Update on IMHA in dogs
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Outline
- Introduction and definitions
- Pathogenesis
- Primary vs Secondary
- Diagnosis
- Prognosis
- Treatment

Great (free!) resources
- Journal of Veterinary Internal Medicine
  - All articles are OPEN ACCESS

What is IMHA?

Understanding your RBCs
- Primary driver: erythropoietin (EPO) from peritubular renal interstitial cells
- Need iron for synthesis of Heme
- Copper for release of iron from storage
- Vitamin B6 for first step of heme synthesis

Normal RBC end of life
- Red blood cell (RBC) lifespan: 120 days
- Humans: 0.03-0.5% turnover daily
- Oxidative injury → phagocytosis by macrophages of mononuclear phagocyte system (spleen, liver, etc.)
Causes of hemolytic anemia

- Intrinsic/Inherited RBC defects
- Hereditary osmotic fragility
- Idiopathic Heinz body anemia
- Methemoglobin-reductase deficiency
- Phosphofructokinase deficiency (ESS, ACS)
- Pyruvate kinase deficiency (basenjis)

Causes of hemolytic anemia

- Chemical/toxin injury
- Zinc (pennies post-1983, diaper cream, sunscreen)
- Garlic/onion
- Propylene glycol
- Methylene blue
- Castor bean

Causes of hemolytic anemia

- Other
- Severe hypophosphatemia (DKA treatment, malnutrition)
- Snake envenomation

What is IMHA?

- Type II hypersensitivity reaction

What is IMHA?

- Type II hypersensitivity reaction
- Extravascular hemolysis

1. Predominantly IgG response
2. Minimal complement activation
3. Destruction by MPS in spleen

1. Hyperbilirubinemia
2. Bilirubinuria
3. Spherocytes

1. Predominantly IgG
2. IgM
3. IgG, IgA
What is IMHA?
- Type II hypersensitivity reaction
- Intravascular hemolysis

1. Hemoglobinemia
2. Hemoglobinuria

1. Severe, primarily IgM response
2. Extensive complement activation

Membrane attack complex leads to destruction in circulation

Does it really matter?
- Maybe

What causes IMHA?
- The vast majority are primary or autoimmune idiopathic IMHA
- No identifiable underlying cause

What causes secondary IMHA?
- Response to non-self antigens adhered to or altering RBC membranes
- Many possible and reported causes

What causes IMHA?
- Infectious
  - Ehrlichiosis
  - Babesiosis
  - A. phagocytophilum
  - Mycoplasma spp.
  - Leptospirosis
  - Dirofilariais
  - Histoplasmosis

What causes secondary IMHA?
- Infectious
- Neoplastic
  - Lymphoma
  - Hemangiosarcoma
  - Histocytic disease
  - Diffuse sarcoma
  - Multiple myeloma
  - Other solid tumors
What causes secondary IMHA?
- Infectious
- Neoplastic
- Drug/toxin
  - Acetaminophen
  - Cephalosporins
  - Heparin
  - Penicillins
  - Procainamide
  - Sulfonamides
  - Topical benzocaine
  - Vitamin K
  - Quinidine

What causes secondary IMHA?
- Infectious
- Neoplastic
- Drug/toxin
- Other
  - Bee-sting envenomation
  - Recent vaccination (14-28d)
  - Systemic lupus erythematosus

Signalment, History & Clinical signs

How do dogs with IMHA present?

Primary IMHA signalment
- Any breed possible
- Cockers, English springer spaniels, poodles, CEs, Irish setters, collies
- Females over-represented
- Median age 6yr
- Seasonality?

History and clinical signs
- History often indicative of hypoxia and anemia
- Weakness
- Collapse
- Lethargy
- Anorexia
- Tachypnea
- Jaundice
Diagnostics
The long road of rule-outs to primary IMHA
Suggested criteria for diagnosis of primary IMHA

- Severe anemia
- Evidence of hemolysis
- Evidence of immune response
- Autoagglutination
- Spherocytosis
- Positive direct Coombs' test
- Lack of identification of an underlying cause
- Response to therapy

Criteria #1: Severe Anemia

- How severe?

Criteria #2: Hemolysis evidence

- Intravascular vs extravascular
- Blood: plasma icteric or hemolyzed

Criteria #1: Severe Anemia

- How severe?

Criteria #2: Hemolysis evidence

- Intravascular vs extravascular
- Blood: plasma icteric or hemolyzed
- Urine: hemoglobinuria versus bilirubinuria
Criteria #3: Immune Response

- Autoagglutination

Criteria #3: Immune Response

- Slide agglutination test
- 1 drop anticoagulated whole blood + 1 drop physiologic saline

Criteria #3: Immune Response

- Autoagglutination
  - Spherocytosis
  - Identified in the majority of dogs with IMHA
  - Suggestive, not diagnostic

Criteria #3: Immune Response

- Autoagglutination
  - Spherocytosis

Evaluate a blood smear!

- Should be done for EVERY dog with anemia
- Can be send-out or in-house
- Ability to assess:
  - Reticulocytosis
  - Polychromasia
  - Anisocytosis
  - Spherocytes
  - RBCs
  - Blood parasites
  - Platelet count
Criteria #3: Immune Response

- Autoagglutination
- Spherocytosis
- Coombs test

Direct Coombs testing

Patient’s washed RBCs + Coombs reagent (antiserum) = Agglutination

Criteria #3: Immune Response

- Autoagglutination
- Spherocytosis
- Coombs test

Direct Coombs
- Detects antibodies on patient’s RBCs
- Positive in 66-75% of dogs with IMHA

Indirect Coombs
- Detects antibodies in patient’s serum

Coombs limitations

False Positive
- Post-transfusion
- Non-specific coating
- Storage artifact

False Negative
- Antibody titer too low
- Prior steroid therapy
- If still having signs, not as big a problem
- Elution of antibody during washing
- Sample aging resulting in antibody loss

What about regeneration?

- Is this anemia regenerative?
  - Dogs >60,000 absolute or greater than 1% corrected
  - Corrected percentage = (Patient’s PCV x Retic %) / 45%
  - Approximately 1/3 are not regenerative at diagnosis

- Why?
  - Too early (recall pathway for RBC production)
  - Non-regenerative
  - Not immune-mediated
  - Precursor directed immune response

Suggested criteria for diagnosis of primary IMHA

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- Lack of identification of an underlying cause
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What causes secondary IMHA?

- Infectious
- Neoplastic
- Drug/toxin
- Other

Limitations of Serology & PCR

- PCR is highly specific (as long as you use a good lab)
- Negative PCR NEVER rules out infection
- Antibiotic therapy can make PCR negative quickly depending upon organism

Serology can be affected by time course of disease
- Acute disease may need convalescent titers
- Time for the body to mount an immune response
- TIME!
- Especially applicable for culture (BAPGM)
- Don’t wait, start treatment

What causes secondary IMHA?

- Infectious
- Neoplastic
- Drug/toxin
- Other

- Chemistry panel
- Urinalysis and culture
- Imaging
- Thoracic radiographs
- Abdominal ultrasound
- Don’t be surprised if the liver and spleen look unusual

Suggested criteria for diagnosis of primary IMHA

- Severe anemia
- Evidence of hemolysis
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- Positive direct Coomb’s test

- Lack of identification of an underlying cause
- Response to therapy
Treatment
Immunosuppressive, supportive care & newly available modalities

Glucocorticoids
- Mainstay of treatment
- Immunosuppressive dose: 2 mg/kg/d (divided)
- Exception: Large and giant breed dogs
- Smaller dogs may need higher doses
- Mechanism of action: many!
  - Stabilize cell membranes
  - Decreased T-cell activation and cytotoxicity
  - Suppress cytokine activity and macrophage function

Glucocorticoid dosing
- Prednisone 1-2 mg/kg BID
- Dexamethasone
  - Potency of 7-10x prednisone
  - Divide dose of prednisone by 7-10
  - 0.1-0.2 mg/kg BID

Glucocorticoids
- Drawbacks: Many!
  - PU/PD/PP
  - Iatrogenic HAC
  - Muscle wasting
  - Lethargy
  - Panting
  - Gastric ulceration

Additional immunosuppressives
- Goal: decrease the amount of prednisone needed
- Minimize drug side effects
- No study has shown a benefit over prednisone alone in treating IMHA
  - Azathioprine
  - Mycophenolate
  - Cyclophosphamide

Additional immunosuppressives
- Not all sunshine and roses
  - Adverse effects with other drugs
  - Serious adverse effects with combined immunosuppressive therapies
  - Fungal infection
  - Bacterial infection
  - Marrow suppression
  - Neoplasia

Immunosuppression
Side effects
Other immunosuppressives

- **Azathioprine**
  - Purine analog
  - Metabolized to ribonucleotide monophosphates
  - Incorporation into DNA leads to faulty transcription
  - Dose: 2.2mg/kg SID for 1 week, then q48hr

**Drawbacks:**
- GI upset most commonly
- Uncertain levels in all dogs
- Differences in metabolism by breed
- Hepatotoxicity
- 15% of dogs in median 14 days
  - ALT > 2-fold increase
  - Bone marrow suppression
  - Thrombocytopenia or neutropenia in 8%
  - Median onset 53 days

**Recommendations**
- Check chemistry panels occasionally
- Recheck full CBC
- Avoid in dogs with known liver/marrow disease

**Mycophenolate**
- MOA similar to azathioprine
- Inhibits inosine monophosphate dehydrogenase
- Affects purine synthesis
- Developed as an alternative to azathioprine
- Dose: 10 mg/kg q12h

**Mycophenolate mofetil**
- Retrospective study
  - n=64
  - Mycophenolate n=30, mean dose 20.5 mg/kg q24h
  - One dog experienced diarrhea
- Mycophenolate with prednisone has similar efficacy to other combinations
  - Cyclosporine, azathioprine, IVIg
- Prospective study
  - n=5, dose 10-15 mg/kg q8h
  - All dogs developed diarrhea
  - One euthanized for GI toxicity
  - Two discontinued drug
  - 3/5 long-term survival
  - Cannot recommend without further studies
**Other immunosuppressives**

- Azathioprine
- Mycophenolate
- Cyclosporine

**Calcineurin inhibitor**

- Supresses T cell function
- Monitoring: whole-blood cyclosporine levels between 400 ng/mL and 600 ng/mL
- Mississippi State
- Dose: 5 mg/kg PO q12h

**Cyclosporine**

- Abstract from 1996
- Effective in combination with prednisone
- Adverse effects
  - GI upset (typically self limiting)
  - Gingival hyperplasia
  - Opportunistic infections
  - Lymphoproliferative disorders
  - Hepatotoxicity
  - Nephrotoxicity

**Other immunosuppressives**

- Azathioprine
- Mycophenolate
- Cyclosporine
- Leflunomide

- Selective pyrimidine synthesis inhibitor
- Inhibits dihydroorotate dehydrogenase
- B and T cells lack pyrimidine salvage pathway
- Dose: 2-4 mg/kg q24h

**Other immunosuppressives**

- Azathioprine
- Mycophenolate
- Cyclosporine
- Leflunomide

- No randomized, controlled studies on IMHA in dogs
- Effective in IMPA
- Generic available now so more affordable

- Adverse effects
  - Lethargy, GI upset (can be severe)
  - Bone marrow suppression
  - Case reports of necrosis
Other immunosuppressives
- Azathioprine
- Mycophenolate
- Cyclosporine
- Leflunomide
- Intravenous immunoglobulin

Modulation of expression and function of Fc receptors
- Interference with activation of B and T cells and complement
- Decrease in immunoglobulin production
- Dose: 0.5-1.0 g/kg once as a slow infusion

Intravenous immunoglobulin
- Blinded, randomized, clinical trial
- n=28 (underpowered)
- hIVG with glucocorticoids did not improve response or survival
- Did not shorten length of hospitalization
- Did not decrease the transfusion requirement
- Drawbacks
  - Can increase thrombotic tendencies
  - Anaphylaxis
  - Expensive
  - Recommendations
    - Nonresponders
    - ITP shows promise
    - Evans’ syndrome dogs?

What kills dogs with IMHA?
- Hypercoagulability
  - Thromboembolism presence estimated 30-50%
  - May be much higher based on necropsy studies
  - Mix of venous and arterial thrombosis (PTE > other locations)
  - Dysregulation of platelets and coagulation
  - What drives the thrombosis?
**Virchow’s triad**

- Blood Stasis
- Endothelial Injury
- Hypercoagulability

**What drives thrombosis in IMHA?**

- Platelet and endothelial activation
- Procoagulant microparticles
- Decreased antithrombin activity
- Treatment of the disease
  - Impaired macrophage function decreases scavenging of MPs
  - Corticosteroids
  - IVIg
  - Cyclosporine?

**How do we prevent it?**

**Platelet inhibition**
- Low dose aspirin
- Inhibits thromboxane A2
- Non-responders (up to 30%)
- Dose: 0.5-1 mg/kg q12h
- Clopidogrel
- Inhibits ADP receptor
- Dose: 2 mg/kg q24h

**Coagulation inhibition**
- Unfractionated heparin
- 1.5x baseline aPTT
- Anti-factor Xa measurement
- Dose: 100U/kg/day as a CRI or divided into 30-200 U/kg TID
- LMWH
- Expensive
- Direct Xa inhibitors
- Rivaroxaban 0.5-1 mg/kg q24h

**What’s the cost? (18kg dog)**

<table>
<thead>
<tr>
<th>Drug class</th>
<th>(mg/kg/d)</th>
<th>Monitoring cost</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspirin</td>
<td>1</td>
<td>N/A</td>
<td>$0</td>
</tr>
<tr>
<td>Clopidogrel</td>
<td>2</td>
<td>N/A</td>
<td>$0</td>
</tr>
<tr>
<td>UFH (90 U/kg/d)</td>
<td>900 U/kg/d</td>
<td>N/A</td>
<td>$0</td>
</tr>
<tr>
<td>LMWH</td>
<td>N/A</td>
<td>N/A</td>
<td>$0</td>
</tr>
<tr>
<td>Rivaroxaban (1 mg/kg/d)</td>
<td>375 ($30, 15mg tabs)</td>
<td>N/A</td>
<td>$375/mo</td>
</tr>
</tbody>
</table>

**Thromboprophylaxis in IMHA**

- We know this improves outcome!
- Individually adjusted dose heparin versus continuous dose
- 5/6 dogs in CD group had TEV
- 1–3/6 dogs in IAD group
- Doses of unfractionated heparin from 150-566 U/kg q12h
What about blood products?

- Necessary in 70-90% of patients
- Most studies based on referral centers
- Indications:
  - Treatment of tissue hypoxia
  - Tachypnea, tachycardia, dyspnea, weakness
  - NOT treatment of PCV
- Packed RBCs
  - 10 ml/kg over 4h
  - 1 ml/kg raises PCV 1%
  - Vol = 90 x BW x (PCV/donor PCV)
- When to crossmatch?
  - No transfusion history? Not necessary
  - Transfusion history? Absolutely
  - Give only DEA 1:1 negative products
  - NB: Autoagglutination may make interpretation of Xmatch impossible

Other supportive care

- Intravenous fluid support if needed
- Dehydration
- Hemoglobin-related nephropathy
- Use caution when also giving pRBCs
- Antileukemics
  - Maropitant 2 mg/kg PO q24h
  - Ondansetron 0.5 mg/kg IV or PO q8h
- Gastroprotectants (high dose prednisone)
- Antibiotics
  - Doxycycline until known VBD status

Plasmapheresis

- Exchange of patient’s plasma and the IgG and IgM fractions within it
- IgM 70% intravascular
- IgG 45% intravascular
- Indicated in humans with IMHA, ITP, myasthenia gravis
- One dog refractory to standard treatment
  - Post exchange 37% and 79% reduction in IgG & IgM
  - No further transfusions needed – BUT only had 3d therapy prior to TPE

More on transfusions ...


Prognosis

Not as clear as we’d like
General IMHA prognosis

- Widely variable mortality rate reported
- Approximately 50% die within first 14 days
- Majority of thromboembolic disease
- Up to 90% survival after first 14 days
- Relapse rates from 15-30%

Prognostic indicators

<table>
<thead>
<tr>
<th>Reported indicators</th>
<th>Not associated with worse outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Increased BUN</td>
<td>Thrombocytopenia</td>
</tr>
<tr>
<td>Increased bilirubin</td>
<td>Leukocytosis with bands</td>
</tr>
<tr>
<td>Reticulocyte response</td>
<td>Anemia, petechiation, hypoalbuminemia</td>
</tr>
<tr>
<td>Degree of anemia</td>
<td>Degree of spherocytosis</td>
</tr>
<tr>
<td>Degree of spherocytosis</td>
<td>Reticulocyte response</td>
</tr>
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Prognostic indicators in IMHA

- Reported indicators
- Not associated with worse outcome
  - Increased BUN
  - Increased bilirubin
  - Thrombocytopenia
  - Leukocytosis with bands
  - Anemia, petechiation, hypoalbuminemia

Decreasing medications

- What I do when the PCV is normal:
  - Nothing. For another month.
- Well, not nothing.
  - Stop heparin when no longer autoagglutinating
  - Continue platelet inhibitor until off prednisone
  - Recheck full CBC and Chemistry panel after 30 days normal PCV
  - Taper prednisone by 25%
  - Recheck PCV/TSH in one week, full CBC three weeks later
  - Repeat until off immunosuppressives
Your plan may vary

- Lots of ways to taper immunosuppressive drugs
- Things I look for before tapering
  - Spherocytes
  - Agglutination
  - Reticulocyte count
  - Medication side effects
    - Physical and biochemical
- These may necessitate a shorter or longer duration of therapy

Questions?
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