Haematology

LECTURE 5. EVALUATION OF BLOOD SMEAR RBCs AND RETICULOCYTES

OVERVIEW

1. RBC morphological evaluation
2. Reticulocyte count and evaluation

1. RBC MORPHOLOGICAL EVALUATION
VARIATIONS IN SPECIES

DOG
- Large erythrocytes
- Size uniformity
- Central pallor

CAT
- Smaller erythrocytes
- Anisocytosis
- Scarce central pallor

VARIATIONS IN SPECIES

HORSES
Rouleaux (sedimentation tendency)

RUMINANTS
Anisocytosis and crenation

EVALUATION OF RBC NUMBER AND SIZE IN DIFFERENT ANIMAL SPECIES

<table>
<thead>
<tr>
<th>Tierart</th>
<th>dogs</th>
<th>cats</th>
<th>horses</th>
<th>pigs</th>
<th>cattle</th>
<th>sheep</th>
<th>goats</th>
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<tr>
<td>n</td>
<td>46</td>
<td>61</td>
<td>61</td>
<td>105</td>
<td>176</td>
<td>141</td>
<td>181</td>
</tr>
<tr>
<td>RBC 10^12/l</td>
<td></td>
<td></td>
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<tr>
<td>MCV fl</td>
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ABNORMALITIES OF RBCs MORPHOLOGY

1. Abnormalities in size
   - MACROCYTOSIS
   - MICROCYTOSIS

2. Abnormalities in colour
   - POLYCHROMASIA
   - HYPOCHROMASIA

3. Abnormalities in shape
   - SPHEROCYTOSIS
   - NON-SPECIFIC FINDINGS

4. Abnormalities in distribution
   - ROULEAUX
   - AGGLUTINATION

5. Inclusion Bodies and infectious agents

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ABNORMALITIES IN SIZE

MACROCYTOSIS

\[
\text{Reticulocytes}\quad \text{FeLV}
\]

MICROCYTOSIS — Iron deficiency

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ABNORMALITIES IN SIZE

Reticulocytes and spherocytes in a dog with immune-mediated haemolytic anemia

Macrocytic (but non-regenerative) anemia in FeLV infected cat
ABNORMALITIES IN COLOUR

POLYCHROMASIA

- Reticulocyte

HYPOCHROMASIA

- Iron deficiency


ABNORMALITIES IN SHAPE

(POIKILOCYTOSIS)

- SPHEROCYTOSIS (MARKER OF IMMUNE-MEDIATED HAEMOLYTIC ANAEMIA)

- NON-SPECIFIC FINDINGS
  - Crenation
  - Acanthocyte
  - Schistocyte/fragmentocyte
  - Target Cell


Spherocytes
Crenation
Numerous pin-point projections

Acanthocytes (few irregular elongations of RBC border with rounded ends often forming a bud)

Schistocytes (erythrocyte fragmentation)
Target cell

Rouleaux
- normal finding in horses
- indicates inflammation in small animals

ABNORMALITIES IN RBC DISTRIBUTION

Agglutination
- can suggest immune-mediated haemolytic anaemia
INCLUSION BODIES AND INFECTIOUS AGENTS

HOWELL-JOLLY BODIES
BASOPHILIC STIPPLING
NUCLEATED ERYTHROCYTES

HEINZ BODIES
INFECTIOUS AGENTS (Babesia, Mycoplasma haemofelis)

Signs of regeneration

Nucleated RBCs and Howell-Jolly Bodies

Basophilic stippling
Infectious agents

* Babesia canis
  
* Haemobartonella felis (Mycoplasma haemofelis)
2. RETICULOCYTE COUNT AND EVALUATION

Reticulocytes are young (immature) erythrocytes prematurely released to blood from the bone marrow in some anaemias.

CLINICAL APPLICATIONS:
Evaluation of erythropoiesis in bone narrow.
Differentiation of regenerative and non-regenerative anaemia.

TECHNIQUES OF DETECTION:
- Romanowsky stains
- Supravalent stains:
  - New methylene blue
  - Brilliant cresyl blue

ROMANOWSKY STAINS

In dogs, an average of >10 polychromatic red cells per OIF suggests a marked regenerative response
NEW METHYLENE BLUE (NMB) STAIN

Reticulocytes are non-nucleated erythrocytes in which NMB stain precipitates in RNA-protein complexes.

ABSOLUTE RETICULOCYTE COUNT

- Manual
- Automated (some haematological analysers)

MANUAL ABSOLUTE RETICULOCYTE COUNT

1. Count number of reticulocytes per 500-1000 erythrocytes (blood smear)
   - e.g. 10 reticulocytes / 500 erythrocytes
2. Calculate % of reticulocytes
   - e.g. Reticulocyte % = 10 x 100 / 500 = 2
3. Calculate absolute reticulocyte count based on erythrocyte count from the haematology analyser
   - e.g. Absolute reticulocyte count (10^9/L) = Reticulocyte % x Erythrocyte count / 100
If absolute reticulocyte count:

Dogs >60x \(10^9\)/L  
Cats >50x \(10^9\)/L  
A sign of regeneration

**RETICULOXYTE PRODUCTION INDEX: CALCULATIONS**

- Corrected reticulocyte percentage (CRP)
  
  \[ \text{CRP} = \frac{\% \text{ reticulocytes} \times \text{PCV of sample}}{\text{normal PCV}} \]

- Reticulocyte Production Index (RPI)
  
  \[ \text{RPI} = \frac{\text{CRP}}{\text{Maturation Index (MI)}} \]
  
<table>
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<tr>
<th>PCV</th>
<th>MI (days)</th>
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<tr>
<td>0.45</td>
<td>1</td>
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<tr>
<td>0.35</td>
<td>1.5</td>
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<tr>
<td>0.25</td>
<td>2</td>
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<tr>
<td>0.15</td>
<td>2.5</td>
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(values for dogs)

- Corrected reticulocytes percentage (CRP)
  
  \[ \text{CRP} = 0.15 \times 0.15/0.45 = 5\% \]

- Reticulocyte Production Index (RPI)
  
  \[ \text{RPI} = 5/2.5 = 2 \]

Dog with PCV=0.15/L and reticulocytes =15%

**RETICULOXYTE PRODUCTION INDEX: PRACTICAL EXAMPLE**
**INTERPRETATION**

RPI > 3  Very good regeneration

RPI = 1-3  Good regeneration

RPI < 1  Inadequate regeneration

**EXAMPLES OF USE OF RETICULOCYTE PARAMETERS IN DOGS**

<table>
<thead>
<tr>
<th>Units</th>
<th>Case 1</th>
<th>Case 2</th>
<th>Case 3</th>
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<tr>
<td>Hct/PCV</td>
<td>0.15</td>
<td>0.15</td>
<td>0.15</td>
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<tr>
<td>RBC count</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
</tr>
<tr>
<td>Reticulocytes %</td>
<td>3</td>
<td>12</td>
<td>25</td>
</tr>
<tr>
<td>Reticulocytes x10⁹/L</td>
<td>60</td>
<td>240</td>
<td>500</td>
</tr>
<tr>
<td>CRP</td>
<td>---</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>RPI</td>
<td>0.4</td>
<td>1.6</td>
<td>3.33</td>
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**Diagnosis**
- non-regenerative anemia
- good regenerative anemia
- highly regenerative anemia

**SPECIES VARIATIONS: IN CLINICALLY HEALTHY ANIMALS**

- **Dogs.** Low number of reticulocytes (<1%), aggregate only

- **Cats.** Two types of reticulocytes:
  - aggregate: blue stained coarse clumping (0.5% of erythrocytes)
  - punctate: small, blue stained dots (1-10%).

- **Ruminants and horses.** Virtually no reticulocytes in blood.
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<th>SPECIES VARIATION: in anaemic animals</th>
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<tr>
<td>- Canine. Strong reticulocyte response in regenerative anaemias.</td>
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<td>Aggregated reticulocytes (indicate recent response)</td>
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<tr>
<td>- Feline. Punctated reticulocytes (indicate response to anaemia occurring 3-4 weeks previously)</td>
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<tr>
<td>- Ruminants and horses. Reticulocytes may not appear even in very severe anaemias</td>
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