EVALUATING BLOOD FILMS
Take 3 minutes to bring abnormalities to light
Many veterinarians and technicians do not routinely evaluate blood films microscopically, largely because they lack confidence in either preparing a well-made blood film or in being able to accurately identify important abnormalities. But blood films should be evaluated whenever a complete blood count (CBC) is requested, regardless of whether the CBC is done in the clinic or at a reference laboratory. Blood film evaluation is as essential to a CBC as a microscopic examination of urine sediment is to a complete urinalysis or two views are to proper radiographic interpretation. No single hematology procedure produces more valuable information yet requires so little additional time (we recommend three minutes) and expense.

Even the most expensive hematology analyzers are not designed to eliminate peripheral blood film evaluation. Morphologic features that instruments cannot identify include left shifts (increased immature neutrophils), neutrophil toxicity, lymphocyte reactivity, white blood cell (WBC) malignancy, red blood cell (RBC) poikilocytosis, RBC inclusions, and platelet abnormalities. A quick blood film review will help validate certain numerical data including platelet counts, WBC counts, WBC differentials, and RBC density, since even reference laboratory analyzers prove inaccurate with some of the more abnormal samples.

In this symposium, we suggest using a systematic method for evaluating blood films. This method—which requires veterinarians and technicians to answer basic questions about RBC, WBC, and platelet numbers and morphology—maximizes hematologic information. And we cover the most common and important morphologic findings in the peripheral blood.

We hope this symposium will provide veterinary practitioners and technicians with the skills to properly prepare, stain, and evaluate a peripheral blood film and will encourage them to use this vital hematology tool in their practices.

—Dr. Fred L. Metzger Jr.
Three-minute peripheral blood film evaluation: Preparing the film

A peripheral blood film evaluation should be part of all complete blood counts (CBCs), regardless of whether hematology is performed in-house or at a reference laboratory. Proper sample collection, slide preparation, and staining are essential to accurately evaluate a blood film, as is the correct use of a high-quality microscope. This article describes the steps in preparing a blood film and the equipment you’ll need.

Components of and indications for a CBC

Important components of a CBC include evaluating the erythron (hematocrit, total red blood cell [RBC] count, hemoglobin concentration, absolute reticulocyte count, and RBC indices), the leukon (total white blood cell [WBC] count, five-part differential count including immature neutrophils), the thrombon (platelet count and platelet indices), and the total protein concentration. As mentioned earlier, always include a peripheral blood film evaluation in the CBC.

A CBC should be included in evaluations of every sick patient, every patient with vague signs of disease, and every patient receiving long-term medications. In addition, a CBC should be performed as part of every preanesthetic workup, for adult wellness and geriatric profiles, and as a recheck test for patients in which RBC, WBC, or platelet abnormalities were previously diagnosed.

Collecting a sample for the blood film

Artifacts must be avoided for proper hematologic interpretation. Causes of artifacts include poor blood collection techniques, inadequate sample volumes, prolonged sample storage, and delayed sample analysis.

Proper blood collection is vital to prevent erroneous results from sample clotting and cellular lysis. Obtain hematology samples from the largest blood vessel possible to minimize cellular trauma and to prevent the activation of clotting mechanisms. For accurate results, discard clotted samples and collect fresh samples. Common venipuncture sites in dogs and cats include the jugular, cephalic, and lateral and medial saphenous veins. Anticoagulants include EDTA, heparin, and citrate. EDTA is the preferred anticoagulant for blood film preparation because it preserves cellular detail better than other anticoagulants do and does not interfere with Romanowsky staining of WBCs.

Inadequate sample volume is a common cause of inaccurate hematologic results. Properly fill anticoagulated blood collection tubes to avoid falsely decreased hematocrits and cell counts and to prevent RBC shrinkage.

Hematologic samples must be analyzed as soon as possible to prevent artifacts created by exposure to anticoagulants and cell deterioration due to storage and shipment. Analyze samples within three hours or refrigerate them at 39.2°F (4°C) to avoid an artificially increased hematocrit, increased mean corpuscular volume, and decreased mean corpuscular hemoglobin concentration. Prepare blood films within one hour of collection to avoid morphologic artifacts. RBC crenation, neutrophil hypersegmentation, lymphocytic nuclear distortion, and general WBC degeneration including vacuolization in neutrophils may occur in aged samples. In addition, monocyte vacuolization, monocyte cytoplasmic pseudopod formation, and platelet agglutination can be encountered in stored samples. If you use a reference laboratory for primary hematologic analyses, we recommend submitting freshly prepared blood films along with the anticoagulated blood.

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Producing high-quality blood films begins by using clean, new slides. Used slides frequently have imperfections such as scratches and other physical defects. Slides must be free of fingerprints, dust, alcohol, detergents, and debris. Using a microhematocrit tube, place a small drop (2 to 3 mm in diameter) of well-mixed EDTA blood about 1 to 1.5 cm from the end of the slide. Next, draw a spreader slide back into the blood drop at about a 30-degree angle (middle) until the spreader slide edge contacts the sample drop and capillary action disperses the sample along the edge. Then, using a smooth steady motion, push the spreader slide away from the blood drop, creating a uniform film across the slide (bottom). Changing the angle of the spreader slide will change the length and thickness of the blood film. For blood samples with low hematocrits (severe anemia), you may need to decrease the angle of the spreader slide to make a good-quality slide. In contrast, for samples with high hematocrits (severe dehydration and polycythemia due to a variety of conditions), you may need to increase the angle of the spreader slide.

Drying is an important step in the production of good-quality blood films. Allow the blood film to thoroughly air-dry before applying stain, or use a heat block (at the low setting) or a hair dryer to hasten the drying time, thus minimizing refractile markings that can distort erythrocyte morphology. In addition, keep formalin and formalin-containing containers away from all blood and cytology smears to prevent staining artifacts such as increased cytoplasmic granularity and basophilia.

Most veterinary practices use a modified Wright’s stain (Romanowsky stain) for both hematology and cytology. Modified Wright’s stains are available as either three- or two-solution kits. We prefer kits with three separate solutions: an alcohol fixative, an eosinophilic staining solution, and a dark-blue staining solution. Veterinary practices should have two separate Coplin stain jar sets—one for hematology and cytology samples and one for contaminated samples such as those collected for ear and fecal cytology. For optimal results, replace staining solutions regularly.

Recommended blood staining protocol includes dipping the air-dried slide five to 10 times in each solution while blotting one edge briefly in between each solution. Rinse the slide with distilled water after the final staining step, and allow the blood film to air-dry before examination. Good staining technique is critical to identifying polychromatophils on a peripheral blood film. If the staining is done improperly, many of the quick Romanowsky-type stains will not give the needed tintorial differences between mature RBCs (orange-red) and polychromatophils (bluish-pink). Typically, poor staining with the various quick stains results in all RBCs having a bluish or muddy tint.

**The microscope**

A high-quality microscope is essential for hematology. We recommend a binocular microscope with a minimum of 10×, 20×, and 100× (oil-immersion) objectives and wide-field 10× oculars. When evaluating the slide, make sure maximum light reaches the blood film. This means positioning the substage condenser as close as possible to the stage with the iris wide open. The light source should be equipped with a variable rheostat to allow maximal control of light intensity. To ensure optimal focused light for the microscopic evaluation, the microscope should be in Köhler il-

**Good staining technique is critical to identifying polychromatophils on a peripheral blood film.**

![Figure 1](image1.png)

1. The steps in preparing a blood film. A small drop of blood is placed near the end of a slide (top). A spreader slide is drawn back into the blood drop at a 30-degree angle (middle). Then the spreader slide is pushed away from the blood drop, creating a uniform film across the slide (bottom).

![Figure 2](image2.png)

2. The components of the peripheral blood film slide.

Evaluating the blood film

- **Good staining technique is critical to identifying polychromatophils on a peripheral blood film.**

- **The microscope**

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**Staining the blood film**

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lumination. Contact your microscope vendor, or review the microscope manual to ensure proper illumination.

The three zones

A good-quality blood film has three zones: the body (near the point of blood application), the monolayer (the zone between the body and feathered edge), and the feathered edge (the area most distant from the point of application) (Figure 2). The definition of the monolayer varies from institution to institution, but the definition used to ensure consistent semiquantification of various morphologic abnormalities is the area where about half of the RBCs are touching one another without overlapping. The monolayer is the area of the blood film where WBCs and platelet numbers are estimated and cell morphology is examined. Although the monolayer is the only zone where morphologic evaluation of individual cells is performed at oil-immersion magnification, systematic blood film evaluation includes assessing of all three zones.

First, examine the blood film at low magnification (10× or 20×), and scan the entire slide to evaluate overall film thickness, cell distribution, and differentiation of the three different zones. Evaluate the feathered edge (Figure 3) for microfilariae, phagocytized organisms, atypical cells, and platelet clumping. Next, examine the body of the blood film (Figure 4) for rouleau formation or RBC agglutination. Then, still using low magnification, evaluate the monolayer (Figure 5), estimate the total WBC count (see the third article in this symposium), and predict the expected WBC differential count. Finally, use the oil-immersion objective (100×) to examine RBC, WBC, and platelet morphology in the monolayer.

Evaluate RBCs for evidence of anisocytosis, poikilocytosis, polychromasia, hemoglobin concentration, and RBC parasites. Important RBC morphologic abnormalities include spherocytes, schistocytes, acanthocytes, and leptoerytes (see the second article in this symposium).

Evaluate neutrophils for toxicity and the presence or absence of a left shift (increased numbers of band neutrophils). Evaluate lymphocytes for reactivity and monocytes for phagocytized organisms (see the third article in this symposium).

With relatively little practice, you can identify most important morphologic abnormalities in the RBCs, WBCs, and platelets with the 20× objective field of view; these can be quickly validated with the 100× oil-immersion view.

Summary

Examining a properly prepared peripheral blood film offers invaluable information about cellular morphologic changes not provided by automated instruments and provides a quality assurance confirmation of CBC data generated by in-clinic or reference laboratory hematology analyzers. We recommend taking about three minutes to view the blood film and, using the questions discussed in the next two articles of this symposium, to systematically evaluate the RBC, platelet, and WBC components of the peripheral blood film.

ACKNOWLEDGMENT

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REFERENCES


FIGURE 3

FIGURE 4

FIGURE 5

3. Look for platelet clumps, microfilariae, and large cells in the feathered edge of the film (purple section).
4. Look for rouleau formation and RBC agglutination in the body of the film (purple section).
5. Estimate platelet and WBC counts and examine cell morphology in the monolayer zone (purple section).
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Three-minute peripheral blood film evaluation: The erythron and thrombon

When examining the red blood cells and platelets in a blood film, ask yourself these questions to quickly identify and further characterize conditions such as anemia and thrombocytopenia.

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Evaluating a peripheral blood film validates cell counts performed by hematology analyzers, plus it offers valuable diagnostic information relayed by erythrocytes, platelets, and leukocytes. The first article in this symposium described how to prepare a peripheral blood film. In this article, we discuss important red blood cell (RBC) and platelet number and morphologic changes. And in the next article, we discuss white blood cell (WBC) alterations.

If a patient’s RBC mass is reduced, then the patient is anemic. Once anemia is recognized, the next concern is bone marrow responsiveness: Is the anemia regenerative or nonregenerative? If the RBC bone marrow precursor cells respond with increased production of appropriate magnitude, the anemia is regenerative (responsive). If RBC production is not effectively increased, the anemia is nonregenerative (nonresponsive). Keep in mind that a lag of 48 to 72 hours exists before bone marrow responsiveness is perceived in the peripheral blood, so be careful in defining nonresponsiveness.

Evaluating the thrombon by first assessing platelet numbers is another important part of every complete blood count (CBC). Thrombocytopenia is more common than thrombocytosis and can be of great clinical importance. Platelet counts below 40,000/µl can lead to spontaneous bleeding. Platelet clumping, which can be seen in any species but is particularly problematic in cats, can give a falsely low platelet count. Platelet clumping frequently interferes with results from impedance cell counters because aggregated platelets may be included in the RBC or WBC counts, resulting in artificially decreased platelet counts.1

Our approach to peripheral blood film evaluation uses a question-based format. This systematic approach allows veterinary practitioners and technicians to maximize important visual information.

### Evaluating the RBCs

**Is there evidence of RBC regeneration? (Is polychromasia or reticulocytosis present?)**

In dogs and cats, polychromasia is the principal feature of RBC regeneration on a blood film prepared with Wright’s or modified Wright’s stain. Polychromatophils are immature RBCs that stain bluish because they contain RNA (Figure 1). Polychromatophils on blood smears prepared with Wright’s or modified Wright’s stain roughly correspond to reticulocytes on smears prepared with new methylene blue stain. If regeneration is still questionable after you’ve evaluated Wright’s-stained blood films, perform a reticulocyte count with new methylene blue stain.

When performing reticulocyte counts in cats, it is important to count aggregate reticulocytes (cells containing diffuse accumulations of reticulum) since they represent recent RBC production.

**Figure 1**

1. Polychromatophils (arrows) in a dog (modified Wright’s stain; 100X).
in response to relatively severe anemia (Figure 2). In cats, aggregate reticulocytes mature into punctuate reticulocytes within 12 hours, and punctuate reticulocytes mature into RBCs in about 10 days. Consequently, elevated punctuate reticulocytes represent RBC regeneration two weeks earlier, whereas aggregate reticulocytes indicate recent regeneration.2 In dogs and cats, peak reticulocyte counts occur four to eight days after the onset of anemia.

As a general guideline, a reticulocyte count above 60,000/µl in cats (aggregate only) and 80,000/µl in dogs indicates a regenerative anemia.3 Dogs with regenerative anemias have reticulocyte counts that are frequently 100,000 to 300,000/µl (dogs with extremely regenerative anemia can have counts of 500,000/µl or greater), while cats typically demonstrate a less dramatic response when only the aggregate reticulocyte counts are evaluated.

Reference laboratories can provide reticulocyte counts, and in some cases, they will automatically add the reticulocyte count to the CBC at an additional charge if anemia is identified. The newer in-clinic, laser-based hematology analyzers automatically provide absolute reticulocyte counts with each CBC.4 Regenerative anemias can be further classified as either blood loss or hemolytic (decreased RBC lifespan) anemias. Whenever you suspect hemolysis, closely examine RBC morphology to detect spherocytes, acanthocytes, schistocytes, RBC inclusions, and parasites (see below). Figure 3 is a flow chart for classifying anemias in dogs and cats.

Are nucleated RBCs present?

Nucleated RBCs, or metarubricytes (Figure 4), are not found in any appreciable numbers in mammalian peripheral blood samples. Most clinicians consider fewer than 4 nucleated RBCs/100 WBCs to be insignificant when the WBC count is within the reference range. Nucleated RBCs may be seen in increased numbers with strongly regenerative anemias, but they should always be less numerous than the polychromatophilic reticulocytes. This finding has been identified as an appropriate nucleated RBC response by some clinicians.

An inappropriate nucleated RBC response occurs when more than 5 nucleated RBCs/100 WBCs are present in the absence of polychromasia. Conditions associated with an inappropriate nucleated RBC response include bone marrow stromal damage, extramedullary hematopoiesis, fractures, hyperadrenocorticism, feline leukemia virus infection, chemotherapeutic drug administration, and lead toxicosis.

Blood loss anemia is a possible cause. Look for external or internal hemorrhage or parasites (e.g. fleas, hookworms). Keep in mind that it takes one to three days to see the hematocrit decrease.

Hemolytic anemia is a possible cause. Consider immune-mediated hemolytic anemia with spherocytosis or Heinz body hemolytic anemia (e.g. onion ingestion, acetaminophen administration).

If bone marrow hypoplasia is present, consider anemia of inflammation, anemia of chronic renal disease, myelophthisis due to bone marrow disease, or chemotherapy toxicosis.

If bone marrow hyperplasia with ineffective erythropoiesis is present, consider a nuclear maturation defect (FeLV infection, drug toxicosis) or a cytoplasmic maturation defect (lead toxicosis, iron deficiency including chronic blood loss).

The anemia is regenerative if the reticulocyte count is more than 80,000/µl in dogs and more than 60,000/µl in cats.

The anemia is nonregenerative if the reticulocyte count is less than 80,000/µl in dogs and less than 60,000/µl in cats. In these cases, perform a serum chemistry profile, urinalysis, and other needed diagnostic tests to rule out underlying diseases. If no such disease is found, perform a bone marrow evaluation.
cosis, among others. Splenic dysfunction (e.g., decreased clearance of circulating nucleated RBCs) is an important cause of an inappropriate release of nucleated RBCs and may occur in patients in which the spleen has been removed and in patients with splenic neoplasms, especially hemangiosarcoma.

**Is autoagglutination present?**

Agglutination is unorganized three-dimensional clumping of RBCs that must be differentiated from rouleau formation. It is common in dogs with immune-mediated hemolytic anemia. When confirmed, autoagglutination suggests an immune-mediated process such as autoimmune-mediated hemolytic anemia or drug-induced hemolytic anemia (e.g., cephalosporins, penicillin) because RBC surface antibodies cause cell cross-linking and the resulting agglutination.

Rouleau formation is a common finding when fibrinogenesis is increased and must be differentiated from agglutination. On routine Wright’s-stained or modified-Wright’s-stained blood films, marked rouleau formation and agglutination may be difficult to differentiate.

Whenever you suspect agglutination on a blood film, mix a drop of the well-mixed EDTA anticoagulated blood on a new glass slide with two or more drops of isotonic saline solution. Add a cover slip, and view the mixture as an unstained wet preparation. Under these conditions, most rouleaux formations dissipate but autoagglutination typically persists and is recognized as clumped RBCs (Figure 5).

**Are poikilocytes present?**

Normal canine RBCs are shaped like biconcave disks with prominent central pallor. Feline RBCs have much less apparent central pallor. Abnormally shaped RBCs are called poikilocytes and include artifactual changes (crenation) as well as true abnormalities (e.g., spherocytes, acanthocytes, schistocytes, leptoocytes).

Crenation (Figure 6) can be confused with important RBC changes such as acanthocytosis. Crenation is a shrinking artifact most commonly seen when less than optimal amounts of blood are collected into the EDTA tube and the peripheral blood film is not made relatively quickly after the anticoagulation process. Some less common causes of crenation include electrolyte disturbances, uremia, and rattlesnake envenomation. A useful differentiating feature for identifying crenation is that crenation typically affects large numbers of cells in a particular area of the slide, whereas true poikilocytosis typically affects relatively lower numbers of cells throughout the peripheral blood film. Crenation can be minimized by preparing blood films immediately after blood samples are collected and properly anticoagulated.

Spherocytes are spherical RBCs that have lost their normal biconcave shape, resulting in more intense staining than normal RBCs. They have no central zone of pallor, and they appear smaller than normal RBCs (Figure 7). More than four to six spherocytes per 100× field is considered elevated. Spherocytes are commonly seen with many of the immune-mediated hemolytic anemias we encounter in veterinary medicine. Be careful when attempting to identify spherocytes in feline RBCs because feline RBCs are much less biconcave than canine RBCs and, therefore, have much less central pallor.

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**FIGURE 5**

A saline wet preparation of canine blood showing autoagglutination (20×).

**FIGURE 6**

Crenation in a dog (modified Wright’s stain; 100×).

**FIGURE 7**

Spherocytes (black arrows) and a polychromatophil (red arrow) in a dog (modified Wright’s stain; 100×).

**FIGURE 8**

Acanthocytes (arrow) in a dog (modified Wright’s stain; 100×).

**FIGURE 9**

Schistocytes (arrows) in a dog (modified Wright’s stain; 100×).
Acanthocytes are abnormally shaped RBCs having two to 10 blunt, fingerlike surface projections of varying sizes (Figure 8). These morphologic changes are related to abnormal accumulation of lipids within the RBC membrane when there is an abnormal plasma cholesterol:phospholipid ratio. Acanthocytes are seen occasionally in normal animals. Conditions most commonly associated with acanthocyte formation include underlying metabolic diseases or diseases affecting normal lipid metabolism. Nonneoplastic and neoplastic (hemangiosarcoma in particular) diseases involving the liver, spleen, and kidney may have associated acanthocytosis in dogs and, occasionally, in cats. Acanthocytosis is most frequently associated with liver disease and splenic hemangiosarcoma.

Schistocytes are RBC fragments formed by mechanical injury (Figure 9). Even in very low numbers (one schistocyte in every three to five 100X objective fields), schistocytes are clinically relevant. Finding even a few schistocytes may help you identify underlying or subclinical disseminated intravascular coagulation (DIC). Microvascular mechanical fragmentation of RBCs associated with diseases such as hemangiosarcoma and dirofilaria are may also result in schistocyte formation.

A leptocyte (codocyte, target cell) is an RBC with excess cell membrane that forms a shape often referred to as having a Mexican hat appearance (Figure 10). Leptocytes are seen occasionally in normal animals. Increased numbers of leptocytes (> 3/100X oil-immersion field) are expected with polychromasia because polychromatophils have excess membranes compared with normal RBCs. Consequently, leptocytosis is common when reticulocytosis is present, so most reference laboratories do not report this morphologic finding. However, laboratories will report leptocytes when they are seen in the absence of polychromasia since the excess lipid membrane compared with cytoplasmic volume is an abnormality. The two primary mechanisms causing this abnormal morphology are either an upset in the cholesterol:phospholipid ratio in plasma resulting in lipid loading (as might be seen with liver disease and other metabolic disorders) or a decrease in cytoplasmic content compared with normal (as might be seen with iron deficiency typically due to chronic blood loss). With iron deficiency, RBC hemoglobin content decreases, and in addition to the leptocytosis, hypochromasia is often observed.

Are RBC inclusions present?

Accurately characterizing various inclusions is an important aspect of blood film evaluation. Inclusions may include Heinz bodies, basophilic stippling, Howell-Jolly bodies, and certain infectious agents.

Heinz bodies are localized accumulations of denatured, oxidized, and precipitated hemoglobin in the RBC. They often affix to the inner RBC membrane and project from the RBC surface. Conditions associated with Heinz body hemolytic anemia include acetaminophen toxicosis in cats and acute onion and zinc toxicosis in dogs. Diabetes mellitus, hyperthyroidism, and lymphosarcoma in cats and the use of oral benzocaine sprays are also associated with increased numbers of Heinz bodies; but anemia is not present in these cases. The Heinz bodies observed in cats may be small or not project from the cell surface, making their identification difficult (Figure 11). New methylene blue staining will help confirm the presence of Heinz bodies; the Heinz bodies stain dark blue compared with the rest of the erythrocyte (Figure 12). In contrast to Heinz bodies in dogs, Heinz bodies can be an incidental finding in cats, and their relevance must be determined by evaluating clinical signs and other hemogram parameters.

Basophilic stippling is seen in Romanowsky-stained films as small, dark-blue punctate aggregates of residual ribosomes in RBCs (Figure 13). It has traditionally been associated with lead toxicity in dogs but may also be associated with highly regenerative anemias in any species. Further diagnostic investigation is important when basophilic stippling is seen in the absence of reticulocytosis.

Howell-Jolly bodies are small nuclear remnants that may be increased with acceler-

10. Leptocytes (arrow) in a dog (modified Wright’s stain; 100X).
11. Heinz bodies, which are noselike RBC projections, (arrows) in a cat (modified Wright’s stain; 100X).
12. A blood film from a cat showing Heinz bodies; they are easier to see with new methylene blue stain (100X).
13. Basophilic stippling (black arrow) and a polychromatophil (red arrow) in a dog (modified Wright’s stain; 100X).
ated RBC regeneration. In healthy animals, the low numbers of RBCs with Howell-Jolly bodies released from the bone marrow are quickly removed from circulation primarily through the action of fixed tissue macrophages in the spleen. If Howell-Jolly bodies are easily identified and no reticulocytosis is noted, investigate underlying splenic disease. This inclusion can be an incidental finding in cats because of the open splenic architecture and decreased erythrophagocytic properties inherent to the feline spleen.

Infectious agents including Babesia (Figure 14), Cyttauxzoon, and Mycoplasma (previously known as Haemobartonella) species may be identified in RBCs. Evaluation for Mycoplasma species (Figure 15) should be performed on freshly collected blood samples that have not been refrigerated to improve identification.

Evaluating the platelets

Are platelet numbers normal, decreased, or increased?

Microscopic validation of platelet counts is an important component of blood film evaluation. Platelet clumping (Figure 16) interferes with accurate enumeration of platelets with all hematology analyzers and with manual counting methods. Clumping occurs in many samples because of platelet activation during the collection process. Increased numbers of large platelets may also result in inaccurate platelet counts when impedance counting methods are used.

On a well-prepared peripheral blood film in which no marked platelet clumping at the feathered edge is present, the average number of platelets observed per 100× oil-immersion monolayer field multiplied by 20,000 provides a good estimate of the number of platelets/µl. As a general rule, you should see a minimum of eight to 10 platelets and a maximum of 35 to 40 platelets per 100× oil-immersion monolayer field of view.9

If the platelet numbers are decreased, can the mechanism be determined?

Thrombocytopenias occur through four basic mechanisms: sequestration, utilization (consumption), destruction, and decreased or ineffective production. While clues as to the underlying mechanism are found in the CBC, in most cases, bone marrow evaluation is needed for complete interpretation.

Sequestration thrombocytopenias are uncommon in veterinary medicine. They are usually the result of hypersplenism and, thus, are characterized by an enlarged spleen on physical or radiographic examination.

Utilization, or consumption, thrombocytopenias are caused by excessive activation of the coagulation cascade. These thrombocytopenias are associated with inflammatory disease and DIC. Utilization thrombocytopenias are usually moderate, with platelet numbers in the 75,000 to 150,000/µl range, but values below 40,000/µl accompanied by petechiae are possible.10 Bone marrow evaluation reveals normal to increased numbers of megakaryocytes.

Destruction thrombocytopenias are immune-mediated thrombocytopenias in which normal circulating platelets are destroyed by circulating antiplatelet antibodies. Destruction thrombocytopenias are often extreme, with peripheral platelet counts well below 50,000/µl. Bone marrow examination is characterized by normal to increased numbers of megakaryocytes.

Decreased or ineffective production is associated with extremely low platelet counts; counts well below 50,000/µl are common. Bone marrow evaluation will vary greatly in most of these cases, although they will share similar end results of deceased effective production by the bone marrow. With decreased production thrombocytopenias, no identifiable to extremely low numbers of megakaryocytes are present. With ineffective production, most bone marrow samples have normal to increased numbers of megakaryocytes, and the production of platelets is ineffective in many cases because of an immune-mediated destructive process directed at an early stage of platelet development or at the megakaryocyte population itself.
If platelet numbers are increased, is the thrombocytosis reactive or neoplastic?

Most thrombocytosis is reactive or neoplastic. Reactive thrombocytosis can occur secondary to exercise, hemorrhage, splenectomy, excitement, fractures, high circulating glucocorticoid concentrations, myelofibrosis, and iron deficiency anemia as well as 24 hours or more after blood loss. When the possible causes of reactive thrombocytosis have been ruled out, then the possibility of primary platelet leukemia must be considered. When extremely high platelet counts are seen (> 1,000,000/µl), thrombocytosis due to neoplasia must be strongly considered.

Are enlarged platelets present?

The presence of enlarged platelets (Figure 17), which correlates with increased mean platelet volume (MPV), is supportive of an increased rate of thrombopoiesis in the bone marrow in response to a peripheral demand. This interpretation can be used with most species; however, in cats, enlarged platelets is an equivocal finding.

Determining if an anemia is regenerative or nonregenerative is critical when developing a list of differential diagnoses. In addition, automated platelet counts should be verified by examining blood films because platelet clumping is common, especially in cats, which results in artifactually decreased platelet counts.

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REFERENCES


Conclusion

FIGURE 17

A blood film from a dog with immune-mediated hemolytic anemia. Note the enlarged platelets (black arrows). Also note the polyehromasia, spherocytosis, and Howell-Jolly body (red arrow) (modified Wright’s stain; 100×).
Three-minute peripheral blood film evaluation: The leukon

Taking a moment to briefly examine white blood cells will help you identify conditions such as inflammation or stress that may indicate serious disease.

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In the previous article, we discussed how to examine the erythron and thrombon components of peripheral blood films. In this article, we again use a question-based format to guide you in evaluating the leukon by assessing white blood cell (WBC) numbers and morphology. All five WBC cell types are assessed. Table 1 lists the general patterns of WBC response under a variety of circumstances.

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<th>Is the total WBC count elevated, normal, or decreased?</th>
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<td>Experience is required to accurately estimate cell counts directly from blood films. Subjective analysis of total WBC numbers may be performed by counting three to five 20× objective fields; 10 to 20 WBCs/20× field is considered normal in dogs and cats. Another method involves counting several 100× oil-immersion monolayer fields and multiplying the average number of WBCs/100× oil-immersion field by 2,000 to obtain a final estimated total WBC count.</td>
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<table>
<thead>
<tr>
<th>Is there a monocytosis?</th>
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<tbody>
<tr>
<td>The monocyte-macrophage continuum represents the second major branch of the circulating phagocyte system (neutrophils are the first). Monocytes (Figure 2), unlike granulocytes (neutrophils, eosinophils, basophils), are released into the peripheral blood as immature cells and then differentiate into phagocytic macrophages (Figure 3), epithelioid macrophages, or multinucleated giant cells. Monocytosis can be another indicator of inflammation. It may be seen in both acute and chronic conditions but may also be a component of stress leukograms. Conditions frequently associated with monocytosis include systemic fungal diseases (histoplasmosis, blastomycosis, cryptococcosis, coccidioidomycosis, aspergillosis),</td>
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<table>
<thead>
<tr>
<th>Is a left shift present?</th>
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<tbody>
<tr>
<td>A left shift may be the only indicator of active inflammation in veterinary patients because total WBC and neutrophil counts are frequently within the reference range. Left shifts are characterized by increased numbers of immature neutrophils (e.g. band cells, metamyelocytes) in circulation. A left shift is a hallmark of inflammation, so accurately identifying band cells is extremely valuable. No hematology analyzer (in-house or reference laboratory instrumentation) has been documented to accurately identify band neutrophils; consequently, they must be identified microscopically. On blood films, the nucleus of a band neutrophil typically has parallel sides (Figure 1), whereas the nucleus of a mature neutrophil is distinctly segmented. One useful approach to differentiate band neutrophils is to estimate the degree of nuclear indentation. First, identify the narrowest and widest portions of the nucleus. If the narrowest portion is less than one-third of the widest portion, the cell is classified as a band cell.</td>
</tr>
</tbody>
</table>
immune-mediated hemolytic anemia, bacterial endocarditis, certain neoplastic diseases (particularly when tissue necrosis is noted), and certain pyogranulomatous diseases (e.g. feline infectious peritonitis, toxoplasmosis, foreign body reactions). The best interpretation of monocytosis in the absence of a stress leukogram is tissue demand for macrophages.

Is a persistent eosinophilia present?

Persistent eosinophilia indicates a systemic hypersensitivity reaction and can be another indicator of inflammation. Conditions associated with persistent eosinophilia include heartworm disease in dogs and cats, feline asthma, canine atopic syndromes, feline eosinophilic granuloma complex (linear plaque form), hypereosinophilic syndrome, mast cell leukemia, and flea allergy dermatitis. Parasitic infections confined to the bowel, such as whipworm infections or ascarid and hookworm infections in adult nonpregnant dogs and cats, do not cause persistent peripheral eosinophilia because the parasites do not have a systemic phase.

Are reactive or atypical lymphocytes present?

The presence of reactive lymphocytes (Figure 4) simply suggests systemic antigenic stimulation. This stimulation could be in response to a recent vaccination, an infectious disease, or another process stimulating the immune system. The most commonly identified morphologic change associated with lymphocyte reactivity is increased amounts of basophilic cytoplasm.

Atypical lymphocytes (Figure 5) are abnormal lymphocytes characterized by increased size with indented or cleft nuclei and large azurophilic cytoplasmic granules. These cells are not disease-specific and may be present with infectious and neoplastic conditions. Atypical lymphocytes require additional investigation by a clinical pathologist. Bone marrow examination and detailed cytologic and possible histologic evaluation of peripheral and internal lymphoid tissues may also be recommended.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Total WBC Count</th>
<th>Segmented Neutrophil Count</th>
<th>Band Neutrophil Count</th>
<th>Lymphocyte Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overwhelming inflammation</td>
<td>Decreased</td>
<td>Decreased or no change</td>
<td>Increased or no change</td>
<td>Decreased</td>
</tr>
<tr>
<td>Acute inflammation</td>
<td>Increased or no change</td>
<td>Increased or no change</td>
<td>Increased or no change</td>
<td>Decreased or no change</td>
</tr>
<tr>
<td>Chronic inflammation</td>
<td>Increased or no change</td>
<td>Increased or no change</td>
<td>Increased or no change</td>
<td>Increased or no change</td>
</tr>
<tr>
<td>Excitement</td>
<td>Increased or no change</td>
<td>Increased or no change</td>
<td>No change</td>
<td>No change</td>
</tr>
<tr>
<td>Stress</td>
<td>Increased or no change</td>
<td>Increased or no change</td>
<td>No change</td>
<td>Decreased</td>
</tr>
</tbody>
</table>

TABLE 1 Common Leukocyte Patterns in Dogs and Cats

1. Canine band neutrophils (modified Wright’s stain; 100×).
2. A canine monocyte (modified Wright’s stain; 100×).
3. A buffy coat smear from a dog showing a macrophage (arrow) phagocytizing Histoplasma species (modified Wright’s stain; 100×).
4. A reactive lymphocyte in a dog (modified Wright’s stain; 100×).
Toxic neutrophils are a result of an accelerated rate of neutrophil production in response to inflammatory signals received by the bone marrow. Toxic neutrophil changes include retention of cytoplasmic features of immaturity and include foamy basophilic cytoplasm (Figure 6) and small basophilic precipitates known as Döhle bodies (Figure 7). Döhle bodies are often present in low numbers in normal cats, so, by themselves, they are a relatively equivocal finding; but Döhle bodies indicate serious toxicity in dogs.5 Other toxic changes include bizarre nuclear shapes and cellular giantism (Figure 8).

Systemic toxemia is often associated with bacterial endotoxins, but noninfectious causes also occur. Infectious diseases commonly accompanied by severe neutrophil toxicity include feline pyothorax, pyometra, and severe canine prostatitis. Noninfectious causes associated with toxemia include immune-mediated hemolytic anemia, acute pancreatitis, tissue necrosis, zinc and lead toxicity, and cytotoxic drug therapy.6

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Conclusion

Evaluating the five different WBC types is critical when you develop a differential diagnosis list. Increases or decreases in neutrophils, eosinophils, basophils, lymphocytes, and monocytes should alert you to investigate certain conditions. For example, neutrophilic left shifts, persistent eosinophilia, and monocytosis are indicators of inflammation: Left shifts (increased numbers of immature, or band, neutrophils in circulation) indicate increased turnover and tissue use of neutrophils. Persistent peripheral eosinophilia indicates a systemic allergic or hypersensitivity reaction. And monocytosis is seen in peripheral blood when a demand for phagocytosis is present.

REFERENCES