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CASES FROM A PRACTITIONERS PERSPECTIVE

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The complete blood count (CBC), urinalysis, and serum chemistry profile are the cornerstones of clinical laboratory assessment. Particularly for the sick patient, it is important to perform all the tests concurrently on presentation. Interpretation of one test or one group of tests without the others is prone to errors.

INDICATIONS FOR HEMATOLOGY

Hematology is a critical component to laboratory evaluation of every sick patient.

Complete blood counts should be included in evaluations of every patient with vague signs of disease, every pre-anesthetic evaluation, every wellness and geriatric profile and as a recheck test for patients previously diagnosed with erythrocyte, leukocyte or thrombocyte abnormalities.

TIMING AND ARTIFACTS

Artifacts must be avoided for proper hematological interpretation. Poor blood collection techniques, inadequate sample volumes, prolonged sample storage, and delayed sample analysis provides opportunities for artifacts to occur. Hematological samples must be analyzed as soon as possible to prevent artifacts created by exposure to anticoagulants and cell deterioration due to storage and shipment. Samples should be analyzed within 3 hours or be refrigerated at 4°C to avoid artificially increased hematocrit (HCT), increased mean cell volume (MCV), and decreased mean cell hemoglobin concentration (MCHC).1 Blood films should be prepared within 1 hour of collection to avoid morphologic artifacts. Erythrocyte crenation, neutrophil hypersegmentation, lymphocytic nuclear distortion and general leukocyte degeneration may occur in aged samples. In addition, monocyte vacuolization, monocyte pseudopod formation and platelet agglutination can be encountered in stored samples.2 Formalin and formalin containing containers must be kept away from all blood and cytology smears to prevent staining artifacts.

METHODOLOGY

Several technologies have been applied to hemogram evaluation. Traditional in-clinic hematological evaluation includes manual counting, electronic resistance (impedance) and quantitative buffy coat analysis. Reference laboratories utilize a more accurate and expensive technology called laser flow cytometry. Recently a new combination technology, which utilizes impedance and laser flow cytometry, has become available in clinic.

HEMOGRAM INTERPRETATION

The CBC comprises the, red blood cell data, white blood cell data platelet data, and total protein concentration. In general, I evaluate red blood cell and protein data first, white blood cells next and the platelet data last.

EVALUATING THE RED BLOOD CELLS

Red blood cell data include the hematocrit (HCT), red blood cell count, hemoglobin (HGB) concentration, mean cell volume (MCV), and mean cell hemoglobin concentration (MCHC). As stated earlier, include total protein concentration as a part of the red blood cell evaluation. The red blood cell data are used to address the following central issue: Is the red blood cell mass normal, reduced, or increased?

Red blood cell mass is evaluated by assessing HCT, HGB, and red blood cell count. For the in-clinic laboratory, one reason for determining all three is to achieve some measure of internal quality control. Any deviation from the reference range should occur in all three tests to the same degree. If results of the three tests do not correlate, the possibility of laboratory error should be considered.

CLASSIFYING POLYCYTHEMIA

If red blood cell mass is increased, then the animal is polycythemic. This then raises the question: Is the polycythemia relative or absolute? Relative polycythemia, the most common polycythemia recognized in veterinary medicine, is the result of hemoconcentration. It is generally established based on the clinical history and signs consistent with dehydration, as well as an elevated total protein concentration. Polycythemia in the absence of these findings is absolute. Absolute polycythemia can be either primary or secondary.

CLASSIFYING ANEMIAS

If red blood cell mass is reduced, then the animal is anemic. The degree of anemia should be further considered in conjunction with plasma protein concentrations. If protein concentrations are elevated, then the animal may be dehydrated, and the anemia may be more severe than red blood cell mass measures indicate.

Once anemia is recognized, the next issue of concern is bone marrow responsiveness: Is the anemia regenerative or nonregenerative? If the red blood cell marrow responds with an increase in production of appropriate magnitude, the anemia is regenerative (responsive). On the other hand, the anemia is nonregenerative (nonresponsive) when there is not an effective increase in the production of red blood cells.

Evidence of red blood cell regeneration can be evaluated on a peripheral blood film. In dogs and cats, the principal feature of regeneration on a smear prepared with Wright’s stain is polychromasia. Polychromatophils are immature red blood cells that stain bluish because they contain RNA. Because polychromatophils are larger than mature red blood cells, another feature of regeneration is anisocytosis (variation in red blood cell size). Nucleated red blood cells may also be present in small numbers, but they should always be much scarcer than polychromatophils. A large number of nucleated red blood cells in the absence of polychromasia usually indicates bone marrow stromal damage; this finding is called an inappropriate nucleated red blood cell response. We discuss it in more detail when we describe the anemia of lead poisoning.

Polychromatophils on blood smears prepared with Wright’s stain correspond to reticulocytes on smears prepared with new methylene blue stain. If the issue of regeneration is still in doubt after stained blood films are evaluated, a reticulocyte count can be done. As a guideline, a reticulocyte count above 60,000 (cats) to 80,000/μl (dogs) indicates a regenerative anemia.3 In many cases, dogs with regenerative anemias have reticulocyte counts that are much higher. Cats show a somewhat less overall regenerative capacity than do dogs. When performing a reticulocyte count for a cat, it is important to count only cells containing diffuse accumulations of
Regenerative anemias can be further classified as either blood loss or hemolytic anemias. Remember that it might take one to three days for the packed cell volume to decrease from blood loss. A patient history of trauma or parasitic infection may suggest blood loss. Furthermore, the total protein concentration may be decreased in such cases. Another useful differentiating factor is that hemolytic anemias are generally much more regenerative than are blood loss anemias. Nonregenerative anemias with ineffective erythropoiesis can result from nuclear or cytoplasmic maturation defects in red blood cell precursors (cytonuclear dissociation). Ineffective erythropoiesis implies that even though the red blood cell marrow is active, increased numbers of normal red blood cells are not being released into circulation. The peripheral blood evaluation suggests the diagnosis, and the combination of peripheral blood findings and bone marrow findings confirms the diagnosis.

EVALUATING THE WHITE BLOOD CELLS

Leukogram data include total and differential white blood cell counts and a description of white blood cell morphology from the peripheral blood film. Differential cell counts should always be expressed and interpreted in absolute numbers, not percentages. White blood cell data are used to answer the following questions: 1) is there evidence of inflammation? 2) Is there evidence of a glucocorticoid (stress) or epinephrine (excitement) response? 3) Is there a demand for phagocytosis or evidence of tissue necrosis? 4) If inflammation is present, can it be further classified as acute, chronic, or overwhelming? And 5) Is there evidence of systemic toxemia?

IS THERE EVIDENCE OF INFLAMMATION?

Neutrophilic left shifts, persistent eosinophilia, and monocytosis are the best indicators of inflammation. Left shifts (increased numbers of immature [band] neutrophils in circulation) indicate increased turnover and tissue use of neutrophils. Persistent peripheral eosinophilia indicates a systemic allergic or hypersensitivity reaction. Monocytosis is seen in peripheral blood when there is a demand for phagocytosis. Blood smears are currently the only way to accurately evaluate band neutrophils; consequently blood smears are the cornerstone of hematology whether performed in-clinic or at an outside laboratory.

IS THERE EVIDENCE OF A GLUCOCORTICOID (STRESS)?

Absolute neutrophil, lymphocyte, eosinophil and monocyte counts are essential for proper hemogram evaluation. High levels of circulating glucocorticoids cause a mild mature neutrophilia, lymphopenia and eosinopenia, and mild monocytosis. Of these changes, lymphopenia is the most consistent and reliable indicator of stress. In dogs and cats, we regard lymphocyte counts of 1,000 to 1,500/µl as marginal lymphopenia, whereas lymphocyte counts below 1,000/µl are absolute lymphopenias. When lymphocyte counts drop below 600/µl, other causes of lymphopenia such as chylos us effusions, lymphangiectasia, and malignant lymphoma should also be considered.

IS THERE A DEMAND FOR PHAGOCYTOSIS OR EVIDENCE OF TISSUE NECROSIS?

Monocytosis indicates a demand for phagocytosis or tissue necrosis. Monocytosis is almost always seen in chronic inflammatory conditions, but can also occur with acute inflammation. Whenever a severe monocytosis is observed (>4,000/µl), a buffy coat smear should be prepared in attempt to identify any particles or causative agents that have been phagocytosed by circulating monocytes (e.g. opsonized red blood cells in immune hemolytic anemia, *Ehrlichia canis, Histoplasma capsulatum*).

IS THERE EVIDENCE OF SYSTEMIC TOXEMIA?

Circulating toxins (i.e. systemic toxemia) can arrest development of neutrophil precursors in the bone marrow. Either cytoplasmic or nuclear development can be affected. The abnormal neutrophils produced in this way are recognized on the peripheral blood film as toxic neutrophils. Cytoplasmic features of toxicity include foamy basophilia and the presence of small basophilic precipitates known as Döhle bodies. Döhle bodies are a sign of mild toxicity in cats (often seen in low numbers in normal cats), but indicate serious toxicity in dogs. Nuclear changes of toxicity include bizarre nuclear shapes and cellular giantism. Systemic toxemia is usually associated with bacterial endotoxins. Infectious diseases commonly accompanied by severe toxicity include feline pyothorax, pyometra, and severe canine prostatitis. Toxemia can also be associated with noninfectious causes such as tissue necrosis, heavy metal toxicosis, or cytotoxic drug therapy.

EVALUATING THE PLATELETS

An assessment of platelets is an important part of every CBC. As with red blood cells, the principal issue is whether platelet numbers are normal, increased (thrombocytosis), or reduced (thrombocytopenia). Thrombocytosis is rarely seen. When the number of platelets in dogs or cats approaches one million/µl, platelet leukemia (primary thrombocythemia, megakaryocytic myelosis) should be considered. More commonly, thrombocytosis is less marked, and reactive responses are probably more likely. For example, stimulation of red blood cell production in any regenerative anemia also stimulates platelet production. Thrombocytopenia is more common than thrombocytosis and can be of great clinical significance. Platelet counts below 50,000/µl can lead to overt bleeding. Platelet clumping can give a falsely low platelet count, particularly in the cat. Platelet clumping frequently interferes with results from impedance counters because aggregated platelets may be included in the RBC count. Thrombocytopenia can result from:

- Decreased platelet production (e.g. with estrogen toxicosis or infection by FeLV or feline immunodeficiency virus)
- Increased peripheral use of platelets (e.g. in DIC, acute ehrlichiosis, or Rocky Mountain spotted fever)
- Peripheral destruction of platelets (e.g. in immune-mediated thrombocytopenia)
- Sequestration of platelets in the spleen (in hypersplenism). Hypersplenism is rare, poorly documented in animals, and generally can be ruled out in the absence of splenomegaly.

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REFERENCES