oral bioavailability of the microemulsion is improved by up to 50% compared to the oil-based preparation. Because of the marked variability in bioavailability of the non-ultramicronized preparation, it is not recommended for oral use in small animals.

Cyclosporine has a high binding affinity for red blood cells and plasma lipoproteins. Because up to 50% of the drug in blood is located in red cells, whole blood is recommended for therapeutic drug monitoring (TDM). Once in the circulation, cyclosporine distributes widely, accumulating in the skin, liver, kidneys, and fat of dogs, resulting in a large volume of distribution. Tissue levels exceed levels in serum by a factor of 3 to 14. Peak blood concentrations generally occurring approximately 2 hours after oral administration of cyclosporine. Blood concentrations then rapidly decrease over the remainder of the dosing interval, reflecting a relatively rapid half-life as the drug is cleared from plasma.

Extensive metabolism of cyclosporine by the hepatic cytochrome P-450 system yields many different metabolites, some of which may retain therapeutic efficacy. In dogs, several drugs that inhibit P-450 enzymes have been given concurrently with cyclosporine in order to decrease the dose needed to maintain adequate blood drug concentrations. Ketoconazole, in particular, has been used to decrease in oral cyclosporine dosages in dogs by as much as 75 percent, although individual responses are variable.

The complexities of cyclosporine disposition in normal animals, coupled with confounding factors associated with disease and differences in drug preparation, may contribute to markedly variable blood drug concentrations both between patients and even within the same patient. Therapeutic management may therefore be facilitated by monitoring blood cyclosporine concentrations. Unfortunately, however, the process of adjusting drug doses based on monitoring cyclosporine blood concentrations is clinically complex, and not necessarily associated with the desired clinical outcome. Currently available methods for TDM include HPLC, a specific monoclonal RIA, and a dimersion cyclosporine immunoassay. HPLC has the advantage that the parent drug can be discriminated from metabolites, although most methods detect only the parent compound. Both RIA and dimension cyclosporine immunoassay, in contrast, measure metabolites as well as the parent drug, and blood cyclosporine concentrations will therefore be higher by a factor of 1.5 to 1.7 compared to the same sample analyzed using HPLC. Although HPLC is considered the gold standard for cyclosporine assays, HPLC is labor intensive and not routinely offered for patient monitoring. TDx and RIA have been the methods most often employed in clinical situations, with the laboratory performing the assay typically providing recommendations regarding ideal target blood drug concentrations. Some laboratories have adjusted target blood concentrations upward to reflect the fact that TDx and RIA results will be approximately double HPLC assay results. Other laboratories have not made this adjustment, with the rationale that the cyclosporine metabolites measured by the TDx and RIA assays may arguably be pharmacologically active and contribute to overall immunosuppressive effects. Much study has gone into determining the most appropriate sample collection time in patients receiving cyclosporine. In human medicine, trough blood concentrations were the initial basis for adjustment of drug dosages. However, multiple studies in people have since suggested that area under the plasma drug concentration time curve (AUC) or 2 hour peak drug concentrations are preferred. With measurement of peak cyclosporine concentrations requiring only a single
sample, adjusting drug doses to attain target peak drug levels has become the single best blood concentration measurement for use during human organ transplantation. In veterinary medicine, measurement of trough cyclosporine concentrations also prevailed for many years based on initial work done in canine and feline renal transplant studies. Recommendations from laboratories offering TDM have often involved measurement of both peak and trough cyclosporine blood levels, although target peak concentrations have not been well established. Individual laboratory recommendations depended on the target ranges determined by each laboratory as well as the assay used to measure cyclosporine concentrations. Currently, the Auburn University Clinical Pharmacology Laboratory is the only veterinary laboratory routinely offering cyclosporine blood level assays.

Pharmacodynamic assays investigate a drug’s effect on target cells. Several pharmacodynamic biomarkers of the immunosuppressive effects of cyclosporine have been studied in human medicine, including lymphocyte proliferation, calcineurin enzyme activity, lymphocyte surface antigen expression, and intracellular cytokine quantification. Through pharmacodynamic monitoring, human studies have shown individually distinct degrees of calcineurin inhibitor sensitivity in patients. Pharmacodynamic monitoring shows great promise for optimizing cyclosporine therapy and delivering individualized therapy. At Mississippi State University, there are ongoing investigations into the pharmacodynamic evaluation of cyclosporine in dogs. We recently measured activated T cell expression of IL-2, IL-4, and IFN-γ via flow cytometry in dogs administered two different oral cyclosporine dosages. The dogs were first administered a high dose of cyclosporine (10 mg/kg orally twice daily), with doses adjusted upwards as needed to attain a target trough drug concentration greater than 600 ng/mL as measured via HPLC, a dosing protocol known to be sufficiently immunosuppressive for canine organ transplantation. With high dose cyclosporine, activated T cell expression of IL-2 and IFN-γ was significantly suppressed. The dogs were then administered the FDA-approved dose of cyclosporine used to treat canine atopy (5 mg/kg orally once a day), a dose which has been considered to be low enough to avoid predisposing to immunosuppression-associated infection. Even with this low dose of cyclosporine, however, T cell expression of IFN-γ and IL-2 was still markedly suppressed in some dogs. Subsequent studies evaluating activated T cell mRNA IL-2 and IFN-γ expression utilizing molecular methods have demonstrated that results using a quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) assay are comparable to flow cytometry, and that the technique shows promise as a pharmacodynamic assay in dogs. One advantage of the qRT-PCR assay compared to flow cytometry is that it can be performed on blood samples mailed in by practitioners. Cyclosporine has been shown to have much the same effect on T cell cytokine production in cats as it does in dogs.

Cyclosporine is FDA-approved for the treatment of canine atopic dermatitis and feline allergic skin disease, and has also been used to prevent transplant rejection and to treat sebaceous adenitis, pemphigus foliaceus, anal furunculosis, feline stomatitis, inflammatory bowel disease (IBD), myasthenia gravis, non-infectious inflammatory meningoencephalitis, pure red cell aplasia, immune-mediated hemolytic anemia (IMHA), immune-mediated thrombocytopenia (IMT), and immune-mediated polyarthritis in dogs and cats. Recent pharmacodynamic research evaluating T cell responses to cyclosporine in dogs has confirmed that canine responses are comparable to the response profile that is well recognized in people: that individual responses to cyclosporine are extremely variable from dog-to-dog, both in dogs receiving the same standard oral dose, and in dogs with oral doses adjusted to attain comparable blood levels. Given that a
high degree of variability of individual responsiveness to cyclosporine has been established in dogs, cyclosporine dosing protocols should be tailored to allow for this patient-to-patient variability. In my opinion, recommended dosing protocols in dogs with chronic, non-life-threatening inflammatory skin and gastrointestinal diseases should be quite different from the protocols used in dogs with more acute and life-threatening immune-mediated diseases.

In chronic inflammatory diseases that are typically not immediately life-threatening, such as skin conditions, anal furunculosis, and mild IBD, cyclosporine is often effective at a standard, relatively low starting dose. Cyclosporine therapy is typically delivered long term, with drug doses adjusted upwards if needed ‘to effect’, based predominantly on clinical signs. Most commonly, however, starting doses do not need to be increased and, in the long-term, the cyclosporine dosage is typically tapered to the lowest effective dosage needed to maintain disease remission. Currently recommended starting cyclosporine doses in dogs are 5 mg/kg once daily for most skin diseases and IBD, and 5 mg/kg once to twice daily for anal furunculosis. In cats with skin conditions such as allergic skin disease, eosinophilic granuloma complex and pemphigus foliaceus, a starting cyclosporine dose of around 5 mg/kg daily is recommended. Cyclosporine blood concentrations are usually not necessary for treatment of these conditions, as remission of disease is the main criterion used to decide whether adequate cyclosporine therapy is being delivered. In fact, for many of these conditions, cyclosporine blood concentrations have been shown to have minimal correlation with disease remission, perhaps because the drug is selectively concentrated in tissues such as the skin. Recent pharmacodynamic studies, however, have shown that, even at standard low FDA-approved doses, some dogs can still develop significant suppression of certain T-lymphocyte biomarkers of immunosuppression despite very low trough cyclosporine concentrations. This could explain the phenomenon anecdotally reported by some dermatologists, that individual dogs treated for atopic dermatitis can develop severe secondary infections, although the ‘atopy’ cyclosporine dose was originally not thought to cause clinically significant immunosuppression. Therefore, even in dogs on low cyclosporine doses, clinicians should remain vigilant for potential signs of systemic infection.

In canine patients suffering from more acute and immediately life-threatening diseases such as severe IMHA and IMT, in contrast, cyclosporine must be targeted to attain effective immunosuppression as rapidly as possible. These animals are somewhat comparable to patients that have recently undergone organ transplantation, in that any delay in attaining effective immunosuppression can lead to a disastrous outcome. In these patients, starting cyclosporine at a low dose and adjusting doses upwards ‘to effect’ is not recommended. Attaining effective oral doses as rapidly and accurately as possible is essential for ensuring adequate immunosuppression whilst avoiding overdosage with associated adverse effects and expense. Currently recommended starting cyclosporine doses for life-threatening diseases range from 5 mg/kg to 10 mg/kg twice daily. Subsequent measurement of blood cyclosporine concentrations and/or assessment of activated T cell mRNA IL-2 and IFN-γ expression using qRT-PCR within one week of commencement of treatment, with dose adjustments as needed, are the best methods that are currently routinely available to assess adequacy of therapy, and are strongly recommended in patients with life-threatening diseases.

Side effects are uncommon with cyclosporine therapy in dogs and cats, with the exception of gastrointestinal side effects such as vomiting, diarrhea, anorexia and nausea. Administering the medication frozen and/or with food can reduce gastrointestinal side effects, although there is a
risk that such measures will also alter drug absorption profiles. Uncommonly, cyclosporine can cause an idiosyncratic hepatotoxicity, which does not seem to be dose dependent. Gingival hyperplasia and hypertrichosis have also occasionally been reported with cyclosporine therapy. Chronic cyclosporine therapy may also predispose to neoplasia such as lymphoma. One advantage of cyclosporine as an immunosuppressive agent is that it is not myelosuppressive. Experimentally, oral cyclosporine has been shown to increase some markers of platelet activation in normal dogs, which may be a concern in patients with IMHA, where hypercoagulability and resultant pulmonary thromboembolism can be a major contributor to patient mortality. However, to date, it has not been demonstrated whether this phenomenon is clinically relevant in IMHA patients with naturally occurring disease.

Cyclosporine is an expensive drug, particularly at higher immunosuppressive doses, and clinicians are therefore tempted to explore cheaper forms of the drug. In human medicine, there are many approved human generic microemulsion preparations similar to the Neoral® formulation, and these generic preparations have been shown to have therapeutic equivalency in people. Studies investigating the pharmacokinetic properties of these generic preparations in dogs have not been performed, and it is not safe to assume that a generic formulation is therapeutically equivalent to the approved canine product (Atopica®). Clinically, there appears to be marked variability seen in individual dogs in the oral bioavailability of these generic products. Use of generic products may therefore have the potential place our patients at risk of either therapeutic failure or toxicity. The proprietary human microemulsified cyclosporine product, Neoral®, currently costs around $2 for a 25 mg capsule and $6 for a 100 mg capsule, while the generic equivalent equivalents cost around $1 and $2 for the 25 mg and 100 mg capsules accordingly. The veterinary product, Atopica®, tends to be priced comparably to the human proprietary products, but has the advantage of being FDA-approved and available in a range of capsule sizes that are convenient for dosing accuracy in our small animal patients (10 mg, 25 mg, 50 mg and 100 mg), as well as a 100 mg/ml oral suspension. Unfortunately, transdermal cyclosporine has been shown to be inadequately absorbed in cats.

**Leflunomide**

Leflunomide is an isoxazol derivative immunosuppressive drug that was developed within the past two decades, initially for treatment of rheumatoid arthritis and prevention of transplant rejection. Leflunomide is a prodrug for its primary active malononitriloamide metabolite, A77 1726 (also known as teriflunomide). Malononitriloamides reversibly inhibit the mitochondrial enzyme dihydroorotate dehydrogenase, a key enzyme in pyrimidine synthesis, with resultant inhibition of the pyrimidine ribonucleotide uridine monophosphate (rUMP), and decreased DNA and RNA synthesis and G1 cell cycle arrest. Leflunomide inhibits B and T cell function, suppresses antibody production and has anti-inflammatory effects, possibly via inhibition of de novo pyrimidine biosynthesis and cytokine-associated and IL-2-stimulated tyrosine kinase activity.

Prior to commercial development, leflunomide was made available for small animal transplant research to Dr. Clare Gregory’s group at the University of California, Davis. Because of the drug’s availability to this group, a small number of canine patients with refractory naturally-occurring inflammatory and immune-mediated diseases such as immune-mediated hemolytic anemia, immune-mediated thrombocytopenia, non-infectious inflammatory meningoencephalitis,
systemic histiocytosis, immune-mediated polymyositis, immune-mediated polyarthritis, and pemphigus foliaceus were also treated, typically with promising success rates. Unfortunately, when these initial promising results were reported at the ACVIM Forum and in the veterinary literature in the late 1990s, the drug was not commercially available. When leflunomide did become available, as the proprietary product Arava®, the drug was so prohibitively expensive that its use was very limited in small animal clinical studies. Even after the generic equivalent was approved in 2005, leflunomide remained expensive for several more years. Only recently did the generic drug become more affordable and, as a result, anecdotal and preliminary reports of leflunomide’s use in small animal patients are beginning to surface. There are therefore currently very few published reports discussing the use of leflunomide in dogs and cats. Recently, a case series describing the use of leflunomide in 14 dogs with immune-mediated polyarthritis reported a high response rate with minimal side effects.

One of the most promising features of leflunomide in dogs is that it appears to be very well tolerated although if, as anticipated, the drug attains more common usage, it is likely that less frequent but more serious side effects will be recognized. The most common side effect observed with leflunomide use in dogs is occasional inappetence, lethargy and vomiting. Serious side effects occasionally reported in people, and thus with the potential to appear in our veterinary population with more common usage, include myelosuppression, cutaneous drug reactions and hepatotoxicity. In humans, traces of the active metabolite teriflunomide can persist for months or even years after drug discontinuation, and in the instance of severe drug reactions, cholestyramine or activated charcoal is needed to rapidly reduce drug levels. In dogs, the terminal half-life of teriflunomide is much shorter than in humans, so the potential for persistent side effects is probably significantly less. Complete blood counts and serum biochemistry (especially ALT) should be regularly monitored in small animal patients on leflunomide.

The initial recommended starting oral dose for leflunomide in dogs is 2-4 mg/kg daily, with doses adjusted to attain a plasma trough A77 1726 level of 20 µg/ml within a few weeks of commencing therapy. For cats with immune-mediated polyarthritis, a leflunomide dose 10 mg (total dose) orally, once daily, in combination with methotrexate, has been suggested, with dose reductions to effect. Measurement of leflunomide levels is available through the Auburn University Veterinary Clinical Pharmacology Laboratory. One advantage of leflunomide is that it comes in tablet sizes (10 mg and 20 mg) that are convenient for dosing our smaller patients. Leflunomide as the proprietary product Arava® currently costs around $40 for a 10 mg tablet and, interestingly, $40 for a 20 mg tablet, although it is rumored to soon be discontinued. The generic leflunomide equivalent is currently priced at around $1 for a 10 mg tablet and $1.50 for a 20 mg tablet. Leflunomide generics, as with many commercially available generic problems, have an ‘AB’ rating by the FDA, meaning that the generic is ‘equivalent’ to Arava®. However, since ‘equivalence’ is often determined by pharmacokinetic data in healthy individuals, an AB rating does not guarantee identical performance in clinical patients.

**Mycophenolate**

Mycophenolate mofetil is the synthesized prodrug form of mycophenolic acid, a selective and reversible inhibitor of inosine monophosphate dehydrogenase, an enzyme that controls the rate of synthesis of guanine monophosphate in the *de novo* pathway of purine synthesis. Mycophenolate mofetil is a fermentation product derived from fungi in the *Penicillium* group.
Mycophenolic acid inhibits B and T cell proliferation, and decreases antibody production. Mycophenolate mofetil is primarily used in human medicine for prevention of rejection of transplanted organs, although it also used to treat immune-mediated diseases such as systemic lupus erythematosus, immune-mediated hemolytic anemia (IMHA), immune-mediated thrombocytopenia and pemphigus vulgaris. Mycophenolate mofetil is often used in the place of azathioprine in human medicine and, since they have similar mechanisms of action, the two drugs should probably not be used together.

The original proprietary mycophenolate mofetil product, CellCept®, and the closely related mycophenolate sodium product, Myfortic®, were expensive, and as a result the products only achieved limited usage in small animal medicine. However, recently, the availability of much cheaper generic alternatives has led to a greatly increased usage of mycophenolate mofetil in small animal patients. A single 250 mg CellCept® capsule currently costs around $7, whereas the equivalent generic 250 mg capsule costs less than 50c. An oral suspension version of mycophenolate mofetil (200 mg/ml) is available for more convenient dosing in smaller patients. Successful usage of mycophenolate mofetil in a small animal patient with naturally-occurring disease was first described in a dog with acquired myasthenia gravis. Much of the subsequent anecdotal usage of mycophenolate mofetil for a variety of different immune-mediated diseases was similar to the dosing reported in this original paper. Mycophenolate mofetil is also available in an injectable form, and the intravenous use of the drug has been described during the successful initial stabilization of three dogs with acquired myasthenia gravis that could not tolerate oral medications. Ironically, a more recent case report of 15 dogs with acquired myasthenia gravis treated with mycophenolate mofetil reported that the drug was ineffective at attaining clinical remission.

A recommended starting dose for mycophenolate mofetil in dogs is 10-20 mg/kg once daily or divided twice daily, although occasionally gastrointestinal signs (particularly vomiting and diarrhea) at the higher end of the dose rate will necessitate dose reductions. Mycophenolate mofetil appears to have variable oral bioavailability in dogs, so variability in response to therapy should probably be expected. A pharmacodynamic study in dogs measuring inosine monophosphate dehydrogenase enzyme activity suggested that mycophenolate mofetil would best be dosed three times daily, but this recommendation has not entered common usage. Mycophenolate mofetil has not been used widely enough in veterinary medicine to establish the frequency of serious side effects but, in people, gastrointestinal signs and, less commonly, marked myelosuppression and a rare and fatal neurologic disease (progressive multifocal leukoencephalopathy) have been reported. Based on the human side effect profile, complete blood counts should probably be regularly monitored in dogs receiving mycophenolate mofetil. In humans, gastrointestinal side effects can be reduced by replacing mycophenolate mofetil with mycophenolate sodium. Mycophenolic acid in humans is primarily excreted conjugated to glucuronide and, since cats lack the glucuronyl transferases responsible for glucuronidation of drugs such as mycophenolate mofetil, the drug should probably used with caution, if at all, in this species, although the use of mycophenolate mofetil has been described at a dose rate of 10 mg/kg twice daily, with no obvious side effects, in two cats with IMHA.
One ‘side effect’ that is common to all immunosuppressive agents, both established and new, is that they can cause significant immunosuppression. This is highly desirable when treating severe life-threatening diseases, but comes with the significant associated risk that immunosuppression also predisposes to infection. Severe infection and even, occasionally, infection-associated deaths have been associated with most of the immunosuppressive agents used in dogs and cats. This is especially true when high doses of potent drugs are used, or when multiple drugs are used in combination, such as the kinds of protocols that are used to prevent transplant rejection. As well as bacterial infections, immunosuppressed patients can develop all kinds of unusual infections, including toxoplasmosis, mycobacteriosis, nocardiosis, and generalized demodecosis.

Although the risk of infection is always going to be present when immunosuppressive agents are used, a few general guidelines can help to reduce this risk:

- Avoid using powerful immunosuppressive therapy to treat minor diseases that are not life-threatening, and instead save the ‘big guns’ for more severe illnesses.
- Use the lowest effective drug doses that are possible.
- Avoid using combinations of multiple different immunosuppressive agents unless absolutely necessary.
- Screen patients very carefully for underlying infectious disease before commencing immunosuppressive therapy, especially when infection can mimic immune-mediated disease (*Babesia gibsoni* masquerading as IMHA, for example).
- Watch patients on immunosuppressive therapy closely for signs of new infection.