Unraveling the Hemogram: What do those numbers mean?  

Quantitative Analysis of Anemia  
- Anemia (reductions in erythrocyte mass)  
  - Hemorrhage  
  - Hemolysis  
  - Decreased production  
- Two areas of quantitative analysis  
  - Evaluation of red blood cell mass (PCV and HCT)  
  - Red blood cell indices

Are PCV and Hematocrit the Same?  
1. Yes  
2. No

Analysis of Red Cell Mass  
- Hematocrit, RBC, PCV  
  - Red cell mass in relation to plasma volume  
  - PCV vs. HCT via automated cell count  
    - HCT calculated based on RBC and MCV  
    - HCT Affected by agglutination
Red Blood Cell Indicies

- MCV, MCH, MCHC
- Assist in the classification of anemia
  - Regenerative
    - Hemorrhage
    - Hemolysis
  - Nonregenerative
    - Decreased production
- Identification of specific diseases or pathological processes

Mean Cell Volume (MCV)

- An evaluation of the average size (volume) of a single RBC
- Can be measured directly (cell counters)
- Calculation: \( \text{PCV} / \text{RBC} \times 10 \)

When is the MCV increased?

1. Nonregenerative anemia
2. Regenerative anemia
3. Iron deficiency anemia
4. Anemia of chronic disease

Mean Cell Volume (MCV)

- Increased (larger than normal RBCs)
  - Regenerative anemias (requires a week or more)
  - FeLV
  - Leukemia / preleukemia (Myeloproliferative disorders)
- Decreased (smaller than normal RBCs)
  - Iron deficiency (requires 1 or more months)
  - Fragmentation of erythrocytes
  - Portosystemic shunts
  - Normal in Akita dogs
Mean Cell Hemoglobin Concentration (MCHC)

- The average Hb concentration
- Calculation: \( \frac{\text{Hb}}{\text{PCV or HCT}} \times 100 \)
- Decreases
  - Regenerative anemia
  - Iron deficiency anemia
- Increases (artifact)
  - Intravascular hemolysis (also in vitro)
  - Heinz body formation
  - Lipemia

Common Abnormalities in the Anemic Pet

- Normocytic, Normochromic
- Macrocytic, Hypochromic
- Microcytic, Hypochromic

Normocytic, Normochromic

- Normal MCV and MCHC
- Nonregenerative anemia
  - Decreased production problem
    - Anemia of Chronic Inflammation
    - Chronic Renal Failure
    - Bone marrow disease
  - Peracute hemorrhage or hemolysis
    - 3 to 5 days for peripheral response
    - May take a week or more to change indices
    - RDW (Red Cell Distribution width)
    - Blood film evaluation

Concurrent Evaluation of Indicies

- Necessary for interpretation
- MCV
  - Normocytic
  - Macrocytic
  - Microcytic
- MCHC
  - Normochromic
  - Hyperchromic
  - Hypochromic
Macrocytic, Hypochromic

- Elevated MCV, decreased MCHC
- Regenerative anemia
  - Hemolysis (most dramatic response)
  - Hemorrhage (less dramatic)
    - Temporal
      - Acutely nonregenerative
      - Regenerative
      - Poorly regenerative

Red Cell Indicies and the Regenerative Anemia

- Study of over 4,000 dogs with anemia
  - (JAVMA, 138:1452-1458, 2011)
- 32.5% had regenerative anemia
  - Of those, only 11.8% had macrocytosis and hypochromasia (11% sensitivity; 98% specificity)
  - Polychromasia on blood smear alone, or with high RDW had 77% and 79% accuracy, respectively

Microcytic, Hypochromic

- Iron deficiency
- Chronic blood loss
- Diameter may be normal
- Cells are flat with reduced hemoglobin

Other Abnormalities of Indicies

- Macrocytic, normochromic (Inc. MCV)
  - Leukemia / preleukemia
    - Dysplastic change (preneoplastic)
    - Neoplastic (leukemia)
  - FeLV infection
- Microcytic, normochromic (Dec. MCV)
  - Portosystemic shunt
  - Normal in Akita and Saluki dogs
What is the most unreliable measurement on the hemogram?

1. Red blood cell count
2. White blood cell count
3. Platelet count

In which species are platelet counts most commonly erroneous?

1. Dog
2. Cat
3. Horse
4. Cow
5. Pig

The Platelet Count

- Low platelet counts should always be confirmed
  - Check tube and stopper for clots
  - Evaluate platelet numbers on blood smear (clumps)
- Beware of the thrombocytopenic cat!
- Very difficult to obtain accurate counts for cats
- Impedence counters vs. Laser technology
  - Size of platelets vs. RBC
  - Platelet clumps

Does the WBC count give you enough information to evaluate the leukogram?

1. Yes
2. No
The Leukogram

WBC (it's not enough!)
- Analyzers that perform an accurate differential are superior
- Clinically significant changes with normal Leukogram
  - Increased numbers of immature neutrophils
  - Toxicity
  - Atypical leukocytes
  - NRBCs
- Laser technology vs. impedence counters
- "Flagging" atypical cells

Microscopic Examination of a Blood Film (When?)
- Any quantitative abnormalities
  - RBC
  - WBC
  - Platelets
- Evaluation of sick patients
- Quality assurance for all samples
  - Quick assessment

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
History

- Annual exam last year was normal
  - Fecal negative, HWT negative
  - On monthly preventative (Revolution®, Pfizer)
- Owner claims Bean appears to be eating normally
- Water intake
  - Not drinking any more or less than normal

Physical Examination

- Quiet, but alert and responsive
- $T = 101.0 \, ^\circ F$, 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

Problem List

- Pale mucous membranes
- Moderate dental tarter/periodontal disease

CBC

<table>
<thead>
<tr>
<th>Test</th>
<th>Value</th>
<th>Flag</th>
<th>Normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC (K/µl)</td>
<td>34.7</td>
<td>H</td>
<td>6-17</td>
</tr>
<tr>
<td>RBC (M/µl)</td>
<td>3.91</td>
<td>L</td>
<td>5-8.5</td>
</tr>
<tr>
<td>HGB (g/dl)</td>
<td>6.7</td>
<td>L</td>
<td>12-18</td>
</tr>
<tr>
<td>HCT (%)</td>
<td>21.6</td>
<td>L</td>
<td>35-55</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>55.3</td>
<td>L</td>
<td>60-77</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>17.1</td>
<td>L</td>
<td>21-27</td>
</tr>
<tr>
<td>MCHC(g/dl)</td>
<td>31</td>
<td>L</td>
<td>32-36</td>
</tr>
<tr>
<td>PLT (K/µl)</td>
<td>$797 \times 10^3$</td>
<td>H</td>
<td>200-500</td>
</tr>
<tr>
<td>Plasma color</td>
<td>clear</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma Protein (g/dl)</td>
<td>4.8</td>
<td>L</td>
<td>6-7.8</td>
</tr>
<tr>
<td>Retic (%)</td>
<td>1.5</td>
<td></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)]

Corrected Reticulocyte Percentage

- Must be adjusted for the degree of anemia
- Corrected Retic. % = (Counted Retic. %) x (Patient HCT / 45)
- Corrected Retic. % = (1.5) x (21.6/45) = .72% (poorly regenerative)
- >1% is considered a regenerative response

Absolute Reticulocyte Numbers

- (% retics.) X (RBC)
- (.015) X (3.91 x 10⁶) = 58,650 / µl
- Good regenerative response should have
  - >80,000 reticulocytes (dogs)
  - >60,000 considered increased

Is microcytosis the typical finding in animals with a regenerative response to anemia?

1. Yes
2. No
### CBC (con't)

<table>
<thead>
<tr>
<th></th>
<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>
<td>3-11.5</td>
</tr>
<tr>
<td>Bands</td>
<td>2</td>
<td>0.7</td>
<td>&lt; 0.2</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>1</td>
<td>0.3</td>
<td>1-4.8</td>
</tr>
<tr>
<td>Monocytes</td>
<td>9</td>
<td>3.1</td>
<td>0.1-1.4</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>1</td>
<td>0.3</td>
<td>0.1-1.2</td>
</tr>
</tbody>
</table>

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

### Erythrocyte Abnormalities

- Microcytosis  
- Hypochromasia  
- Poikilocytosis  
  - Changes reflect iron deficiency anemia  
  - Poorly regenerative

### Bean’s Smear

![Bean’s Smear](image)

### Normal Cells with Hypochromic Cells

![Normal Cells with Hypochromic Cells](image)
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 . . .

Thrombocytosis and Iron Deficiency

- Seen in over one-half of the dogs with iron deficiency anemia
- In people, only seen when iron deficiency is due to chronic blood loss
- Pathogenesis unclear
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.

What is the most likely (common) cause of chronic blood loss in this case?
1. Epistaxis
2. GI bleeding
3. Hemorrhage into body cavity
4. Urinary tract

Which of the following biochemical analytes can assist with the identification of GI bleeding?
1. AST and CPK
2. ALT and ALP
3. BUN and Creatinine
### Chemistry Panel (Significant results)

<table>
<thead>
<tr>
<th>Test</th>
<th>Result</th>
<th>Ref. Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mg/dl)</td>
<td>110</td>
<td>75-115</td>
</tr>
<tr>
<td>ALP (IU/L)</td>
<td>140</td>
<td>&lt;135</td>
</tr>
<tr>
<td>ALT (IU/L)</td>
<td>110</td>
<td>&lt;100</td>
</tr>
<tr>
<td>AST (IU/L)</td>
<td>53</td>
<td>&lt;60</td>
</tr>
<tr>
<td>T. Bili (mg/dl)</td>
<td>0.2</td>
<td>&lt;0.4</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>141</td>
<td>130-240</td>
</tr>
<tr>
<td>BUN (mg/dl)</td>
<td>42</td>
<td>H 6-22</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.9</td>
<td>0.4-1.5</td>
</tr>
<tr>
<td>Calcium (mg/dl)</td>
<td>8.2</td>
<td>L 8.7-11.2</td>
</tr>
<tr>
<td>Phosphorus (mg/dl)</td>
<td>3.1</td>
<td>2.5-5.5</td>
</tr>
<tr>
<td>T. Protein (g/dl)</td>
<td>3.9</td>
<td>L 5.7-7.4</td>
</tr>
<tr>
<td>Globulins (g/dl)</td>
<td>1.8</td>
<td>L 2.1-4.1</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>2.1</td>
<td>L 2.9-4.0</td>
</tr>
</tbody>
</table>

### Problems
- Evidence of chronic intestinal hemorrhage

### Plan
- Fecal exam with Hemocult test
- Serum iron
- Coagulation profile
- Abdominal imaging

### Additional Tests
- **Fecal exam** - negative for intestinal parasites
- **Hemocult** - 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>
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<th>Reference Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMBT</td>
<td>2 min 30 sec</td>
<td>( &lt; 4 minutes)</td>
</tr>
<tr>
<td>ACT</td>
<td>60 sec</td>
<td>(60-90 seconds)</td>
</tr>
<tr>
<td>PT</td>
<td>6.5 sec</td>
<td>(5-8.5 seconds)</td>
</tr>
<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>
Serum Iron Status

- Serum iron (µmol/L) 8 L (15-42)
- TIBC (µmol/L) 69 (51-102)
- Saturation (%) 12 L (19-59)

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

25% 25% 25% 25%
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

Leiomyosarcoma (GILMs & GISTs)

- Biological behavior
  - Usually slow growing
  - Affected animals often anemic
  - Metastasis sporadic (<30% of cases)
  - Histopathology needed to detect invasive potential
  - Immunohistochemistry needed for stromal tumors of GI tract (LaRock & Ginn, Vet Pathol 1997, 34:301-311)
  - Vascular smooth muscle, myofibroblasts
    - Negative for desmin
    - More aggressive, may metastasize
  - Intestinal wall smooth muscle
    - Positive for desmin
GILMs vs. GISTs
Russell et al., JAVMA 2007, 230:1329-1333
- Histologically and cytologically indistinguishable
  - Need immunohistochemistry
- GILMs / GILMS
  - 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 sulphate (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.