Hematology Case Challenges

Or, Maybe Not?

Sadie

- 7 year old, female, spayed golden retriever
- Presentation (Emergency)
  - Episodic weakness (twice) over 2 week period
  - Acute collapse day of presentation, but recovered

Physical Exam Findings
- Pale mucus membranes, tachycardia (120 BPM)
- CRT prolonged @ 4 sec.
- Abdominal distension
  - Effusion and/or mass was difficult to determine on abdominal palpation
- PCV 19%
- TPP 5.4
- Plan: CBC

CBC Results

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Value</th>
<th>Reference Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC</td>
<td>19.1</td>
<td>(6.0 – 17.0) x 10³</td>
</tr>
<tr>
<td>Neuts</td>
<td>15.2</td>
<td>(3.0 – 11.5) x 10³</td>
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<tr>
<td>Bands</td>
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<td>Lym.</td>
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<td>Mon.</td>
<td>2.3</td>
<td>(0.15 – 1.35) x 10³</td>
</tr>
<tr>
<td>Eos.</td>
<td>0.0</td>
<td>(0.1 – 1.25) x 10³</td>
</tr>
<tr>
<td>RBC</td>
<td>2.51</td>
<td>(5.4 – 7.8) x 10⁶</td>
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<tr>
<td>HGB</td>
<td>6.8</td>
<td>(13.0 – 19.0) g/dL</td>
</tr>
<tr>
<td>HCT</td>
<td>18.2</td>
<td>(37.0 – 54.0) %</td>
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<td>MCV</td>
<td>76.2</td>
<td>(66 – 75) fL</td>
</tr>
<tr>
<td>MCHC</td>
<td>36.3</td>
<td>(34.0 – 36.0) g/dL</td>
</tr>
<tr>
<td>Plts</td>
<td>25.0</td>
<td>(150 – 430) x 10³</td>
</tr>
</tbody>
</table>

Reticulocyte count (6%) = 150,600 / µl (>80,000 = regenerative)

Blood film evaluation
Findings from Blood Film Evaluation

- Regenerative anemia (polychromasia)
- Poikilocytosis
  - Acanthocytes
  - Schistocytes
- Thrombocytopenia

Schistocytes

- Hallmark of fragmentation hemolysis
- Fragmentation of cells passing through tortuous or abnormal vessels
  - DIC
  - Neoplasia (HSA, Thyroid ACA)
    - Up to 50% of dogs with HSA
  - Vasculitis
  - Thromboembolism (Cushing's, HWD)
  - Caval Syndrome
  - Glomerulonephritis
- Increased fragility of erythrocytes
  - severe iron deficiency anemia
  - Doxorubicin
Abdominal Ultrasound
- Free abdominal fluid
- Large mass in cranial abdomen (14 cm)
  - Cavitated with mixed echogenicity
  - Appeared to be associated with the spleen

Abdominal Fluid
- Color – opaque and red
- PCV – 15%
- WBC – 12,500 cells / µl
- Interpretation – hemorrhagic effusion
  - Erythrocytes, macrophages, mesothelial cells, neutrophils
  - No neoplastic cells seen

No firm diagnosis?
- Hemangioma, hemangiosarcoma, hematoma, lymphoma, others?
  - Imaging and fluid analysis not helpful
- Hematological abnormalities indicate HSA
  - Anemia seen in 80% of dogs with splenic HSA
  - Dogs with splenic masses and evidence of anemia, fragmentation hemolysis and thrombocytopenia
  - **Significantly greater risk of having HSA (90%)**
Hemoabdomen and HSA

- Spontaneous and may be sporadic
- DIC – causes bleeding tendencies
- Thrombocytopenia
- Rupture of neoplastic vessels

CBC findings that support Dx of HSA

- Anemia – (80%) of dogs
  - Hemolysis and/or hemorrhage
- Thrombocytopenia – (75%) of dogs
  - DIC or microangiopathic disease in fibrin filled neoplastic vessels
- Schistocyte formation – (up to 50%) of dogs
  - Hallmark of red cell fragmentation
  - DIC or microangiopathic disease in fibrin filled neoplastic vessels

Hemostasis Profile

- PT and APTT – normal
- FDPs - negative
- D-dimers – (ref. range < 250 ng/ml)
  - Not useful in this case due to hemoabdomen
  - Can result in d-dimer levels > 1,000 ng/ml in dogs without evidence of TE disease

Next Step?

- Need to confirm diagnosis
  - Take the dog to surgery
  - Ultrasound guided FNA of mass
Fine-needle Aspiration of Splenic Mass

- Potential for definitive, presurgical diagnosis
- Potential for complications
  - Seeding the abdomen with tumor cells
  - Hemorrhage
  - Dog is already bleeding likely due to rupture of neoplastic vessels, not DIC

Cytologic Features of HSA

- Low to moderate cellularity
- Hemorrhage
- Oval to spindle-shaped cells, arranged individually or in small clusters
- Pale, lightly basophilic, veil-like cytoplasm
- Small, punctate, clear cytoplasmic vacuoles
- Cytomegaly and bizarre nuclear features of malignancy

Plan for Sadie

- Owners elected surgery and chemo if possible
- Sadie was transfused (PCV 26%)
- Surgery was performed and a 14 cm x 16 cm mass was identified in the spleen
- Multiple, red-purple, raised nodules were present in all lobes of the liver (not seen on ultrasound)
- The spleen and biopsies taken from the hepatic masses were submitted for histopathology
- Final Dx: hemangiosarcoma
Treatment
- Chemotherapy was initiated approximately 1 week post-op (once histopath confirmed a diagnosis) (PCV 35%)
- 21 day cycle of VAC
  - Vincristine 0.75 mg/m² BSA (IV) (Day 8 & 15)
  - Doxorubicin 30 mg/m² BSA (IV) (Day 1)
  - Cyclophosphamide 200 - 300 mg/m² BSA (PO) (Day 10)
- Sadie received 4 cycles of therapy

Prognosis
- Long-term prognosis extremely poor
- Death from exsanguination from rupture of metastatic site
- Surgery alone rarely curative with MST of 1 to 3 months
- Multi-drug chemotherapy MST 6 to 9 months

Sadie
- Sadie was found dead in her bed 9 months after splenic surgery
- Likely the result of ruptured metastatic lesion

Penny: 8 year old, F/S
Long-haired Dachshund
Rhinocerocyte
Presentation to Referring Veterinarian

- 3 week history of lethargy
- Eating and vomiting grass and grass roots
- Sleeping a lot
- Presently on monthly heartworm medication
- Vaccinations current

Penny’s Exam

- Temp. 101.5°F
- Respiration 35 PM
- HR 90 BPM
- Pale mucus membranes
  - No history of trauma or evidence of hemorrhage
- Fecal parasites check negative
- Heartworm test negative
- CBC & Biochemical profile

CBC Results

- HCT 12.2% (37-55)
- RBC 2.0 M/µl (5.5-8.5)
- Hgb 4.9 g/dl (12-18)
- MCV 62.0 fl (60-77)
- MCH 24.5 pg (18-30)
- MCHC N/A
- RDW 15.9% (14.7-17.9)
- Platelet 819 K/µl (175-500)

- WBC 6.58 K/µl (5.5-16.9)
- Neut 4.23 K/µl (2-12)
- Lym 1.52 K/µl (1.0-4.9)
- Mono 0.67 K/µl (0.3-2.0)
- Eos 0.13 K/µl (0.1-1.5)
- Baso 0.04 K/µl (0-0.1)

Reticulocyte 0.6%
Absolute retic 11.6 K/µl
Biochemical Profile

- WNL

Problem List

- Severe nonregenerative anemia
- Thrombocytosis
- Pica
- Vomiting
- Plan

Problem List

- Severe nonregenerative anemia
- Thrombocytosis
- Pica
- Vomiting
- Plan
  - Refer to UF-VMC for further evaluation
**Penny’s Presentation**

- 1 month history of lethargy and decreased appetite
- Eating grass and grass roots and vomiting them
- Physical exam
  - BW 4.6 kg
  - Quite but alert
  - Temp 102.1
  - HR 80, Resp. 32
  - MM white with no CRT available
- Normal fecal color
  - Fecal occult blood test (Hemocult) negative

**Diagnostic Plan**

- MDB
- CBC, UA, Biochemical profile
- Reticulocyte count
- Coombs’ test
- Cross match

**CBC Results**

- HCT 15.1% (37-55)
- RBC 2.31 M/µl (5.4-7.8)
- Hgb 5.4 g/dl (13-19)
- MCV 66.4 fl (66-75)
- MCH 23.4 pg (18-30)
- MCHC 35.8 g/dl (34-36)
- RDW 12.1% (11-13)
- Platelet 735 K/µl (150-430)
- Plasma Protein 6.9 g/dl

- WBC 6.92 K/µl (6.0-17)
- Neut 4.5 K/µl (2-12)
- Bands .21 K/µl (0-.3)
- Lym 1.80 K/µl (1.0-4.9)
- Mono 0.45 K/µl (0.3-2.0)
- Eos 0.1 K/µl (0.1-1.5)
- Baso 0.0 K/µl (0-0.1)

- Reticulocyte 0.1%
- Absolute retic 2.3 K/µl

**Iron Levels**

- Serum iron 374 ug/dl (70-264)
- TIBC 559 ug/dl (246-504)
- % saturation 67 (19-79)
Biochemical Profile

- If Penny was bleeding (e.g. chronic hemorrhage) what abnormality would you see in the profile?
  - Low total protein (albumin, pos. globulin)
  - High BUN, normal creatinine
  - Penny was normal, no abnormalities seen

Diagnostic Plan

- Cross-matched transfusion
  - Adapted to chronic anemia
  - Concern for anesthesia
- General anesthesia
- Imaging
  - Thoracic and abdominal radiographs (neoplasia?)
  - Ultrasound of abdomen
- Bone marrow collection and evaluation

Results

- Pre transfusion PCV 15.1%
- Post transfusion PCV 20.0%
- Imaging: no abnormalities seen
- Bone marrow aspirate
  - Dry tap (Why?)
  - Core biopsy of marrow
    - Role core on slide for cytological evaluation
    - Submit core for histopathology
- Sent patient home awaiting biopsy results
Bone Marrow Evaluation

- Erythroid hyperplasia with left-shifting
- Maturation arrest at rubricyte/metarubricyte stage
- Erythrophagocytosis of precursor cells
- Tentative diagnosis: Nonregenerative (Central) immune-mediated anemia

Core Biopsy

- *Myelofibrosis*, multifocal, moderate
- Erythroid hyperplasia
- Hemosiderosis
- Megakaryocytic hyperplasia with left-shift
Tentative Diagnosis and Plan

- Nonregenerative IMA
- Direct Coombs’
- Tick titers
- Await results and begin therapy

Recheck

- Return visit 1 week after diagnostic evaluation
- Penny more active after transfusion, but getting lethargic again
- PCV 15% with low number of spherocytes on blood smear
- Direct Coombs’ negative at 1:2
- Serology for *E. canis* and *A. phagocytophilum* - negative

Treatment Plan

- Azathioprine 2mg/kg for two weeks
- Prednisone 1 mg/kg BID
- Famotidine 5 mg per day (pepcid AC)

2 week Recheck

- Penny more alert than before
- PCV 22%
- No spherocytes noted on smear
- Continue on current dose until PCV normal
Nonregenerative IMHA

- Estimated 33% to 58% of IMHA are nonregenerative
- Anemia typically severe, (median PCV 11%) with majority being <20%
- Age 10 mo. to 12 yr. (median 6.5 yr.)
- Female over-represented in most studies
- **Dachshunds over-represented at UF (> 10 cases)**
- Two forms
  - PRCA: more severe less common form
  - Evidence of erythropoiesis in bone marrow (>90%)

Stokol et al., JAVMA 216:1429-1436, 2000. (43 cases)

Chronicity of Anemia

- Important in establishing diagnosis
- Most dogs have clinical signs of 7 or more days
  - Lethargy, anorexia, pallor, weakness, pica, vomiting
- **Animals tolerant of very low red cell mass**

Laboratory Analysis

- Normocytic, normochromic anemia
  - Spherocytes seen in small percent (16%), numbers vary
- 0 to 7 NRBC’s / 100 WBC (no / rare polychromasia)
- Normal leukocyte count
  - 50% had mild left-shift
- Platelets most often increased
  - Decreased in 22% of cases
- Direct Coombs’ test positive in 30 to 50% (low titer 1:4)
- ANA positive in 23%

Bone Marrow Evaluation

- Bone marrows difficult to aspirate in 21 of 43 dogs
  - No spicules
  - Dry tap
- **16 dogs had core biopsies, all had myelofibrosis (reversible)**
  - Erythroid precursors may be absent (5% PRCA), normal numbers, or increased (most common)
  - Erythroid hyperplasia was common among dogs with myelofibrosis
  - Maturation arrest at rubiricytes and metarubricytes with few to no polychromatophilic cells
  - Most dogs had large amounts of iron

(Stokol et al.)
Treatment for NRIMHA

(Stokol, et al., JAVMA 216:1429-1436;2000)

- Combination chemotherapy
  - Pred. & Cytoxan (73% remission)
  - Pred. & Azathioprine (52% remission)
  - Pred. alone (25% remission)

- Response rate
  - Complete remission (55%)
  - Partial remission (18%)
  - Poor response (27%)

Response Time and Mortality

- Response to treatment seen in 1 to 10 weeks
  - Median 2 weeks
- 18 cases with extensive follow-ups
  - 5 off all medication within 2 years
  - 9 on alternate day pred / azathioprine for 3 years
  - 6 relapsed when drug dosage / frequency was reduced
  - If drugs are reduced, maintain at reduced level for prolonged period of time (60 days?)

- Mortality rate 28%

Summary

- NR-IMHA should be considered in dogs with severe, chronic, nonregenerative anemia
- Normal WBC and normal or increased platelets
- Bone marrow evaluation may aid in confirming diagnosis
  - Fibrosis, maturation arrest, erythrophagia
- Myelofibrosis may complicate marrow aspiration
- Treatment should include combination chemotherapy
- Response to therapy may take weeks to months

Signalment

“Bean”
12 year old female/spayed mixed breed dog
Presenting Complaints

- Sleeping more than she used to
- O' claims she is not as active as she was a year ago
- Lethargy more pronounced over past 2-3 months
  - O’s thought she was just getting old

Physical Examination

- Quiet, but alert and responsive
- T = 101.0 F, HR=128 b/m (N= 70 – 120), Resp=40 br/m (N= 18 – 34)
- Pulse strong, CRT < 2 sec
- MM color pale pink
- Moderate dental tarter
- Rest of PE unremarkable

CBC

<table>
<thead>
<tr>
<th>CBC</th>
<th>Flag</th>
<th>Normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC (K/µl):</td>
<td>34.7</td>
<td>6-17</td>
</tr>
<tr>
<td>RBC (M/µl):</td>
<td>3.91</td>
<td>5-8.5</td>
</tr>
<tr>
<td>HGB (g/dl):</td>
<td>6.7</td>
<td>12-18</td>
</tr>
<tr>
<td>HCT (%):</td>
<td>21.6</td>
<td>35-55</td>
</tr>
<tr>
<td>MCV (fl):</td>
<td>55.3</td>
<td>60-77</td>
</tr>
<tr>
<td>MCH (pg):</td>
<td>17.1</td>
<td>21-27</td>
</tr>
<tr>
<td>MCHC(g/dl):</td>
<td>31</td>
<td>32-36</td>
</tr>
<tr>
<td>PLT (K/µl):</td>
<td>797 x 10³</td>
<td>200-500</td>
</tr>
<tr>
<td>Plasma color:</td>
<td>clear</td>
<td></td>
</tr>
<tr>
<td>Plasma Protein (g/dl)</td>
<td>4.8</td>
<td>6-7.8</td>
</tr>
<tr>
<td>Retic (%)</td>
<td>1.5</td>
<td></td>
</tr>
</tbody>
</table>

Erythrocyte Indices

- Hypochromasia
  - MCHC 31 g/dl (N = 32 - 37)
    - Often indicates a regenerative response
- Reticulocytes 1.5% (uncorrected)
- Microcytic [MCV 55.3 fl (N= 60 – 77)]
Absolute Reticulocyte Numbers

- (% retics.) \( \times \) (RBC)
- \( (.015) \times (3.91 \times 10^6) = 58,650 / \mu l \)
- Good regenerative response should have >80,000 reticulocytes (dogs)
- >60,000 considered increased

CBC (con’t)

<table>
<thead>
<tr>
<th>%</th>
<th>x1,000/µl</th>
<th>normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seg Neutrophils:</td>
<td>89</td>
<td>30.3</td>
</tr>
<tr>
<td>Bands</td>
<td>2</td>
<td>0.7</td>
</tr>
<tr>
<td>Lymphocytes:</td>
<td>1</td>
<td>0.3</td>
</tr>
<tr>
<td>Monocytes:</td>
<td>9</td>
<td>3.1</td>
</tr>
<tr>
<td>Eosinophils:</td>
<td>1</td>
<td>0.3</td>
</tr>
</tbody>
</table>

nRBC 3/100 WBC
Polychromasia, anisocytosis
Several Howell-Jolly bodies
Few schistocytes seen

Bean’s Smear

Erythrocyte Abnormalities

- Microcytosis
- Hypochromasia
- Poikilocytosis
  - Changes reflect iron deficiency anemia
  - Poorly regenerative
Poikilocytosis (Abnormal Erythrocyte Shapes)

- Iron deficiency results in poorly structured erythrocytes
  - reduced red cell membrane deformability
- Various shapes identified
  - schistocytes, acanthocytes, keratocytes
- Difference from fragmentation hemolysis (DIC, hemangiosarcoma)
  - Hypochromic erythrocytes
  - and . . .

\[
\text{Ave. } \# \text{ of platelets per } 100\times \text{ oil field } \times 15,000 = \# \text{ of platelets / } \mu\text{l.}
\]

To Summarize:

The hematologic findings seen in this patient all indicate the presence of an iron deficiency anemia and an associated stress/inflammatory leukogram.

The most common / only cause of iron deficiency anemia in dogs and cats is:
To Summarize:

The hematologic findings seen in this patient all indicate the presence of an iron deficiency anemia and an associated stress/inflammatory leukogram.

The most common / only cause of iron deficiency anemia in dogs and cats is: **chronic blood loss**?

<table>
<thead>
<tr>
<th>Chemistry Panel (Significant results)</th>
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</thead>
<tbody>
<tr>
<td><strong>Results</strong></td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
</tr>
<tr>
<td>ALP (IU/L)</td>
</tr>
<tr>
<td>ALT (IU/L)</td>
</tr>
<tr>
<td>AST (IU/L)</td>
</tr>
<tr>
<td>T. Bili (mg/dl)</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
</tr>
<tr>
<td>BUN (mg/dl)</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
</tr>
<tr>
<td>Phosphorus (mg/dl)</td>
</tr>
</tbody>
</table>

Problems

- Evidence of chronic intestinal hemorrhage

Plan

- Fecal exam with Hemoccult test
- Coagulation profile
- Abdominal imaging

Additional Tests

- Fecal exam – negative for intestinal parasites
- Hemoccult – positive for blood

- Abdominal radiographs - normal; gas present in the stomach and the colon.
- Abdominal ultrasound - normal; the complete evaluation of the small bowel and stomach is inhibited by gas present in the stomach and colon.
Coagulation Profile

<table>
<thead>
<tr>
<th>Test</th>
<th>Value</th>
<th>Normal Range</th>
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</thead>
<tbody>
<tr>
<td>BMBT</td>
<td>2 min 30 sec ( &lt; 4 minutes)</td>
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<tr>
<td>ACT</td>
<td>60 sec</td>
<td>(60-90 seconds)</td>
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<tr>
<td>PT</td>
<td>6.5 sec</td>
<td>(5-8.5 seconds)</td>
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<tr>
<td>aPTT</td>
<td>10.5 sec</td>
<td>(9-14 seconds)</td>
</tr>
<tr>
<td>FDP</td>
<td>&lt;20</td>
<td>(&lt; 20 µg/ml)</td>
</tr>
</tbody>
</table>

Chronic Hemorrhage

- GI bleeding
  - Gastrointestinal neoplasia
  - Gastroduodenal ulceration
  - Gastrointestinal parasitism (Ancylostomiasis)
  - Thrombocytopenia
  - Liver disease
    - Coagulation abnormalities

- Plan – anesthesia and endoscopy

While under anesthesia

- Gastroduodenoscopy – no abnormal findings.
- Exploratory laparotomy – 3 cm small intestinal mass (resected)
  - Cytology
  - Histopathology

Which G I tumor (in the dog) most commonly results in chronic blood loss and iron deficiency without causing significant clinical evidence of gastrointestinal disease?

1. Adenocarcinoma
2. Mast cell tumor
3. Leiomyosarcoma
4. Lymphoma
Cytology from mass

Diagnosis

• Intestinal Smooth Muscle Tumor (Leiomyosarcoma)
• Characteristic cytologic features
  – elongated cells with lightly basophilic cytoplasm
  – fragile cells with indistinct cytoplasmic borders
  – oval to cigar-shaped nuclei
  – strap cells
  – cytologic criteria for malignancy not reliable indicators of biological behavior

GILMs vs. GISTs

Russell et al., JAVMA 2007, 230:1329-1333

• Histologically and cytologically indistinguishable
  – Need immunohistochemistry
• GILMs / GILMSs
  – Originate from smooth muscle of GI tract, more often stomach and small intestine (jejunum)
  – c-kit expression negative (CD-117)
• GISTs
  – Originate from interstitial cells of Cajal
    • Pacemakers of the GI tract
  – Positive for c-kit expression (Palladia Therapy, tyrosine kinase inhibitor)
    • Believed to be responsible for oncogenesis
  – More often occur in cecum and large intestine
  – May be more aggressive than GILMs with higher potential for metastasis, local invasion and perforation of GI tract
Which other neoplastic process can have a c-kit mutation and be responsive to Palladia?

1. Lymphoma
2. Adenocarcinoma
3. Mast cell tumor

Follow-up

- Ferrous sulfate (10 mg/kg po bid with meal)
  - continued until PCV and red cell indices return to normal. This can take weeks to months
- Bean responded well to iron supplementation. The tumor did not recur.

Tabby Thompson

- 9 year old, F/S DSH
- Presented with complaint of listlessness, anorexia and rapid respiration
- Physical exam findings
  - Muffled heart and lung sounds
  - Cranial thorax not easily compressed

Tabby Thompson

- Radiographs
  - Very large mass in cranial mediastinum
DDx for most common cranial mediastinal mass in the cat
- Lymphoma
- Thymoma

Problems
- Drastically different therapies
- Both contain lymphocytes
Thymic Lymphoma

- Lymphocyte is the neoplastic cell population
- T-cell in origin
- Large blast cells with occasionally clefted nuclei
Thymoma

- Neoplasia of the epithelial cell population of the thymus
- Small lymphocytes predominate in most aspirates
- Low numbers of neoplastic epithelium are often seen
- Mast cells frequently observed in feline thymomas

- 60% in the cat are cystic
- Benign forms well encapsulated (80%)
- Malignant forms invasive
  - Rare to metastasize
  - Cytology incapable of distinguishing forms
- Paraneoplastic syndromes
  - Myasthenia (40% dogs, rare in cats)
  - Megasophagus and or aspiration pneumonia
  - Immune-mediated anemia, polymyositis
  - Dermatitis (reported in cats)

The End?