Providing the best quality care and service for the patient, the client, and the referring veterinarian.

Immune-Mediated Diseases: Overview and Alternate Therapies

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Major Immune Mediated Diseases

- Immune Mediated Hemolytic Anemia
- Immune Mediated Thrombocytopenia
- Immune Mediated Polyarthritis
- Glomerulonephritis

Other Immune Mediated Diseases

- Immune Mediated Neutropenia
- Granulomatous Meningoencephalitis
- Keratoconjunctivitis Sica
- Pemphigus Foliiaceous
- Immune Mediated Myositis

Overview of Immune Mediated Diseases

- Regardless of the type of immune mediated disease, goal of diagnostics is to determine if disease is primary or secondary.
- Primary disease is IDIOPATHIC
Overview of Immune Disease

- Secondary Immune mediated disease
  - Neoplasia
  - Drugs: Cephalosporins, Penicillins, Sulfonamides
  - Infectious
    - Rickettsial
    - Parasitic: Babesia, Leishmania, Dirofilariasis
    - Bacterial: Leptospirosis, Mycoplasma felis
    - Viral: FeLV, FIP, FIV
    - Systemic infections: abscesses, pyometra, pyelonephritis, etc..
- Vaccines
  - Vaccine-associated immune-mediated hemolytic anemia in the dog. Duval D, et. al. JVIM, 1996

Immune-mediated Hemolytic Anemia

- Confirming the diagnosis: Spherocytosis
  - Spherocytes are formed as a result of antigen-antibody reaction on surface of RBC. This reaction is recognized by (predominantly) splenic macrophages, which partially phagocytose the RBCs, leaving spherocytes behind.
  - Spherocytosis occurs most commonly with IMHA however genetic mutations, hypophosphatemia, toxins (zinc), and DIC can also cause spherocytosis

Spherocytosis

Immune-mediated Hemolytic Anemia

- Confirming the diagnosis
  - Auto-agglutination
**Immune-mediated Hemolytic Anemia**

- Confirming the diagnosis
  - Coombs
    - Used to detect antibodies or complement on a RBC
    - Species-specific antiglobulins against IgG, IgM, and C3b are used
    - This test does not discriminate between primary and secondary IMHA

**Immune-mediated Thrombocytopenia**

- Confirming the Diagnosis
  - Blood smear showing thrombocytopenia
  - Platelet count less than 50,000

**Immune-mediated Thrombocytopenia**

- Manual Count: 1 platelet per hpf ~ 15,000

**Immune-mediated Polyarthritis**

- Confirming the diagnosis
  - Non-degenerative neutrophils on joint cytology
Immune-mediated Polyarthritis

- Cytology confirmed in multiple joints
  - May also see decreased viscosity

Glomerular Nephropathy

- Diagnostics
  - Proteinuria
  - Elevated UP:C (>1.5)
  - Hypoalbuminemia

Glomerular Nephropathy

- Nephrotic Syndrome
  - Proteinuria
  - Hypoalbuminemia
  - Pitting Edema
  - Hypercholesterolemia

Glomerular Nephropathy

- Confirming the diagnosis
  - Renal Biopsy
**What is Systemic Lupus Erythematosus?**

- Multi-systemic immune-mediated disease
- Diagnosis requires documentation of multi-systemic involvement

**Major Signs**
- Skin lesions
- Glomerulonephritis
- Polyarthritis
- Hemolytic anemia
- Polymyositis
- Leukopenia
- Thrombocytopenia

**Minor Signs**
- Fever of unknown origin
- Central nervous system signs, seizures
- Oral ulcerations
- Lymphadenopathy
- Pericarditis
- Pleuritis

**Serologic evaluation**
- Antinuclear antibody (ANA)
- Lupus erythematosus cell preparation
What is Systemic Lupus Erythematosus?

- **Probable SLE**
  - One major sign with a positive serologic test
  - One major sign, two minor signs and a positive serologic test

- **Definite SLE**
  - Two major signs with a positive serologic test
  - One major sign, two minor signs and a positive serologic test

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What is Systemic Lupus Erythematosus?

- In general SLE is a very rare phenomenon

- Evans is the most common “SLE” disease
  - But even then, may not be a true secondary ITP
  - Thrombocytopenia may be secondary to consumption, not true destruction...
  - Usually consumption is associated with mild to moderate thrombocytopenia, rarely <50,000

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“Sabrina” Bibbero

- 10 yo FS Cocker Spaniel
- Acute Epistaxis

- rDVM labwork showed:
  - CBC: Neutrophils 5994, HCT 46.7%, PLT <50,000
  - Chemistry: All values within normal limits
  - PT/PTT: 6.9/14.3

- Transferred to ASEC for further care and diagnostics

- Past History: Mild heart disease; tx with enalapril and lasix
On presentation to ASEC, 11/16

- T: 102.2, P: 130, R: pant, CRT: <2
- Ecchymoses present on her ventral abdomen
- Grade III/VI systolic murmur

Problem List:

- Thrombocytopenia
- Grade III/VI systolic murmur
  - Previously diagnosed chronic mitral valve disease

Thrombocytopenia

- Increased Loss
  - Hemorrhage
- Consumption
  - Vasculitis, Burns, DIC
- Sequestration
  - Splenic disease
- Increased Destruction
  - ITP, R/O Primary vs. Secondary
- Decreased Production
  - Myelophthisis, myelodysplasia, toxicity

Plan and Recommendations:

- 4DX, RMSF RealPCR
  - Negative for all
- Thoracic Radiographs
  - Cardiomegaly, normal pulmonary vasculature. No evidence of pulmonary nodules or masses
- Abdominal Ultrasound
  - Mild splenomegaly, moderate hepatomegaly with no evidence of neoplastic infiltration
- Other dx to consider:
  - Urinalysis and urine culture – can’t get cysto!!
Plan and Recommendations:

- Owner opted to take Sabrina home

- Medications:
  - Prednisone 1 mg/kg PO BID
  - Cyclosporine 5 mg/kg PO BID
  - Doxycycline 5 mg/kg PO BID (prior to 4Dx results)
  - Pepcid 0.5 mg/kg PO BID

Re-presented to ER, night of 11/16

- Hematemesis

- PE: BAR, melena. PCV, TS: 26%, 5.8

- Hospitalized for overnight monitoring and possible transfusion

- PCV dropped from 26% to 17% overnight

11/17/11

- Transferred to Internal Medicine

- 0-1 platelets per high powered field noted

- Continued on previous medications in injectable forms

- Given 12 grams immunoglobulin (IVIG)

1/18/11

- PCV stable at 17%

- No additional melena on rectal examination

- Increased platelets on smear (3-6 platelets per hpf), good appetite, no additional vomiting

- Transitioned back onto oral medications
11/19

- PCV 20% this morning
- Patient was discharged
  - Prednisone 1 mg/kg PO BID
  - Cyclosporine 5 mg/kg PO BID
  - Pepcid 0.5 mg/kg PO BID

Human Intravenous Immunoglobulin

- IVIG antibodies act through a variety of mechanisms:
  - Neutralize autoantibodies
  - Down-regulation of antibody production
  - Suppression of cytopathic cytokines (interleukin 1a, 6, TNF-alpha, et)
- Can cause significant improvement in thrombocytopenia in 24-48 hours, faster than any other therapy available.

Human Intravenous Immunoglobulin

- Which patients are good candidates?
  - For owners without cost concerns
  - Always recommended for Cocker Spaniels
- What at the contraindications/side effects
  - Cost (Can be upwards of $1000 for drug alone)
  - Possible transfusion reaction

Human Intravenous Immunoglobulin

- Dosing
  - 0.3-1.5 g/kg IV
  - Given as a slow infusion over 6 hours
  - Start infusion at 0.5 ml/kg/hr, if, after 15 minutes there is no evidence of reaction, okay to increase rate to 4 mls/kg/hr for duration of transfusion.
- Trade Names:
  - Carimune
  - Gammagard
Comments:

- Human IVIG was well tolerated and appeared to be associated with rapid platelet count recovery and amelioration of clinical signs in most dogs with IMT.
- Compared with corticosteroids alone, adjunctive emergency therapy of a single hIVIG infusion was safe and associated with a significant reduction in platelet count recovery time in dogs with IMT.

- A Prospective, Randomized, Double-Blinded, Placebo-Controlled Study of Human Intravenous Immunoglobulin for the Acute Management of Presumptive Primary Immune-Mediated Thrombocytopenia in dogs. Bianco D, et al. JVIM, 2009

Comments:

- Dogs with primary IMHA have hypercoagulability as demonstrated by thromboelastography at the time of initial diagnosis and prior to treatment. Such hypercoagulability may be a precursor to clinically evident of thrombosis as a complication of the disease process.


Comments:

- Administration of hIVIgG promotes hypercoagulability and an inflammatory state. This should be considered when using hIVIgG in dogs with IMHA or other prothrombic conditions.
- Makes this medication relatively contraindicated in treatment of IMHA, as can predispose to emboli formation.


Comments:

- Vincristine – single dose at 0.02 mg/kg IV ONCE
  - No significant difference in recovery time in dogs treated with hIVIG and vincristine
  - Should be the first-line adjunctive treatment for acute management of canine ITP, because of lower cost and ease of administration compared to IVIG

Follow up

- Once discharged, Sabrina did very well.
- We saw her once every two weeks for recheck CBCs (which showed resolution of thrombocytopenia and anemia)
- Her Prednisone and Cyclosporine were slowly weaned over the course of 2 months, without recurrence of her ITP
- CBC: Neutrophils 6,786, HCT 46.9%, PLT 468,000

“Shawn” Solomon

- 12 yo MN Maltese
- Generalized Pain

Shawn Solomon

- Referred to ASEC in April for lethargy, anorexia, generalized pain
- rDVM labwork:
  - CBC: Neutrophilia 17,182, HCT 38.5%, PLT 592.00
  - Chemistry: All values within normal limits
  - T4: 1.3
- Historically has had intermittent (suspect back) pain, which responded well to short courses of prednisone

At ASEC

- T 100, P 160, R pant
- Some discomfort on flexion and extension of carpi bilaterally
- Mild spinal pain elicited
- No palpable joint effusion
- Mild discomfort on hip extension bilaterally
Problem List

- Pain on flexion and extension of carpi
- Mild spinal pain
- Mild discomfort on extension of hips bilaterally
- Mild neutrophilia

Radiographs

- Radiographs of the spine, pelvis, carpi, tarsi, hips and elbows were obtained
- All films within normal limits

Next day

- Sedated for Joint Taps (both carpi and tarsi tapped)
- Grossly the fluid was not viscous
- 4Dx,RMSF PCR: Negative
- Abdominal ultrasound within normal limits

Diagnosis

- Joint cytology consistent with IMPA
  - Marked suppurative inflammation in multiple joints
Breaking the Trends

- No joint effusion
- No pyrexia
- Generalized pain

Plan and Recommendations

- Prednisone at 1 mg/kg PO BID, and Pepcid 0.5 mg/kg PO BID
- Doxycycline was started initially at 5 mg/kg PO BID, but discontinued when 4Dx was negative x 4.

4/25/10 – 7/25/10

- At first recheck, doing well, Prednisone was decreased to 5 mg in am, 2.5 mg in pm.
- 5/8/11, repeat joint tap cytology was within normal limits.
- However, owner reported significant prednisone related side effects: pu/pd, muscle wasting, lethargy, etc…

4/25/10 – 7/25/10

- By 6/18/10, Prednisone had been decreased to 2.5 mg PO SID, and Shawn had no evidence of lameness of pain.
- Despite tapering of Prednisone, Shawn continued to experience prednisone related side effects (pu/pd, muscle wasting…)
- By 7/25/11, Shawn was receiving Prednisone at 1.25 mg PO every other day and had no lameness or pain. His prednisone was discontinued completely.
8/11/10

- Represented for lameness and discomfort.
- Physical examination revealed recurrence of discomfort in tarsi and carpi, spine and hips.
- Repeat joint taps showed inflammatory joint fluid consistent with a relapse of his IMPA
- Due to our concern over previously noted side effects and long term prednisone therapy, we considered alternate therapy

New therapy

- He was started on Cyclosporine 5 mg/kg PO BID and Carprofen 2 mg/kg PO BID

Alternate therapy for IMPA

- Rimadyl and Cyclosporine: anti-inflammatory and immunosuppressive therapy.
  - Biggest pro is that therapy can be administered long term
  - Cons of Rimadyl: Liver and kidney dysfunction
  - Cons of Cyclosporine: Cost, GI upset and possible gingival hyperplasia
- Azathioprine
- Other therapy to consider: GOLD SALTS

Gold Salts

- Aurothioglucose, sodium aurothiomalate
  - Auranofin (Ridaura), oral preparation
- Mechanism of Action: Not completely understood, but medication is thought to have:
  - Anti-inflammatory activity
  - Anti-rheumatic activity
  - Immuno-modulating activity
  - Anti-microbial activity
Gold Salts

- Dose: 1-5 mg/kg IM as a test dose, then 1 mg/kg IM once weekly.
  - May take 6-8 weeks to take effect
- Side effects: skin lesions, thrombocytopenia and toxic epidermal necrolysis.


Leflunomide

- Oral administration of leflunomide is a safe and effective alternative to oral administration of corticosteroids for treatment of IMPA in dogs
- Starting dose of leflunomide 3 to 4 mg/kg PO SID


"Ancel" Schwarting

- 8yo MN Dachshund
- Severe anemia

Ancel Schwarting

- 2 weeks progressive lethargy noted at home
- Presented to ASEC for anorexia and pale mucous membranes
- Previous history of numerous vaccine reactions; therefore no recent vaccinations were administered, instead titers were performed to confirm immunity
- History of intermittent back pain
At ASEC on 4/10/11

- Physical examination:
  - T: 99.8, P: 200, R: 30
  - 5% dehydrated
  - VERY pale mucous membranes
  - Grade III/VI systolic murmur

Labwork at ASEC

- PCV/TS: 6%, 6.0
- CBC: Neutrophils: 6,100, HCT: 5.6%, PLT count 268,000, Retics: 9,000
- Chemistry panel within normal limits
- Saline Agglutination: POSITIVE

Problem:

- Non-regenerative Immune Mediated Hemolytic Anemia
  - Acute hemolysis (first 3-5 days of disease)
  - At the level of the bone marrow

Diagnostic Plan:

- RMSF Tick PCR and 4Dx
  - Negative
- Urinalysis and Culture
  - WNL, No growth
- Thoracic Radiographs, Abdominal Ultrasound
  - Both within normal limits
Therapeutic Plan:
- Administered 14 mls/kg pRBC overnight; transferred to IM for continued care
- PCV following the transfusion: Increased to 30% and remained stable over the next day
- Ancel is BAR the next day, eating and drinking well. Switched onto oral medications

4/11/11
- Discharged on 4/11/11:
  - Prednisone 1 mg/kg PO BID
  - Cyclosporine 5 mg/kg PO BID
  - Aspirin 0.5 mg/kg PO BID
  - Pepcid 2.5 mg PO BID

Recheck:
- 4/15/11
  - Neutrophils 9,800, HCT 28.7, PLT 364, Reticulocytes: 26,280
- 4/18/11
  - CBC: Neutrophils 6,900, HCT 27.8, PLT 555, Reticulocytes: 29,500
- 4/22/11
  - CBC: Neutrophils 15,800, HCT 23.1, PLT 392, Reticulocytes: 6,980

Questions?
- Why not change therapy sooner?
  - Prednisone take 3-5 days to start working
  - Cyclosporine takes 5-7 days to start working
- Azathioprine takes 10-14 days to start working
- Why not start a third immunosuppressive?
  - Risk for opportunistic infection
4/25/11, Bone marrow aspirate:
- Diagnosis: Mild hemodilution with only few myeloid and erythroid precursor cells. Severe non-regenerative anemia.
- Clinical Impression: Severe red cell apalsia consistent with IMHA at the level of the bone marrow
- Prognosis is overall GUARDED.

Follow up:
- Between 4/25/11 and 5/3/11, Ancel’s PCV decreased from 24% to 18%, both times without regenerative response.
- On 5/3/11 he received a pRBC transfusion, which increased his PCV to 28%.
- Recheck on 5/8/11 showed progressive anemia, PCV decreased to 20% without regenerative response.

Concerns:
- Persistently non-regenerative
- Already on immunosuppressive therapy for over 1 month
- Transfusion dependent despite immunosuppressive therapy
- Time to consider alternate therapy…

Other therapy for IMHA:
- Leflunomide:
  - Mechanism of action: Inhibition of T cell proliferation and autoantibody production by B cells.
  - Side effects include thrombocytopenia, neutropenia and GI upset.
  - Arava
  - DOSE: 2-4 mg/kg PO SID
Other therapy for IMHA:

- **Azathioprine:**
  - Helpful to spare prednisone in large breed dogs.
  - Mechanism of action: Inhibition of RNA and DNA synthesis
  - Side effects include hepatotoxicity, pancreatitis, GI upset, BM suppression.
  - DO NOT use in cats: Can cause more severe bone marrow suppression; additionally, they are more likely to develop opportunistic infections
  - *Imuran*
  - DOSE: 2 mg/kg PO SID x 1 week, then decrease to 1 mg/kg PO EOD

Other therapy for IMHA:

- **Mycophenolate:**
  - Mechanism of Action: Can inhibit leukocyte recruitment to inflammatory sites and tissues.
  - Side effects include GI upset
  - Can be costly
  - *Cellcept, MMF*

Other therapy for IMHA:

- **Human Intravenous Immunoglobulin:**
  - Possibly contraindicated
  - May perpetuate coagulopathic state

  Prothrombic and Inflammatory Effects of Intravenous Administration of Human Immunoglobulin G in Dogs. Tsuchiya, Et. Al. JVIM, 2009;23: 1164-1169

Back to Ancel:

- Leflunomide was started on 5/15/11 – 4 mg/kg PO SID
- Recheck PCV on 5/20/11 showed progressive non-regenerative anemia and so Ancel received a third transfusion
- Over the next several weeks he continued to have progressive non-regenerative anemia and received a fourth blood transfusion
Progress:

- 5/27/11
  - Neutrophils 10,005, HCT 20.9%, PLT 740, Retic 67,160
- 6/15/11
  - Neutrophils 8789, HCT 25%, PLT 483, Retic 143,190!
- 6/25/11
  - Neutrophils 5740, HCT 26.2%, PLT 482, Retic 121,110
- 7/6/11
  - Neutrophils 11858, HCT 30%, PLT 145, Retics 124,410

7/27/11 – Present:

- Continued very slow taper of medications. Prednisone was weaned first, followed by a long, slow wean of Ancel’s Leflunomide.
- When it was finally discontinued, the dose had been decreased to 2.5 mg (<1 mg/kg) PO every other day.
- Recheck CBC 3 weeks after finishing Leflunomide (2/18/12):
  - Neutrophils 4,902, HCT 44.3%, PLT 598,000

Key Points:

- Cannot rule out IMHA based on a non-regenerative anemia
- Other therapies to consider for IMHA, especially when disease is at the level of the bone marrow, include Leflunomide
- Therapy can still take time to become effective
- Poor overall prognosis for IMHA at the level of the bone marrow

SILVER CASSETA

- 9 yo FS DSH
- Generalized Pain
Silver Casetta

- Owner reports progressive lameness over the two weeks prior to presentation.
- Treated by RDVM with pain medications (tramadol, buprenorphine, meloxicam, antibiotics, short course of prednisolone)- no improvement
- Patient laterally recumbent at home, however eating well
- rDVM labwork:
  - CBC: Neutrophils 17,700, HCT 35.1, PLT 386
  - Chemistry: TP 9.2, Globulins 6.7, Albumin 2.5
  - Urine Culture: Negative
- History: Asthma previously treated with prednisolone

At ASEC

- T: 103.4, P: 160, R: 45, wt: 6.47kg, BCS 8/9
- Laterally recumbent
- Orthopedic examination: Very painful on manipulation of carpi, moderate spinal and cervical pain
- Neurologic examination: Poor placing reactions in TL, cranial nerves and remainder of exam within normal limits.

Problem List

- Generalized pain
- Pyrexia
- Hyperglobulinemia
- Historic Asthma
- Moderate Neutrophilia

Plan and Recommendations:

- Neuro consult revealed no significant neurologic deficits, however MRI was still recommended and decline by owners
- Arthrocentesis for cytology and culture performed.
- Additionally, FIV/FeLV performed
Plan and Recommendations:
- Joint Cytology: Inflammatory joint fluid with increased cellularity.
  - Cells were all neutrophils, no organisms visualized
- Joint Culture: Negative
- FIV/FeLV: Negative
- Owner declined workup for secondary causes of IMPA, including abdominal ultrasound, thoracic radiographs, urinalysis and urine culture.
- PLAN: Prednisolone 1 mg/kg PO BID for Immune-mediated polyarthritis

Follow up
- Silver did not significantly improve on Prednisolone alone over the first week of therapy.
- He remained laterally recumbent and extremely painful at home (although he continued to have a good appetite).
- Recommended starting Cyclosporine 5 mg/kg PO BID.

Repeat Joint Taps
- Showed persistent mild inflammation within the joint.
- Recommended to continue all medications.
  - Cyclosporine 5 mg/kg PO BID
  - Prednisolone 1 mg/kg PO in am, 0.5 mg/kg PO in pm.

Follow up
- The owner reports significant improvement 2-3 days after starting concurrent therapy with cyclosporine.
- Silver is much better and able to walk around house!
- Recommended decreasing Prednisolone to 1 mg/kg PO in the am and 0.5 mg/kg PO in the evening
Follow up:

- Silver developed signs consistent with URI, which we suspect arose due to immunosuppressive therapy.
- Over the next two months, Silver was on two different antibiotics with little improvement noted to URI signs:
  - Clavamox 14 mg/kg PO BID x 2 weeks
  - Doxycycline 5 mg/kg PO BID x 2 weeks
  - Lysine

Follow up:

- During this time, we also further decreased his prednisolone:
  - Decreased to 0.5 mg/kg PO BID
  - Further decreased to 0.5 mg/kg PO SID
- After one month of tapering, Prednisolone was discontinued, Silver was continued on Cyclosporine 5 mg/kg PO BID

Follow up:

- Over the next 2 months, the cyclosporine was also gradually tapered due to persistent mild URI signs:
  - First decreased to 5 mg/kg PO SID
  - Then decreased to every other day therapy
  - Then twice weekly therapy
  - Then discontinued completely

Follow up:

- Over this period the URI signs completely resolved, and Silver had no relapse of lameness or pain. He continued to ambulate normally.
**Key Points**

- IMPA is not just for dogs!
- Presentation can be highly variable
- Opportunistic infection, like URI, is a possible sequela of immunosuppressive therapy
- Adjunctive therapy with Cyclosporine is often indicated in cats with immune mediated disease.

**Conclusions**

- IVIG should be considered in dogs with ITP, specifically Cocker Spaniel dogs or dogs refractory to therapy.
- Cyclosporine and Rimadyl can be used to treat cases of IMPA where Prednisone cannot be weaned, or in patients having severe side effects to Prednisone.

**Conclusions**

- Leflunomide is effective alternate for IMHA at the level of the marrow
- IMPA is not a disease specific to dogs; cats with refractory immune mediated disease can respond very well to adjunctive therapy with cyclosporine

**Conclusions**

- SLE
  - Over-diagnosed condition
  - Very rare phenomenon
Questions?

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