HEMATOLOGY IN THE EMERGENCY SETTING: THE VALUE OF THE BLOOD SMEAR EXAM

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This presentation consists of an initial short lecture on the basics of blood smear evaluation followed by a review of real clinical cases. The main objective is to demonstrate a systematic approach to examining a blood smear in order to gain valuable information for patient assessment and management in the emergency setting.

LEARNING OBJECTIVES:
Perform a thorough blood smear review with the following objectives:
1. Differentiate between a regenerative and a non-regenerative anemia
2. Identify signs of inflammation
3. Identify abnormal cells in circulation
4. Recognize severe thrombocytopenia and identify patients at high risk for bleeding

LECTURE OUTLINE:
1. Introduction
2. Smear preparation and staining
3. Anatomy of the blood smear
4. Low power assessment of the blood smear
5. High power assessment of the blood smear
6. Interpretation of key features of the blood smear

1. Introduction

Although many clinics now have on-site hematology analyzers, these instruments are not infallible. Assessment of a well prepared fresh blood smear is recommended to verify the findings of the automated analyzer and to identify other abnormalities not detected by the analyzer, such as changes in cell morphology or the presence of infectious agents or abnormal cell populations. When patients present in critical condition, the review of a fresh blood smear can be a valuable tool for patient assessment and management. Today we will outline a step-by-step procedure for blood smear review, concentrating on key features of the blood smear that can help guide you in the management of the critical patient.

2. Smear preparation and staining

A well made blood smear prepared from fresh (within 2-3 hours) EDTA blood is critical to maximizing our ability to identify and interpret any abnormalities on the smear. The goal is to make a thin smear with a tapered feathered edge resulting in adequate spreading of the cells to assess morphology, and a uniform distribution of cells to estimate cell density. It is important to
make the smear as soon as possible after sample collection, as delays can result in changes to the cell morphology as well as other artifacts such as cell lysis.

Most stains used in veterinary medicine are Romanowsky-type stains such as Diff-Quick®. Slides being stained in-clinic or being submitted to a reference laboratory should simply be air-dried; prior fixation is not needed (including heat fixation) and may interfere with staining quality. In general follow the recommendation of the manufacturer for the staining procedure, though the following hints may be of use:

Hints:

- Ensure stain reagents are fresh and well filtered, with time and repeated use precipitates may form in the stain making interpretation difficult, the stain may ‘fatigue’ leading to understaining, and organisms may contaminate the reagents.
- Ensure slides are well dried before staining; water may still be present when the slide appears dry, use a hair dryer (especially in warm humid months) or leave for sufficient time for adequate drying.

3. Anatomy of the blood smear

All areas of the blood smear are not created equal! Specific areas of the smear are better for identifying certain abnormalities. For example, large cells or structures (such as large parasites or platelet clumps) are often found at the leading edge of the smear, whereas thicker areas of the smear must be examined to identify spherocytes.

The main areas of the smear include:
- Feathered edge: The leading edge of the smear.
- Monolayer: Approximately 1-2 fields (10X obj) in from the inner boundary of the feathered edge.
- Deeper areas of the smear further away from the feathered edge.

4. Low power assessment of the blood smear (10X objective)

Blood smears should be scanned using a low power objective (10X) to assess RBC and WBC densities and to look for the presence of abnormal cells, infectious agents, or other abnormalities
that may be missed during the differential cell count and higher power assessment of the smear. Low power viewing should start with a review of the feathered edge, followed by a few sweeps of the monolayer and the deeper areas of the smear.

- **Feathered edge**: Scan the entire feathered edge under low power and move to high power for closer examination of any abnormal cells or structures. Look for the following at the feathered edge:
  - Platelet clumps: These will falsely decrease the platelet count by automated analyzers and the platelet estimate in the monolayer. Common in cats.
  - Abnormal cells: These may include neoplastic cells, reactive lymphocytes or other cells not normally seen in peripheral blood such as mast cells.
  - Parasites: e.g. microfilaria.

After review of the feathered edge scan the monolayer and deeper areas of the smear under low power to estimate RBC and WBC densities:

- **RBC density**:
  - Normal: In dogs and cats a normal RBC density generally translates to an even distribution of RBC in the monolayer where the red cells are close together, but not overlapping.
  - Increased (polycythemia) or decreased (anemia) density of RBCs should be confirmed and quantified by spinning a microhematocrit tube to measure a PCV. General guidelines in dogs and cats:\(^1\):
    - Dogs: PCV = 37-55
    - Cats: PCV = 24-45

- **WBC density**: Estimation of WBC density is often done subjectively by examining several areas of the smear or one of several calculations can be used (see example below). WBC estimation is crude and is dependent on an even distribution of cells on the smear but it is useful to identify marked increases or decreases in WBC numbers.
  - 10X Objective: WBC estimate/μL = average number of WBCs in 10 fields x 150

A recent study showed that agreement between WBC estimates by emergency room personnel and automated WBC counts was poor but most reliable and useful in cases of severe leucopenia. \(^3\)

5. **High power assessment of the blood smear (40X, 50X and/or 100X Objectives)**

When examining the smear under high power, the area of the smear you assess is very important. A differential count as well as assessment of cell morphology should be done in the monolayer.
- **Differential count**: Cells are categorized as segmented neutrophils, band neutrophils, lymphocytes, monocytes, eosinophils, basophils and other WBCs (or unclassified cells). Identification of band neutrophils to detect a left shift is somewhat subjective but the following criteria are recommended: nuclei have parallel sides, a smooth outline and lighter/less clumped chromatin.

- **RBC morphology**: You will use the low power view and a PCV to identify anemia and the degree of anemia. It is then important to establish if the anemia is regenerative or non-regenerative in order to determine the mechanism of the anemia.
  - Polychromasia: The regenerative response is best quantified using the absolute reticulocyte count determined by the automated hematology analyzer; however, regeneration can be estimated by evaluating the degree of polychromasia on the smear using the 100x objective:

<table>
<thead>
<tr>
<th>Degree of Polychromasia</th>
<th># polychromatophils/100x objective field</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dog</td>
</tr>
<tr>
<td>1+</td>
<td>2-7</td>
</tr>
<tr>
<td>2+</td>
<td>8-14</td>
</tr>
<tr>
<td>3+</td>
<td>15-29</td>
</tr>
<tr>
<td>4+</td>
<td>&gt;30</td>
</tr>
</tbody>
</table>

- Other findings that are often seen in regenerative anemias include macrocytes, Howell-Jolly bodies and nucleated RBCs, though without polychromasia, these findings alone do not indicate a regenerative response.
- The following abnormalities in RBC morphology can aid further in the identification of the mechanism of the anemia:
  - Spherocytes: Appear smaller and denser than normal RBCs and lack central pallor. They usually indicate immune mediated hemolytic anemia but low numbers can be seen due to RBC fragmentation.
  - Hypochromasia: Increased central pallor of the RBCs suggest decreased hemoglobin content and iron deficiency from chronic external blood loss.
  - Acanthocytes, keratocytes, schistocytes: These abnormally shaped RBCs can indicate fragmentation injury of the RBCs and are seen in conditions such as DIC, hemangiosarcoma or vasculitis. They can also be seen in liver disease, in particular in cats with hepatic lipidosis.
  - Heinz bodies and eccentrocytes: Indicate oxidant injury. Oxidants may be exogenous compounds such as onions, acetaminophen or zinc or may result from endogenous production of reactive oxidant compounds (seen in a variety of inflammatory conditions). Oxidant injury of RBCs causes reduced RBC lifespan and can result in hemolytic anemia.
  - Parasites: e.g. Mycoplasma haemofelis/haemocanis, Babesia spp.
**WBC morphology:** The key features you will look for in the WBC exam are evidence of toxic change and the presence of abnormal cells.

- **Toxic change of neutrophils:** Normal segmented neutrophils in the dog and cat have clear to light pink cytoplasm with faint pink granules. When the bone marrow is stimulated by inflammatory cytokines to increase neutrophil production, morphologic changes referred to as “toxic change” develop. These changes include basophilic streaks in the cytoplasm, foamy cytoplasmic vacuolation and the presence of Dohle bodies. These changes are usually accompanied by an increase in band neutrophils (left shift) in the differential count.

- **Abnormal cells:**
  - **Reactive lymphocytes:** These cells are often larger than normal lymphocytes, have clumped nuclear chromatin and deep blue smooth cytoplasm. Increases in reactive lymphocytes indicate non-specific antigenic stimulation.
  - **Blast cells:** Have single round nucleus with a high nuclear to cytoplasmic ratio, fine nuclear chromatin which may or may not contain visible nucleoli. These may be of lymphoid or myeloid origin and are often indistinguishable. The presence of blast cells suggests hematopoietic neoplasia (lymphoma or leukemia) and should be confirmed by a clinical pathologist.
  - **Mast cells:** Round cells with round to oval nuclei and deep purple granules. Note that some mast cell granules do not stain well with Diff-Quick® stains. Mast cells can be seen in neoplastic conditions and in non-neoplastic inflammatory conditions.

Caution: Even highly trained technicians and clinical pathologists may sometimes have difficulty differentiating between neoplastic blast cells and reactive lymphocytes. When abnormal cells are identified on a smear, it is best to send the sample for review to a veterinary diagnostic laboratory.

- **Platelet estimate:** The platelet estimate should always be done in the monolayer of the smear and the following calculation is used:
  - Estimated platelets per uL (100X objective) = average number of platelets in 10 fields x 15,000
  - General guideline for dogs and cats is approximately 200,000 – 500,000/ul (or about 13 – 33 per 100x field)

6. **Interpretation of key features of the blood smear**

   **Learning Objective 1:** Is the anemia regenerative?
   The degree of polychromasia is the best indicator of the regenerative response when looking at the blood smear alone. The finding of polychromasia in an anemic animal suggests that the
anemia may be regenerative, though the severity and duration of the anemia needs to be considered in your interpretation (e.g. a severe anemia with mild polychromasia is likely not regenerative and very acute anemia may not have had sufficient time for regeneration). Once an anemia is determined to be regenerative it indicates hemorrhage or hemolysis as the underlying mechanism. Identification of other morphologic changes on the blood smear may aid to further identify the cause of the anemia, such as spherocytes in immune-mediated hemolytic anemia or hypochromasia in chronic gastrointestinal bleeding. A non-regenerative anemia on the other hand suggests bone marrow dysfunction either from primary bone marrow injury/disease or suppression of marrow erythropoiesis by inflammation or kidney disease.

Learning Objective 2: Is there evidence of inflammation?
The typical inflammatory leukogram consists of a neutrophilia with left shift and toxic change and a monocytosis; however, if the inflammation is mild or ongoing a left shift and toxic change may not be evident. In these situations it can be difficult to differentiate from a stress response which can also result in a neutrophilia (without a left shift or toxic change). Severe overwhelming infection or inflammation can result in a neutropenia accompanied by a left shift (band neutrophils may outnumber segmented neutrophils) and toxic change. A neutropenia without a left shift and toxic change (especially if accompanied by non-regenerative anemia and thrombocytopenia) should be viewed as a flag for possible bone marrow dysfunction.

WBC estimates and differentials can appear similar with mild inflammation and a stress leukogram. The presence of a left shift or toxic change supports inflammation, as would either a very low or a very high estimated neutrophil number.

Learning Objective 3: Are there any abnormal cells on the smear?
Low numbers of abnormal cells may not be detected and usually requires extensive training to identify. The presence of numerous abnormal cells on the smear can usually be identified by less experienced personnel and although the lineage and origin of these cells may not be readily apparent, recognition of the need for review of the hemogram by a clinical pathologist can expedite patient diagnosis and treatment.

Learning Objective 4: Is there evidence of severe thrombocytopenia? Is the patient at a high risk for bleeding?
There is inherent inaccuracy to platelet estimates from a blood smear, but there is generally better agreement between platelet estimates and automated platelet counts when values are very low.³ Very low platelet counts puts patients at risk for bleeding as seen in the following guidelines:
- Risk of spontaneous bleeding: <30,000/uL (i.e. <2/100x field)
- Risk of increased surgical/traumatic bleeding: <50,000/uL (i.e. <3-4/100x field)

The above are only guidelines. Bleeding risk depends on many factors, including platelet number, platelet function, and coagulation factor concentrations. Animals with platelet counts over 30,000 may bleed spontaneously if they have defects in platelet function or clotting factors, and some animals with lower platelet counts may not show evidence of bleeding.

References:


Acknowledgment: Many thanks for help from Dr. Heather Priest, DVM, DACVP (Veterinary Clinical Pathology), Cornell University, Dept of Population Medicine and Diagnostic Science