Why Perform the CBC, and How Can the Information Be Used to Manage Cases?

Kim A. Sprayberry, DVM, Dipl.DACVIM

This article revisits the components of the single most commonly run lab test and reviews the wealth of information about an equine patient's health that is contained within the basic complete blood count. Author's address: c/o Haygard, Davidson, McGee, & Associates, 4250 Iron Works Pike, Lexington, KY 40511. © 1999 AAEP.

1. Introduction

Next to performing an expert physical examination, a blood sample submitted for a complete blood count is the most basic tool available to veterinary practitioners. The CBC is commonly performed by equine veterinarians as the foundation of diagnostic evaluation, for serial monitoring of a patient’s response to therapy, for presurgical screening, as an adjunct to insurance or prepurchase examinations, and as part of a routine well-horse care program. Many of us fall into a pattern of taking into account only the hematocrit, total protein, and total leukocyte count, thus depriving ourselves of much important and relevant information pertaining to our understanding of the state of health or disease present in the patient that stands before us. The purpose of this article is to revisit the components of a CBC, review what the information means, and discuss how it can augment the examiner’s evaluation of the equine patient.

Because blood is a fluid tissue that circulates through the body and comes into intimate contact with every capillary bed and cell population that the animal possesses, it is not surprising that evaluation of the various components of blood can yield much valuable information regarding an animal’s health status. In addition to the functions of oxygen delivery and waste removal, blood also contains necessary buffer systems, provides nutrients, maintains intravascular pressure and circulation, and carries cells and other elements of the immune system. Physicochemically, blood is a suspension. The fluid phase of whole blood is plasma. Cellular elements such as erythrocytes, leukocytes, and platelets are suspended within the plasma. Also contained within plasma are electrolytes, enzymes, vitamins, gases, pigments, and hormones. Serum is the fluid that remains when fibrinogen and fibrin have been removed from plasma. The dynamics of blood flow are similar, but not identical to, fluid dynamics, a fact that has relevance to the white cell count and the phenomenon of leukocyte margination. The CBC is a tool for consideration of two primary cell systems, the erythron (circulating erythrocytes and their bone marrow precursors) and the leukogram. Platelet numbers are also included in the CBC. Erythrocyte parameters provide information about anemia and, indirectly, trace mineral sufficiency (Cu, Fe), while assessment of the leukogram may inform the examiner of abnormal conditions such as inflam-
mation, neoplasia, allergy, parasitism, immune responses, endotoxemia, and ehrlichiosis.¹

A. Methods of Assessing the Erythron and Leukogram

- Erythron parameters: RBC, PCV, Hb, MCV, RDW, MCHC, MCH
- Leukocyte parameters: WBC, percentage and absolute counts of neutrophils, lymphocytes, monocytes, eosinophils, basophils
- Total protein, fibrinogen: parameters not strictly involved with the erythron or leukogram, but often included with the CBC. Values provide ancillary information regarding inflammation.

2. Results

A. Red Blood Cell (RBC) Parameters

RBCs: RBCs are no. of cells in units of millions/µl; measure the number of circulating erythrocytes. Values are influenced by breed of horse (hot breeds have a higher RBC, PCV, Hb than cold and pony breeds), age of horse (highest RBC from 1–9 months; at same time, Hb, PCV, and MCV are lowest), and level of excitement, stress (splenic contraction affects significantly increases in RBC, PCV, Hb; effect lasts for approximately 60 minutes).

PCV (packed cell volume): used synonymously with “hematocrit”: measures that space in the blood which is taken up by RBCs. Determined by both the number and size of cells. Can be artifically increased if insufficient centrifugation occurred (as might be a problem in species with smaller RBC diameters than the horse) or if many macrocytes are present. Five minutes’ centrifuging time is adequate for most species; at HDM, lab samples are spun for 2 minutes. Because equine RBCs are not small and because they form rouleaux, equine blood samples pack readily with minimal centrifugation. Increase in PCV indicates volume contraction or dehydration, splenic contraction, or rarely, polycythemia. Decreased PCV indicates anemia.

Hb: measures g hemoglobin per 100 ml (dl) blood. Red cells are lysed, and free hemoglobin is converted to the more stable cyanmethemoglobin, which is then quantified with a spectrophotometer. Reading will be spuriously increased by conditions that increase serum turbidity, such as lipemia, increased cholesterol, high blood sugar, and hemolysis. Some of these conditions are obviously more important in blood testing of carnivorous animals and are less important in herbivores. Decreased Hb indicates anemia. Hemoglobin measurements approximate 30% of the PCV in health.

MCV, MCHC, and MCH: These parameters are collectively termed the erythrocyte indexes. Because they are derived from measurements of other values, their usefulness is only as good as the accuracy of the parameters used in their derivation.

MCV (mean corpuscular volume): a mathematical value derived by dividing the packed cell volume by the red cell count.

\[ MCV (fl) = \frac{PCV}{RBC} \times 10^2 \]

Value is given in units of femtoliters (fl) and represents the mean volume or relative size of each erythrocyte. This measurement is meaningful because of the fact that cells become smaller in volume as they mature; thus, if an animal’s mean corpuscular volume is increased, it is deduced that younger cells are appearing in the peripheral circulation in response to need and the condition is, by definition, regenerative. If MCV is less than normal, the cells are said to be “microcytic.” Microcytic anemia is most often seen with Fe deficiency. Though primary Fe deficiency is rare in horses, chronic blood loss secondary to endo- or ectoparasitism, gastric ulceration, erosive gastric neoplasia, or ethmoid hematomas can lead to iron depletion and result in characteristic changes in the erythron. In situations of chronic low-volume bleeding, anemia develops slowly, and shock associated with hypovolemia and cardiovascular collapse do not occur. Physiologic adaptations occur as the PCV drops, so that clinical signs of anemia (pallor, exercise intolerance, lethargy, tachycardia) may not be apparent until the PCV is profoundly low. Acute, external hemorrhage results in irretrievable loss of protein and heme iron, and a slower regenerative bone marrow response. Gastrointestinal hemorrhage should be considered a form of external hemorrhage. When internal hemorrhage occurs, some erythrocytes enter lymphatic vessels and are returned to the circulation. Others undergo phagocytosis and lysis, and their components of protein and heme are reutilized, permitting more prompt regeneration.

Two additional measurements, MCHC and MCH, are employed to assess the Hb content of erythrocytes.

MCHC (mean corpuscular hemoglobin concentration): This value is also mathematically derived, from known values for Hb and PCV. MCHC is the most sensitive erythrocyte parameter.

\[ MCHC (g/dL) = \frac{Hb}{PCV} \times 100 \]

MCHC informs the examiner of the concentration of hemoglobin within a population of cells, taking the size and number of red cells into account. The test procedure for measuring Hb detects both intracellular and extracellular hemoglobin, but the formula assumes that all measured Hb originated from inside erythrocytes. When MCHC is increased, it is usually secondary to intra- or extravascular hemolysis and release of free Hb into the circulation. No true increase in MCHC is recognized, as there is no overproduction of hemoglobin by RBCs. The utility of this parameter lies in the detection of decreased values, which indicate anemia.

MCH (mean corpuscular hemoglobin): This value differs from MCHC in representing the total amount of hemoglobin in red cells, without consider-
Horses differ somewhat from other large animal species in their response to anemia. Bone marrow response is slower, with an estimated increase in PCV of 0.4% per day, even following severe, acute hemorrhage. In other species, circulating reticuloocytes, erythrocytes in the last stage of maturity before becoming RBCs, are used as an index of regenerative bone marrow activity. However, reticuloocytes are absent from the peripheral circulation of horses, both in health and in regenerative anemias. Equine erythrocytes lack the appearance of central pallor that characterizes other biconcave RBCs, so it is difficult to appreciate hypochromasia of cells from the appearance of the blood smear.

RDW (red cell distribution width): This value is provided with most automated hematology analyzers. RDW is the coefficient of variability of the size distribution of the RBC population in a blood sample. It is an index of anisocytosis (variability in cell size) but is a more informative assessment of anisocytosis than notation by human laboratory personnel. The red cell distribution width will increase when either micro- or macrocytosis is present. Visualization of the distribution curve is necessary to determine whether the population of cells elongating the width of the curve lies to the right (macrocytosis) or left (microcytosis) of the figure for mean cell size. In the horse, increases in MCV and RDW are useful in detecting a regenerative anemia.

Total Protein: This parameter is typically estimated using a refractometer. Conditions causing turbidity in serum will result in spurious elevations in the measured value for total protein. Increased total protein occurs in conditions of dehydration or other causes of intravascular volume contraction, increased globulin concentration secondary to chronic antigenic stimulation or neoplasia, or increased fibrinogen concentrations secondary to inflammation.

The final step in assessment of the erythron is examination of erythrocytes under magnification. Erythrocyte findings noted by laboratory personnel on a blood smear might include the following:

- Rouleau: accumulations of RBCs arranged like a stack of coins (normal finding);
- Howell-Jolly bodies: small, basophilic nuclear remnants near the cell periphery (H–J bodies in ≤0.1% of equine RBCs is normal finding);
- Heinz bodies: precipitates of denatured Hb, indication of oxidation injury to RBCs such as occurs with exposure to the toxins in red maple leaves, onions, and phenothiazine tranquilizers;
- Schistocytes (small, irregular RBC fragments): indicate changes in the microcirculation, especially associated with endotoxemia, DIC, inflammatory conditions in highly vascular tissue, neoplasia;
- Autoagglutination: RBCs form grape-like clusters because of “sticky” cell membranes in conditions of antibody-mediated hemolytic anemia;
- Spherocytes: small RBCs that have lost their biconcave configuration and assumed spherical shape. Also associated with hemolytic anemia, spherocytes are created when membrane blebs are removed while cells are in passage through the spleen.

B. Leukocyte Parameters

WBC (white blood cells): No. of leukocytes, given in units of millions per µl.

Increases in white cell numbers (leukocytosis) may be pathologic, as in response to infection, or physiologic, as occurs secondary to endogenous catecholamine or corticosteroid release. A decrease in white cell count to below normal range (leukopenia) is always a pathologic event. Absolute counts of the different leukocyte populations are more meaningful than percentage values.

Since neutrophils are the first line of defense against microbial infection, changes in their numbers or morphology are most notable. The ability of the bone marrow to respond to a bacterial infection is measured by the total leukocyte count (particularly neutrophils), and the intensity of the response is gauged by the extent of the left shift. Description of the neutrophil population is further characterized by the terms “regenerative left shift” or “degenerative left shift.” A left shift refers to the appearance of more immature cell forms in the circulation. When the immature forms are more numerous than mature neutrophils, a degenerative left shift is present. This condition is degenerative because it indicates that production of neutrophils in the bone marrow is unable to keep up with the need for the mature cell forms in tissue sites of inflammation. A regenerative left shift exists when immature cell forms are present in peripheral blood, but their number is still exceeded by that of mature neutrophils. This condition suggests that the rate of production of neutrophils is sufficient to meet tissue demands. The general response to bacterial infection is neutrophilia with or without a left shift, but bacterial infections can be attended by increases in the neutrophil count, normal neutrophil numbers, or neutropenia. Some bacteria elicit a more intense pyogenic response than others, and mycotic soft tissue infections are generally associated with marked elevations in white cell counts. For example, the extreme neutrophilic response seen in Streptococcus equi infections is a result of the presence of the antiphagocytic M protein molecule on the surface of the bacterial cell wall. Large numbers of neutrophils are beckoned to infected lymph nodes by chemotactic factors but are unable to engulf and destroy the bacteria, leaving many organisms free to signal continued need for neutrophil production.

Not all kinds of infection cause elevations in neutrophil or leukocyte counts. Some chronic infections may be accompanied by a normal leukocyte count. Pyogranulomatous conditions result in for-
nlation of abscesses, and the walling off of antigen can result in a modulating effect on further recruitment of neutrophils. Gram-negative bacterial infections increase the animal’s exposure to endotoxin. Endotoxin is a structural component of the cell wall of gram-negative bacteria and is released when a bacterium undergoes lysis. Endotoxemia causes increased expression of adherence molecules on endothelial cells, making them “stickier” so that neutrophil margination increases, decreasing cell numbers in peripheral blood samples. Gram-negative sepsis is thus one type of bacterial infection that can result in neutropenia rather than neutrophilia.

Leukopenia associated with lymphopenia is associated with the acute phase of disease caused by viral infections. Viruses invade and destroy both primary and secondary lymphoid tissues via various mechanisms, causing lymphocyte depletion and immunosuppression. Other causes of lymphopenia include stress, administration of exogenous corticosteroids, endotoxemia, neoplastic infiltration of lymphoid organs, and severe malnutrition or starvation. The finding of persistent lymphopenia in Arabian foals should prompt a concern for CID. Lymphocytosis is unusual in the horse and is most often the result of sympathetic nervous system discharge secondary to stress or emotional excitement. Elevated circulating catecholamine levels cause increased lymph flow in the thoracic duct and other lymphatic conduits, with the effect of returning more lymphocytes to the peripheral circulation. In horses, it is important to consider the age of the animal when interpreting lymphocyte numbers, because the percentage of the total white cells that are lymphocytes is significantly higher in young horses. The neutrophil/lymphocyte ratio (N:L) in normal neonatal foals, for example, is 2–3:1, whereas in weanlings and yearlings that ratio approximates 1:15; thus lymphocyte counts may be relatively higher and will be similar to neutrophil counts, but they would not be interpreted as lymphocytosis. Some chronic viral infections (EIA) and hematopoietic neoplasia can also be associated with high lymphocyte numbers, although the latter condition is very rare in horses. Lymphosarcoma is generally not associated with increased circulating lymphocyte numbers.

**Monocytes:** Monocytosis occurs together with neutrophilia as the hallmark of a stress leukogram. Disease states that require increased tissue macrophage numbers will elicit increased production of monocytes, as monocytes are the precursor cell type for macrophages. Monocytes derive from the same cell progenitor cell line as neutrophils but are less voracious in their ability to phagocytize pathogens.

**Eosinophils:** Circulating eosinophil numbers are increased in a variety of conditions primarily associated with parasitic or allergic disease. Persistent eosinophilia indicates a chronic disease process that involves ongoing degranulation of mast cells and thus is usually associated with disease referable to organ systems that contain high significant numbers of mast cells. The skin, lung, and gastrointestinal tract are tissue sites of high mast cell numbers, and diseases in these organs may result in high local numbers of eosinophils. High peripheral eosinophil counts develop in response to parasitism only if sensitivity to parasite antigen has developed, and continuous production and release of eosinophils is stimulated. Parasitic infections with Oxyuris equi, Parascaris equorum, S. vulgaris, S. westeri, and Tricinella spiralis have been associated with eosinophilia. Eosinopenia is primarily attributed to endogenous corticosteroid or catecholamine release.

**Basophils:** Basophilia is rare in the horse. Basophilia sometimes accompanies eosinophilia, and the diagnostic effort in such cases should be directed toward defining the cause of the elevated eosinophil count.

Notation of the visual characteristics of white blood cells on an impression smear significantly augments the information gained by simple quantification of their numbers. For instance, the appearance of toxic changes in neutrophils suggests the cells’ bone marrow maturation process was disrupted by the presence of severe systemic inflammation, perhaps from bacterial infection, drug toxicity, or endotoxemia. Toxic changes generally involve the cytoplasm, while degenerative changes are noted in the nuclei of neutrophils. The presence of degenerative changes indicates the cells have been activated by a septic or inflammatory process. Leukocyte findings noted by laboratory personnel on a blood smear might include the following:

- **Cytoplasmic changes:** Toxic changes include Doehle bodies (retained aggregates of endoplasmic reticulum), basophilic cytoplasm, foamy vacuolation of the cytoplasm, and toxic granulation.
- **Nuclear changes:** Pyknosis (condensation of nuclear material), karyorrhexis (fragmentation of a pyknotic nucleus), karyolysis (fragmentation of nuclear chromatin), hypersegmentation. These changes in general occur following cell exposure to bacteria or products of sepsis. Hypersegmentation and pyknosis can also be normal aging changes, seen in old or senescent neutrophils.
- **Immature forms:** The appearance of less differentiated cell forms is a left shift. It indicates enhanced bone marrow production of neutrophils in response to increased need at tissue sites. Up to 1% band cells in equine peripheral blood is normal.
- **Inclusions:** The characteristic inclusion bodies of Ehrlichia equi are present in neutrophils and, occasionally, eosinophils. A buffy coat smear is helpful in detecting the inclusions when the number of infected neutrophils is low, as in the early stages of clinical disease.

C. **Total Protein and Fibrinogen Concentrations**

In some chronic or subtle cases of inflammation, a left shift or leukocytosis may not be present; in such
instances the examiner’s ability to identify inflammation is enhanced by the determination of plasma fibrinogen. Fibrinogen and other positive acute-phase proteins are produced by the liver as a nonspecific response to a variety of inflammatory stimuli. The same cytokines that mediate increased acute-phase proteins also signal the liver to decrease production of the negative acute-phase proteins albumin and transferrin. The reference range for fibrinogen concentration in equine blood at most laboratories is 100–400 mg/dl. Two methodologies are utilized for determining fibrinogen concentration. Heat precipitation is commonly employed because it is technologically easy to perform and does not require expensive laboratory equipment. The alternative, more sensitive method is to derive fibrinogen concentration using the fibrin clot method, which requires measurement of thrombin clotting time and extrapolation of fibrinogen levels from a standard curve. In some cases it is further useful to calculate the plasma protein/fibrinogen ratio:

\[
\frac{\text{Total plasma protein (g/dl)} - \text{fibrinogen (g/dl)}}{\text{fibrinogen (g/dl)}}
\]

This ratio helps identify changes in fibrinogen concentration that are not due to inflammation. The utility of the protein/fibrinogen ratio may be appreciated in the case of dehydration. Plasma fibrinogen concentration will be elevated in a volume-contracted patient, but to the same degree as the concentration of albumin. The ratio of the two proteins, then, would remain in the normal range of greater than 15. Values in the 10 to 15 range suggest an absolute increase in plasma fibrinogen levels.\(^7\)

Over 1000 different proteins have been identified and described, but for ease in classification, these proteins are collectively regarded as either albumin or globulins. On most serum chemistry analyzers, the globulin concentration is derived by subtraction of albumin concentration from the figure for total protein.

Total protein determination can be useful in the assessment of acute hemorrhage. During the first 4–6 hours following an episode of hemorrhage, PCV is maintained at near-normal levels because all blood components are lost in equal proportions and because the splenic response to hemorrhage is to contract, injecting high-hematocrit blood (PCV 80%) into the circulation to preserve oxygen-carrying capacity. After 4–6 hours, the plasma protein levels will fall, reflecting interstitial fluid translocation into the vascular space. Anemia associated with the hemorrhage is often not demonstrable until 12 hours following the hemorrhagic event. In horses, bone marrow response to the anemia is suggested by an increase in MCV and RDW, although such changes do not begin to appear until the 4 days required for erythrocyte production have elapsed. Incremental increases in hematocrit will then be evident in a regenerative patient if serial blood monitoring is performed. Although regenerative changes in peripheral blood are useful in confirming an appropriate response to anemia in other animals, evaluation of a bone marrow aspirate provides the most convincing evidence of regeneration in the horse. The normal M:E ratio in horses ranges from 0.5 to 1.5. Valid interpretation of a decreased M:E ratio as reflective of a responsive anemia can only be made if it is known that the population of myeloid precursors is normal, a difficult supposition in many cases. The presence of increased reticulocytes (≥3%) is a favorable finding, and more reliably indicates a responding bone marrow.\(^7\) Although reticulocytosis is not observed in the peripheral blood of a horse responding to anemia, increased reticulocytes in the bone marrow is a likely finding in such cases.

3. Discussion

Some conditions familiar to equine practitioners are associated with typical changes on the complete blood count. Disease entities that will be profiled and reviewed from the standpoint of the CBC include the following:

- Stress leukogram
- Acute hemorrhage
- Anemia associated with EPM therapy
- Severe interstitial pneumonia
- Anemia of chronic disease
- Neonatal isoerythrolysis
- Autoimmune hemolytic anemia

References