

Immune Mediated Hemolytic Anemia

Approach to Diagnosis, Treatment

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Learning objectives

- Recognize where IMHA fits into anemia classification scheme
- Know the clinical signs for IMHA
- Be familiar with laboratory evaluation for IMHA
- Be familiar with treatments
- Be aware of coexistent thromboembolism prevention and treatment

Causes of anemia

- Nonregenerative anemias
 - Most common
 - Chronic disease (ACD)
- Regenerative anemias
 - Hemorrhage
 - Internal hemorrhage
 - External hemorrhage
 - Hemolysis
 - Non-immune
 - Immune

Approach to immune-mediated anemia

- Establish the presence of hemolysis
 - Physical findings
 - Laboratory findings
- Determine if anemia is immune-mediated
 - Rule out non-immune caused
 - Laboratory indicators
 - Common PE findings
- Determine if IMHA is primary or secondary
 - Rule out secondary causes
 - Specific laboratory findings

IMHA (immune mediated hemolytic anemia)

- Most common immune blood disease
- Difficult and frequently unrewarding disease (mortality 21-83%)
 - Most die or are euthanized in acute phase
 - Death from anemia or thromboembolism
- Usually regenerative unlike other anemias
- May be primary or secondary

Primary IMHA

- 60-70% of IMHA are primary
- No recognized underlying disease
- More Common in dogs than cats
- Cockers (3-12X), English Springers, Poodles, OESD
- Young animals predominate
- Equal sex distribution, but neutered more common
- Geographic and seasonal variability

Secondary IMHA

- Important to rule out to treat appropriately
- More common in cats (FeLV)
- Any age, breed, sex may be affected

Secondary IMHA

- Medications
 - TMS, Penicillins, Cephalosporin's, Levamisole
 - PTU, Methimazole
 - NSAIDs (Phenylbutazone), Dipyrone, Quinidine, Chlorpromazine, cyclosporins
 - Phenobarbital
- Immunological
 - SLE
 - Transfusion reactions
 - Neonatal isoerythrolysis (cats)
 - Antilymphocyte globulin (transplants)
- Infectious/parasitic (up to 28%)
 - FeLV
 - Hemobartonellosis (cats)
 - Babesia
 - Bartonella
 - Ehrlichia
 - Dirofilaria
- Neoplasia
 - Lymphoproliferative
 - Hemangiosarcoma
- Miscellaneous
 - Post-vaccine
 - Elapid snake bites (dogs)
 - Bee stings (dogs)
 - Pancreatitis (cats)

Pathogenesis of IMHA

- Breakdown of immune tolerance
 - Failure to recognize self
 - Altered self
- Type 2 autoimmune response (antibody mediated RBC destruction)
- Heterogeneous immunological response
 - IgG>IgM, both, +/- complement
 - IgG + IgM = lower hematocrits, more agglutination
 - Attack various RBC membrane components (glycophorins)
 - Other RBC proteins (calpain, complement component 3)

Intravascular RBC destruction

- Less common than extravascular
- Complement fixation
 - IgM better at complement fixation
 - High levels of antibody attachment
- RBC membrane damage direct osmotic hemolysis (ghost cells)

Extravascular RBC destruction

- Antibody attachment to RBC membrane=opsinized
- Macrophages bind Fc portion of antibody
- Accelerated removal by tissue macrophages and MPS
- Spleen > liver
- Incompletely phagocytized RBCs=spherocytes
- Antibody may be warm or cold reacting
- High antibody binding leads to agglutination
 - Vascular occlusion, hypoxia, inflammation

Human categories of IMHA based on thermal reactivity

- Warm antibody type, agglutination (acute severe anemia)
- Warm antibody type, intravascular hemolysis (acute severe anemia)
- Warm antibody type, incomplete antibody (subacute to chronic, mild to severe)
- Cold antibody type, agglutination (agglutination at extremities)
- Cold antibody type, non-agglutinating hemolysis (transient hemoglobinemia/uria)

Intramedullary RBC destruction

- Uncommon form of IMHA (33%)
- Antibodies are directed against RBC precursors in bone marrow
- May involve any bone marrow stage
- Difficult to diagnose, atypical
 - Not clinically typical (picked up on wellness testing, or late in disease)
 - Poorly to nonregenerative prior to therapy (red cell aplasia)
 - Possible to have a “functional iron deficiency” (increase C-reactive protein)
 - Subacute to chronic course
 - No pigmentemia/uria

Intramedullary RBC destruction

- Presumptive diagnosis
 - Response to therapy (include erythropoietin?)
 - Bone marrow examination
 - Anti RBC antibodies
 - Maturation arrest
 - Erythrophagocytosis

IMHA clinical findings

- Signs vary from peracute to chronic
- Hypoxia signs
 - Lethargy, weakness, pale mucus membranes, murmur
- Sympathetic stimulation signs
 - Tachypnea, tachycardia, bounding pulse
- Immune/inflammatory reaction signs
 - Pyrexia, anorexia, lymphadenopathy
- Hyperbilirubinemia/uria, hemoglogulinemia/uria
- hepatosplenomegaly

Laboratory diagnostics-CBC RBCs

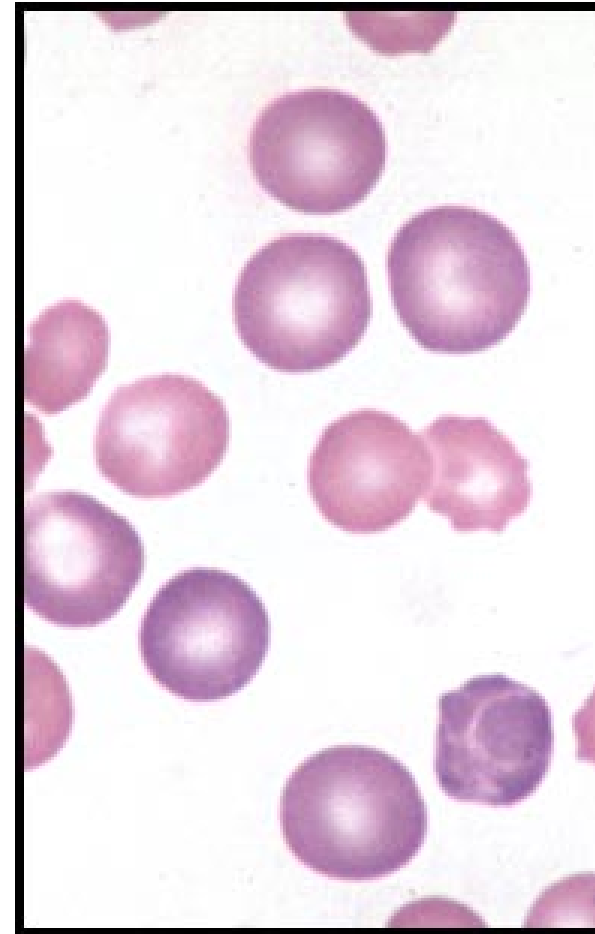
- Moderate to severe anemia (mild anemias go undetected)
- Generally regenerative (reticulocytes, +/- nRBCs) with sufficient duration (3-5 days)
- Erythrocyte indices (macrocytic (MCV), hypochromic (MCHC), rarely spuriously increased MCHC)
- RBC morphology (specific clues)
 - Spherocytosis, difficult call in cats
 - Autoagglutination vs roulette
 - Ghost cells

Autoagglutination

- Positive slide agglutination
 - Seen in 35-78%
 - May persist with washing in 9.5-20%
- Persistent slide agglutination obviates a Coombs Test

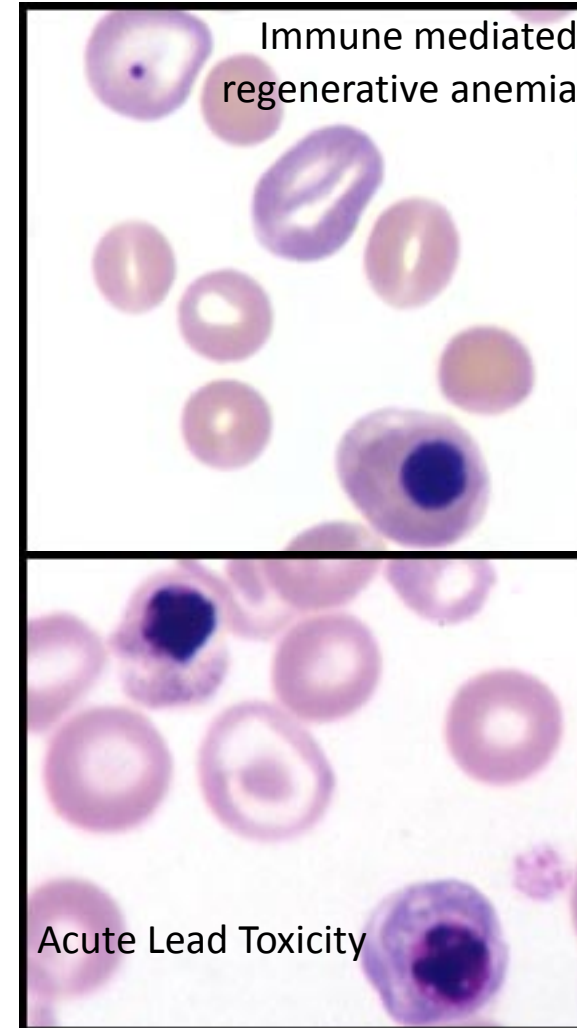
Polychromasia

- Variation in color among cells
- Bluish color in erythrocytes (RNA)
- Cells generally larger than mature RBCs
- Represent reticulocytes
- Few are normal in dogs and cats
- Good morphologic evidence of a bone marrow response to a peripheral demand



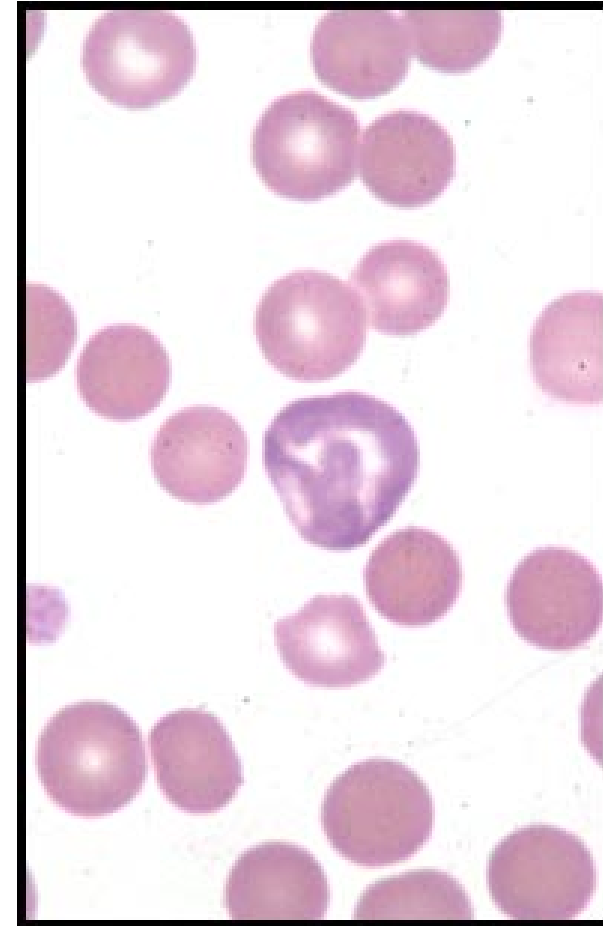
Metarubricytosis

- Metarubricytes are late stage nucleated red blood cells
- Typically restricted from movement into the blood
- Potentially present during:
 - Marked erythroid hyperplasia in “regenerative” marrow response
 - Marrow stromal damage without marked erythroid hyperplasia
 - Acute lead toxicity
 - Marrow infiltrative disease
 - Septicemia



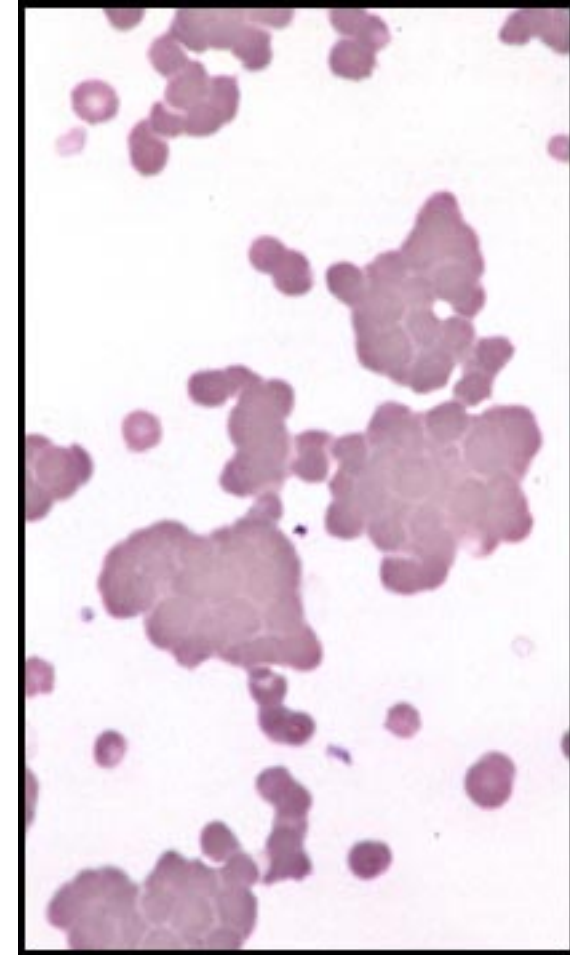
Spherocytosis

- Appear smaller than normal mature erythrocyte
- More dense staining than normal mature erythrocyte
- No central zone of pallor
- As name suggests, they have lost their normal biconcave disc shape and are “spherical”
- Supportive of extravascular immune mediated destruction
 - Partial phagocytosis

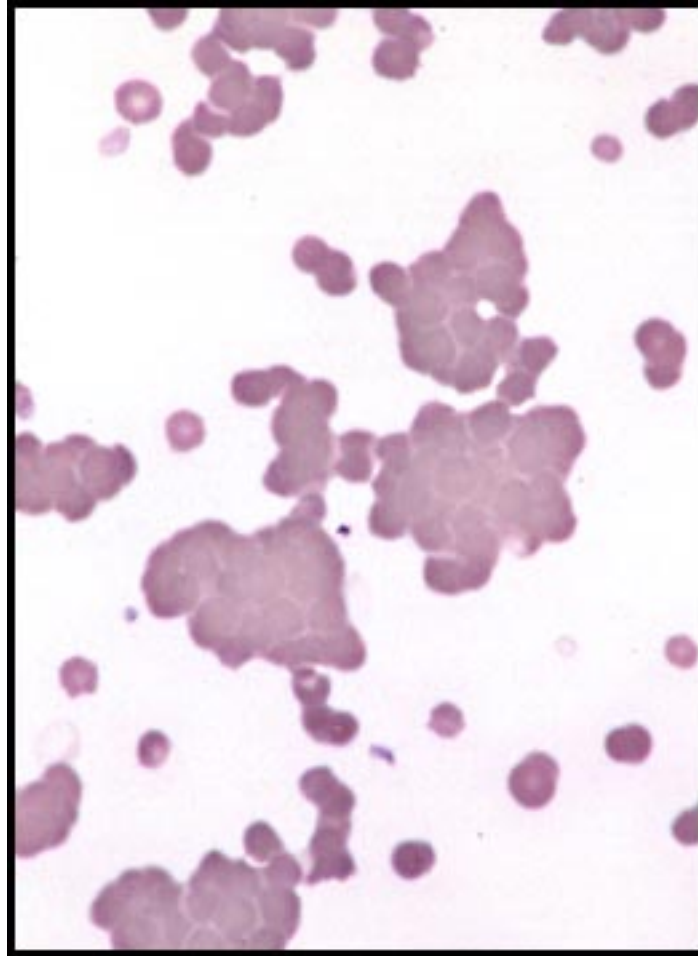


Agglutination

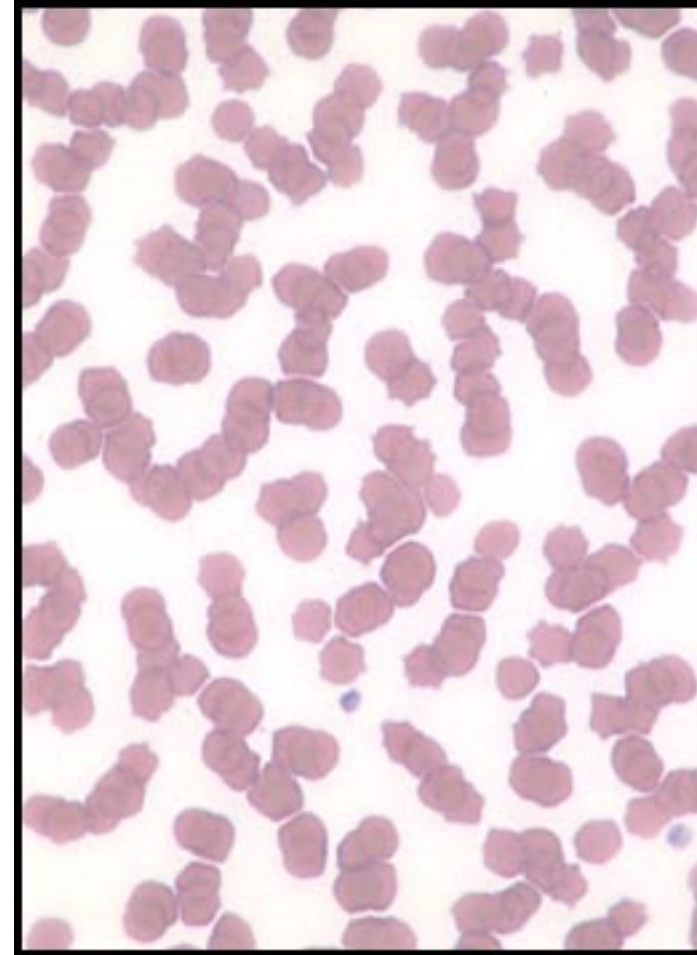
- Three dimensional clumps of erythrocytes
 - Unorganized in contrast to “stack of coins” as with Rouleaux
- Relatively strong binding between cells because of cross-linking of antibodies on surface
- Supportive of an immune-mediated mechanism



Agglutination

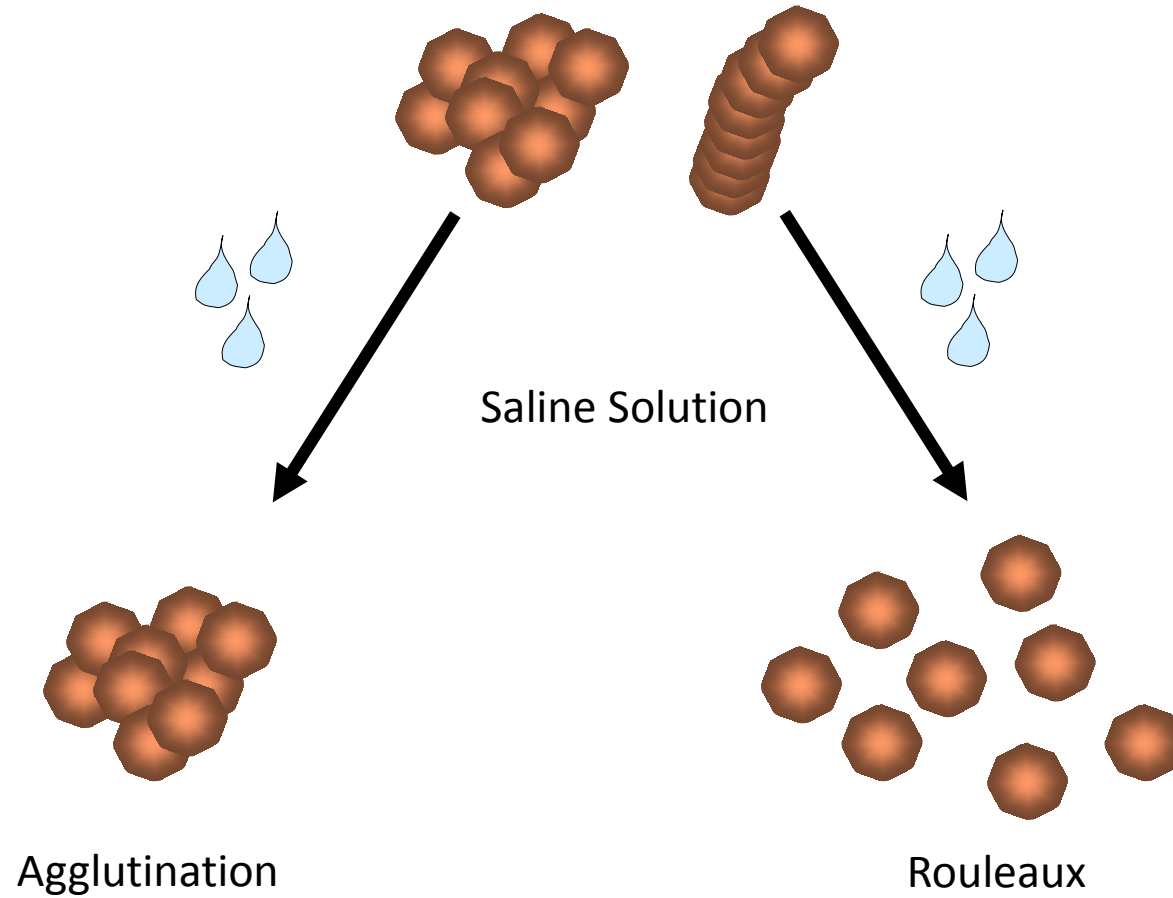


Agglutination



Rouleaux

Saline Agglutination Test



Laboratory diagnostics-CBC WBC, and platelets

- Leukocytosis/leukemoid response
 - +/- bands
 - +/- toxicity
- Concurrent thrombocytopenia (25%)
 - Thromboembolism/DIC
 - Evan's syndrome (10%)

Laboratory diagnostics-remaining MDB

- Serum chemistry
 - Increased liver enzymes (hypoxia)
 - Increased bilirubin
 - Possible azotemia (hemoglobin nephropathy)
 - Metabolic acidosis
- Urinalysis
 - Pigmenturia
 - Possible hyperbilirubinemia
 - Possible hematuria

Immunological testing

- Coombs test 37 degrees
 - Polyvalent (detects IgG, IgM, complement)
 - Multiple dilutions (1:2-1:2048)
 - False positive and negative results
- Antinuclear antibody & rheumatoid factor

Laboratory diagnostics-Infectious disease testing

- FeLV
- 4Dx (HW, Ehrlichia spp., Anaplasma, Lyme)
- PCR panels (Hemotrophic Mycoplasma, Babesia spp., Bartonella [BAPGM])
- Serology (acute and convalescent)

Ancillary diagnostics

- Abdominal imaging
 - Rule out neoplasia
 - Rule out gastric/intestinal metallic foreign body
- Bone marrow examination (needle aspirate and core biopsy)
 - Pure red cell aplasia
 - Erythroid maturation arrest
 - Erythrophagocytosis
 - Anti-RBC antibody staining

Treatment

- Life support
 - Fluids
 - Transfusion (cross matched)
- Immunosuppression (goal may not be completely normal Erythron)
 - Glucocorticoids
 - Only proven therapy (decrease phagocytosis, decrease T-lymphocyte function)
 - Dual therapy OK, triple therapy not recommended
 - Adjunctive immunosuppressant drugs (often started concurrent with steroids)
 - Severity, breed, \$\$\$

Immunosuppression

- Glucocorticoids
 - Prednisone
 - Routine oral: 1-2mg/kg daily
 - Prednisolone
 - Preferred glucocorticoid in cats
 - Routine oral: 1-2mg/kg daily
 - High dose pulse IV: PSS 5-10mg/kg daily, 2-3 days
 - Dexamethasone 1/5-1/3 calculated prednisone dose

Historic adjunctive immunosuppressant drugs

- Cyclophosphamide
 - Once the “big gun”, fallen from favor
 - Dosing difficulties, can't split tablets
 - No better than Prednisone alone
 - Toxicity
 - Hemorrhagic cystitis, may persist
 - Neutropenia, possible reversible, must monitor CBC
- Chlorambucil (0.1-0.2mg/kg SID-EOD)
 - Similar to Cyclophosphamide with fewer side effects (monitor CBC)
 - With glucocorticoid start EOD
 - Recent cost increase

Adjunctive immunosuppressant drugs

- Azathioprine (2mg/kg SID)
 - Along with Prednisone additive affect (Reimer, 1999)
 - Not for cats
 - 7-14 day delay of action
 - Side affects; liver, GI, Avoid with history of pancreatitis
 - Monitor CBC, chemistry panel

Adjunctive immunosuppressant drugs

- Cyclosporine (5-10mg/kg BID)
 - Inhibits T-cell function->blunting immune response
 - Peak absorption 2 hours post dosing
 - Ketoconazole may reduce dose by 75%
 - Marked individual efficacy
 - Blood values don't correlate with clinical outcome
 - Cyclosporine biotherapy assay available at Mississippi State College of Veterinary Medicine Pharmacodynamic Laboratory (5 levels of immune suppression)
 - Caution with Toxoplasma+ cats
 - Start with Atopica or Sandimmune, transition to generic (ultramicrosized)
 - Side effects; infection, gingival hyperplasia, neoplasia, DM, GI upset, papillomas

Adjunctive immunosuppressant drugs

- Mycophenolate mofetil, CellCept (5-12mg/kg PO, IV BID)
 - Purine inhibitor, inhibits B and T-cells
 - Similar to Azathioprine but 10x more potent and more rapid onset
 - Fewer side effects; GI (1-2 weeks), has been used safely in cats
- Leflunomide (2-6mg/kg PO daily)
 - Pyrimidine inhibitor, suppressed B and T-cells
 - ACVIM abstracts report success in various immune-mediated diseases
 - Side effects; liver, GI, bone marrow suppression may persist
 - Monitor CBC, chemistry

Adjunctive immunosuppressant drugs

- IV immunoglobulin 0.5-1 gm/kg IV over 4-6 hours
- Danazol (5-15mg/kg SID or divided BID-TID)
 - Reduces glucocorticoid dose
 - Inhibits macrophage Fc binding

Adjunctive therapies

- Splenectomy
- Voodoo
 - Folate
 - Melatonin
- Gastric protectants (if at risk factor for GI mucosal erosion/ulceration (sepsis, shock, etc.))
- Anti-nausea medication
- Antibiotics?

Pulmonary (splenic) vein thromboembolism (VTE)

- Common in acute disease in animals receiving glucocorticoids (HAC)
- IMHA results in a hypercoagulable state
 - Increased procoagulant
 - Decreased anticoagulant
 - Impaired fibrinolytic activity
- Intrinsic factors (breed, HAC)
- Extrinsic (infectious, vascular injury or stasis)
- Acute often severe dyspnea inappropriate for degree of anemia

Laboratory markers of thrombosis

- CBC (decreased platelets)
- Coagulation panel (shortened or prolonged PT/PTT, FDPs, D-dimers, decreased fibrinogen)
- Antithrombin III
- Other markers
 - Increased C-reactive protein
 - Increased Phosphatidylserine
 - Increased tissue factor (TF)
- Newer biomarkers (products of thrombosis)
 - Microvesicles
 - MCP-1 (monocyte activating cytokine)
 - Cell free DNA
 - Neutrophil extracellular traps (NETS)

Prevention of thrombosis

- Antiplatelet drugs
 - Aspirin 0.5-5 (2) mg/kg PO daily (most common antiplatelet drug in dogs)
 - Works via G-protein receptor coupler (70% of dogs are deficient)
 - 7-10 day inhibition
 - Clopidogrel Plavix
 - Dogs 2-4 mg/kg PO SID loading, 1mg/kg PO SID maintenance
 - Cats 10 mg/cat PO SID
 - 8 day inhibition
 - Melana

Prevention of thrombosis

- Anticoagulation
 - Direct oral anticoagulants (DOAC)
 - The advantages of the DOACs over the vitamin K antagonists are
 - a. Their wide therapeutic window, allowing fixed dosing without the need for anticoagulant level monitoring.
 - b. The lack of influence of oral vitamin K intake on the DOACs' anticoagulant effect.
 - c. A short time to full anticoagulant effect after oral intake of 1.5–3 hours compared to warfarin's 5 or more days, allowing DOACs to be started without the need for a parenteral anticoagulant.
 - d. A short half-life (in the range of 7–14 hours), making preoperative interruption of anticoagulation easier than with warfarin.
 - e. Less drug interactions.

Prevention of Thrombosis

- Direct oral anticoagulants (DOAC)
 - factor Xa inhibitors
 - Rivaroxaban, Xarelto, Bayer (0.5-2mg/kg SID-BID)
 - Rivaroxaban can be used in cats (1.25mg/cat SID)
 - Oral direct
 - Monitoring with Rivaroxaban not essential
 - Monitor with factor Xa inhibition, or PTT
 - Others: Edoxaban, Betrixaban, Apixaban
 - Dabigatran
 - anti-IIa (thrombin)

Treatment for Thrombosis

- Heparin
 - Binds to ATIII->thrombin inhibition
 - Unfractionated Heparin
 - Dogs 200-250 IU/kg SQ TID-QID
 - Cats 100-400 IU/kg SQ TID-QID
 - Ideally monitor with anti-Xa activity
 - Typically monitor with PTT 1.5-2.5x INR
 - Low molecular weight heparin (fractionated)
 - less concern with hemorrhage
 - Endoparin 0.8 mg/kg QID (dogs)
 - Delteparin 150 IU/kg BID (dogs)
- TPA
 - Catheter delivery
 - Systemic administration

General considerations

- Be aggressive initially (prednisone+)
- Do not taper too early (wait for PCV to normalize)
- Taper slowly (2 week minimum)
- Assess affect of last taper before next
- If relapse, restart at beginning
- Some dogs are never drug free

Prognosis

- No conclusive prognostic factors

IMHA case (from Hell)

- Osa was initially evaluated for a seizure like event end of June, 2015. Blood work revealed low red blood cell volume (13%). Osa received her first blood transfusion on 6/27/15. She was started on immunosuppressive doses of steroids and did improve at PDVM.
Diagnostic tests included repeat CBC (non regenerative, no spherocytes, no agglutination), 4Dx (negative), ultrasound possible hepatic nodule VM not cavitated
Thoracic radiographs NSF
- She was initially evaluated here 7.9.15- she was already receiving azathioprine, prednisone, pepcid, melatonin, baby aspirin.
- B12 was added 7.9.15
Folic acid added 7.23.15
- End of July 2015 presented to pdvm after falling out of the car- noted to have left hock swelling, rads- heavy osteophytosis, ST swelling no fx noted. joint effusion present.
cruciate some laxity
She was treated with rest and tramadol

IMHA case

- 8.12.15 evaluated here again for weakness. PCV 17%. She received a 2nd transfusion. Following which her PCV 30. Bone marrow evaluation performed at that time and cyclosporine added to her therapies. Bone marrow consistent with red cell aplasia.
- 8.25.15 presented to ER with acute onset of lethargy, fever and vomiting. A series of diagnostic tests performed her hospitalization stay including VRUS ultrasound, thoracic radiographs, urine culture, blood cultures, and repeat labs. US reported:
 - compatible with emphysematous cystitis.
 - Gas bubbles in vein adjacent to bladder.
 - Urine culture- e.coli and enterococcus
 - Blood cultures- staph PSI
 - Treated in hospital with IV antibiotics and resolved.
 - She received 2 IVIG transfusions 10/11/15 for a total 40 grams
 - Her CBC post IVIG therapy was markedly improved.

AIHA case

- At her visit in February 2016 we had decreased melatonin and folate slightly and PCV decreased to 25 so we adjusted meds back to previous. Follow up labs revealed continued decreased HCT we increased pred to 20/day, immuran to EOD. subsequent increase to 300 mg/day Neoral.
- Osa presented 4/28/16 painful left fore leg- joint effusion present. Started on tramadol- presented 4/29/6 for joint tap. She was started on moxifloxacin based on culture results and did well.
- She rechecked July 12, 2016 for lameness and increased swelling her left hock- MR staph- restarted moxifloxacin.
- Progressive skin disease- started ~July 2016. Calcinosis cutis, started on DMSO, shampoos.

AIHA case

- Recent CBCs:

- 4/18/17 CBC: WBC 17.1 Hct 33.4 retic 1.2/53 PMN 14313 plts 527
- 5/24/17 CBC: WBC 17.4 PMN 14616 Hct 32.9 mcv 78 plts 419 retic 1.3/55
- 6/20/17 CBC: WBC 12.7 Hct 31.2 mcv 79 plts 432 PMN 9614 retic 1.3/51
- 10/20/17 CBC: WBC 10/4 PMN 8538 Hct 20.7 mcv 78 retic 1.1/29 plts 371
- At her last recheck in October 2017 her Hct was significantly decreased and we adjusted her medications. Spherocytes present.
- 11/2/17: CBC: WBC 14.6 Hct 18.6 mcv 78 retic 1.5/36 PMN 12658 slight spherocytes plts 275

AIHA case

- November 10, 2017. Osa is doing well clinically, no concerns other than her calcinosis cutis on her head and neck are increasing again with the increase in prednisone.
- She is a 35 kg dog, current Medications:
 - Prednisone 10mg: 1 tab PO BID
 - Azathioprine 50mg: 1 tab PO EOD
 - Cyclosporine (Neoral) 100mg: 2 cap PO AM and 1 cap PO PM
 - Melatonin 6mg: PO BID
 - Folic Acid 800mcg: 1 tab PO TID
 - Vitamin A 1000 IU PO BID
 - Vitamin B12 injection: 0.8ml SQ every other week
 - DMSO facial TX
 - Chlorhexidine shampoo/mousse PRN
- Current CBC - WBC 13.3 Hct 16.8 MCV 80 retic 2.2/46 PMN 11372 plts 243, no spherocytes noted.
- Suggestions from ACVIM list serve: splenectomy, HBOT
- My suggestions: if on Phenobarbital then change, change to Sandimmune, Vector borne PCR and serology, add Darbopoietin

Questions

