

Interpretation of the Equine Leukogram

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Abstract: The systematic study of total number of leukocytes or white blood cells and their morphological characteristics offers valuable information for the diagnosis, prognosis, response to treatment and control of a wide variety of infectious or inflammatory disorders. This study provides a brief description of the physiological and morphological characteristics of the various types of leukocytes, including neutrophils, lymphocytes, eosinophils, monocytes and basophils and their quantitative and qualitative changes in response to diseases in the horse.

Keywords: Hematology, horse, leukogram.

1. INTRODUCTION

The term leukon refers to the set of data derived from total and differential count of white blood cells (WBC) and the analysis of WBC morphology. Circulating WBC represents the results of the dynamic production of the bone marrow, the release of the cells to the peripheral blood and the storage in different organs or pools. Cells can coexist in different stages of maturation, being fully mature cells such as neutrophils (NEU), eosinophils (EOS), monocytes (MON), lymphocytes (LYM) and basophils (BAS) and immature cells such as band neutrophils (NEUband), metamyelocytes, myelocytes and progranulocytes [1,2].

Although diverse physiological factors as breed, moment of day, gender, training, exercise and reproductive status can affect the leukogram in the horse (Revised by Satué *et al.*, [3]), there are two main WBC responses: physiological leukocytosis and stress leukocytosis. Physiological leukocytosis refers to changes in circulating WBC associated with the activation of the sympathetic-adrenal axis, leading to splenic contraction in cases of fear, excitement, or high intensity exercise. In addition, in the physiological leukocytosis, there is a mobilization of the marginal pool of NEUs and/or LYM, because of a reduction in NEU adherence capacity, increased blood flow through the microvasculature and splenic contraction [4]. These events result in leukocytosis with neutrophilia and/or lymphocytosis. In some cases eosinophilia and

monocytosis are also found. These changes are transient and the marginal pool of NEUs is restored in 20-30 min after the onset of the response and the LYM counts returned to baseline after 1 hr [1].

Stress leukocytosis is associated with cortisol release under certain stressful situations. This hormone induces mature neutrophilia, lymphopenia and eosinopenia. As neutrophilia derives from the mobilization from the marginal pool, the reduced ability to migrate from the blood to the peripheral tissues and the increased mobilization of the population of the bone marrow reserve, it is considered a mature neutrophilia or neutrophilia with right shift. Lymphopenia is the result of LYM sequestration from lymphoid tissues and the eosinopenia derives from the marginalization of EOS in the blood vessels and the decreased release from the bone marrow [1,5]. This response appears between 2 and 4 hours after the elevation of the endogenous cortisol concentrations or after exogenous administration of corticoids [6]. Normal values are recovered in 24 hrs. This response has been also found after an endurance exercise and in response to a great variety of pathological processes [1].

2. INTERPRETATION OF LEUKOGRAM

The reference values for the leukogram in horses are presented in Table 1.

Abnormality in the total WBC concentration is useful only to alert the clinician to look for and interpret abnormalities in the cell distributions in the differential count. When the total WBC is abnormal, one or more distributional abnormalities in the differential are likely. As a result, changes in the concentrations of specific

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leukocyte types are more important for clinical interpretation purposes [11].

Table 1. Reference values for the leukocyte parameters in adult healthy horses, in absolute values and in percentage (modified from Carrick and Begg [7]; Satué *et al.* [8]; [9]; Muñoz *et al.* [10]) (WBC, white blood cells).

Parameters	Reference Range	
WBC	6,000-14,300 $10^3/\mu\text{L}$	
	Units	
	$10^3/\mu\text{L}$	%
Band neutrophils	0-0.100	0-8
Segmented neutrophils	2,500-7,500	22-72
Lymphocytes	2,500-5,000	17-68
Monocytes	0,000-1,000	0-14
Eosinophils	0,000-0,500	0-10
Basophils	0,000	0-4

2.1. Morphological Characteristics and Physiology of Neutrophils

The release of NEU from the bone marrow into the circulated depends on the tissue demands and the production of different pro-inflammatory cytokines. After passing into the blood, NEU can be in the circulating pool or stored in the marginal pool (in the endothelium of several organs i.e., lungs or intestine). Three main locations of the NEU can be described: 1) Bone marrow, there is a proliferating population of NEU, including promyelocytes, myelocytes, metamyelocytes, and mature NEU, prepared for release into peripheral blood; 2) Blood, in the blood compartment, mature NEU appear as round cells, 10-15 μm of diameter, with clear cytoplasm, neutral or granules stain pink and with the nucleus polymorphic and segmented and the chromatin arranged in the form of knots; 3) Tissues, in inflammatory processes, there is a release of chemotactic substances that promotes NEU migration from the vascular bed into the tissues. Marginal pool of NEU adheres to the vascular endothelium, mainly in the small vessels. This fact facilitates the migration to the tissues, while serving as a reserve, so there is a continuous exchange between circulating and marginal pools [1,2]. Barr bodies (sex chromatin lobe/drumstick) can be recognized in females and resemble a small purple body attached to the nucleus by a thin chromatin strand. Further, in peripheral blood, both mature NEU and immature or NEUbands can be found. Equine NEUbands are less frequently seen because horses do

not exhibit marked left shifts during inflammatory insults compared to dogs and cats. In cases of bacterial infection, the NEUbands might represent between 1 and 10% of the total WBC differential count. NEUbands have a polymorphic nucleus, without constrains, with a less condensed chromatic pattern than the segmented NEUs. The cytoplasm is similar to this of the NEU [1,12]. Hypersegmented NEUs are rarely seen in healthy and they have five or more lobes separated by filaments. Prolonged storage of blood may lead to the artifacts development of hypersegmented NEUs. It is the result of a time of increased blood neutrophil transit and the most times corticosteroid administration or endogenous release. Idiopathic hypersegmentation of NEUs have been described in Quarter Horses that lacked evidences of clinical disease. Hyposegmented NEUs have also been reported in apparently healthy Arabian horses, which were diagnosed as Pelger-Huët anomaly [13].

Circulating NEUs have a half-life of 10.5 hours, renewing approximately 1.5 times per day [1,7]. Then, they leave the bloodstream and migrate into the tissues. It is a unidirectional movement, because they do not return to the peripheral circulation. In the tissues, NEUs are functional for 1 to 2 days, and then they are phagocytized by the monocyte-macrophage system or by the mucosal surfaces [1]. The mechanisms involved in phagocytosis of senescent neutrophils in horses have not been specifically investigated, although the processes described for other species have been extrapolated to the horse. Apoptosis or programmed cell death of neutrophils could lead to a recognition by macrophages, increasing phagocytosis [1,7].

The toxic changes are associated with general, toxic states, such as systemic bacterial infections and severe, acute inflammatory lesions. Toxic change is best defined as a set of morphologic changes observed on the blood film that are a result of accelerated marrow production of NEUs. The morphologic abnormalities acquired during maturation under conditions that intensely stimulate neutrophil production and shorten the maturation time in marrow secondary to cytokine stimulation. Morphologic changes include (in order of frequency) diffuse cytoplasmic basophilia, Döhle bodies, and fine cytoplasmic vacuolation. Döhle bodies, blue-gray cytoplasmic inclusions, are aggregates of endoplasmic reticulum. The presence of cytoplasmic toxic granulation indicates presence of gram negative bacterial infections or intestinal alterations that generates elevated absorption of

endotoxins. More rare changes include increased prominence of cytoplasmic azurophilic granules, cellular gigantism, and binucleation [11].

Degenerative changes results of altered cell membrane permeability and include hydropic degeneration of nucleus and a spreading out of the chromatin so that it more completely fills up the cytoplasm of the cell. Aged NEU most commonly in tissues fluid can exhibit hypersegmented pycnotic nuclei with round tightly clumped chromatin [11].

In anaplasmosis, morulae of *A. phagocytophilum* can be detected in cytoplasm of NEU although they may be found occasionally in other cell types, as EOS. These morulae appear as stippled dark blue or purple inclusions, Wright, Giemsa or Diff-Quik stained peripheral blood, bone marrow smears, lymph node smears or other impression smears from fresh tissues may be used to search for morulae. They are most likely to be found early in the acute illness, and disappear from the blood soon after starting tetracycline treatment. The probability of detecting morulae in blood smears is up to 30-40% of the granulocytes in horses that contain these inclusions [14].

2.1.1. Quantitative Alterations of Neutrophils

2.1.1.1. Neutrophilia

The neutrophilia or increased number of circulating NEUs, can occur in physiological (stress response, spleen contraction) and pathological situations (inflammation, infection, neoplasia). Endogenous or exogenous glucocorticoids or epinephrine, excitement, exercise or stress may result in neutrophilia [15,16]. In main lines, neutrophilia is considered when the number of NEUs exceeds $6.000/\text{mm}^3$ of blood. A deviation of the left denotes a value greater than $300/\text{mm}^3$ of immature forms [2]. The regenerative left shift indicates the presence of increased numbers of NEUband cells with mature NEUs greater than the number of NEUband cells, reflection of acute inflammation, and the degenerative left shift the presence of increased numbers of immature cells as myelocytes and metamyelocytes in addition to band cells, indicating severe inflammatory condition.

Clinical neutrophilia occurs when the output of the NEU formed in the bone exceeds migration into tissues. In this circumstance, the total pool of NEU undergoes an expansion and the half-life of circulating population is normal or slightly increased. The magnitude of the neutrophilia is higher in infectious

processes localized, with abscess formation, compared to widespread inflammatory disorders [17,18].

The presence and magnitude of the deviation to the left depends on three factors: 1. Number of NEU in the bone marrow pool, 2. -Release from the bone marrow and 3. -The flow velocity of NEUs from the mitotic compartment of the bone marrow towards the non-mitotic compartment of maturation and storage. Therefore, the severity of the condition is reflected in the intensity of the left shift and the presence of toxic changes in NEUs [2,19].

In some horses, neutrophilia can be observed but the diagnosis of the affected tissue can be tricky. Under these clinical conditions it should be excluded widespread affection of dermal tissues, gastrointestinal and genitourinary systems, joints, tissue planes or hidden abscesses [20,21]. On the other hand, we must bear in mind that hemorrhagic and hemolytic anemias present with neutrophilia [22,23].

The convalescence of a pathological condition is characterized by a decrease in the intensity of the shift to the left, because the production of NEUs from the bone marrow fills the non-mitotic pool. Furthermore, the reduction in tissue demands promotes regression toward baseline values. The persistence of neutrophilic leukocytosis with a minimum deviation to the left reflects the existence of a chronic suppurative disease, usually associated with non-regenerative anemia of chronic disease and hyperfibrinogenemia [2,17,24].

2.1.1.2. Neutropenia

Reduced number of NEUs in peripheral blood, below $3.000/\text{mm}^3$, also called neutropenia, is a consequence of certain pathological processes. Neutropenia can derive from the individual or simultaneous actuation of one of these three mechanisms: defective production in bone marrow, temporary exchange between the marginal and peripheral pools, and faster migration to the tissues, with a speed greater than the ability of the bone of replacing those cells. Always bear in mind that the neutropenia is a serious clinical problem that can result from a severe bacterial process [4].

Reduced NEUs production in the bone marrow has been reported in horses and ponies. Exposure to ionizing radiation and chemotherapy might result in severe neutropenia [25]. In bone marrow diseases, the neutropenia is a manifestation of pancytopenia, but the etiology is often difficult to establish, despite the

comprehensive assessment of medical record, physical examination, laboratory diagnosis and even pathological study. Furthermore, neutropenia secondary to core mass reduction is rare, but can be seen in myelophthisis [2,26], immune-mediated neutropenia and myeloproliferative disorders, including granulocytic leukemia, leading to chronic neutropenia [11,27].

Although medullary destruction and pancytopenia are found in cattle after mastitis and metritis, this fact has not been reported in horses. However neutropenia arising from medullary destruction must be considered in any equine patient, after excluding other causes of neutropenia, especially if there is a concomitant sepsis of severe intensity [2,4].

Medullary space obliteration appears secondary to stromal reactions or infiltrative diseases, even though neutropenia is accompanied by pancytopenia. Stromal reactions include myelofibrosis or replacement of normal bone marrow cells by fibrous connective tissue and osteopetrosis or osteoid deposition. The most common infiltrative diseases are disseminated granulomatous inflammation and cancer [28]. In horses, most of myelophthisic processes with secondary neutropenia are attributed to neoplastic conditions, mainly lymphoma and leukemia [25,29].

The temporary exchange between the circulating and marginal pools is reflected in the leukogram by neutropenia, although the total amount of blood NEUs may remain unchanged. This mechanism together with increased tissue demand, are the primary considerations in the differential diagnosis of neutropenia in horses. This happens mainly in cases of septicemia in neonate foals and endotoxemia in adult horses, in a variety of gastrointestinal disorders such as right dorsal colitis syndrome, strangulating obstruction, peritonitis, enteritis and salmonellosis [30,31]. In these cases, the presence of neutropenia suggests a diminished lifetime of the NEUs and a migration from blood vessels into the tissues severely damaged. Intravascular or intrasinusoidal destruction or sequestration of these cells is found [4]. In addition, neutropenia below $1.500/\text{mm}^3$, is a common finding in hyperadrenocorticism, by administration of exogenous corticosteroids or acute infections of diverse etiology, as bacterial, rickettsial and viral infections, associated with left deviation, toxic cellular degeneration and leukocyte changes [32,33].

2.2. Morphological Characteristics and Physiology of Lymphocytes

LYM are the second largest population of circulating WBCs, after NEUs and the main components of the immune system. They are smaller than NEUs and the other granulocytes, with a dark-staining nuclei, coarse chromatin pattern and scant amount of blue cytoplasm. The mature cell has a diameter of 7-12 μm , an eccentric, round nucleus with a notch on some occasions [34]. LYMs are made up to 38-66% T cells, 17-38% B cells with the remaining being null cells [35]. Occasionally, larger LYMs are present, and they have smooth chromatin patterns and large amounts of pale blue cytoplasm [36]. The half-life of LYMs varies between 20 and 200 days [37], with a mean duration of transit through the blood of 30 hrs. Blood LYMs have the ability to recirculate in the blood, lymphatic channels, lymphoid and peripheral tissues and they are able to have mitosis, allowing amplification of the immune response [1,4,36]. Most of the LYMs are originated in the peripheral lymphoid tissues, and only a small percentage comes from central lymphoid tissues, i.e. bone marrow and thymus. The circulation time depends on the LYM subtype and the tissue of origin. T cells circulate more rapidly than B cells and migration through the splenic parenchyma is faster than through the lymph nodes [38].

Reactive lymphocytes (immunocytes) are rarely seen in health. They are slightly larger than small lymphocytes, have scalloped nuclear margins, moderately aggregated chromatin, occasionally have discernible nucleoli rings, scant to moderate amounts of intensely basophilic cytoplasm, and occasionally have a pale - staining Golgi zone [2,4]. These cells are produced in antigenic stimulation.

They may sometimes be identified morulae of *E. risticii* in cytoplasm of LYMs and MONs. They appear as pleomorphic inclusion bodies gray-blue to dark blue [39].

2.2.1. Quantitative Alterations of Lymphocytes

2.2.1.1. Lymphocytosis

Lymphocytosis, an increased number of circulating LYM, implies an elevation above $5.000/\text{mm}^3$ and is attributed to physiological stress, chronic antigenic stimulation or lymphoid malignancies. As mentioned above, physiological lymphocytosis comes from sympathetic increased release of hormones, mainly

adrenaline, whose release induces an increase in circulating LYM up to 14.000 cells/mm³ [10,15].

Moreover, chronic infections and inflammatory lesions occur with lymphocytosis. Typical examples of these circumstances are bacterial infections and alterations following immunization [40]. This increase in the number of LYM is related to the presence of immune cells in blood smears. Lymphoid neoplasms are not a frequent cause of lymphocytosis in horses. However, it can be seen in lymphoma in the leukemic phase, in lymphocytic leukemia and in multiple myeloma or plasmocytoma [41,42,43].

2.2.1.2. Lymphopenia

Lymphopenia is associated with glucocorticoid administration, stress and *Anaplasma* infections. It is also a common finding in the early days of viral infections, such as herpes virus type I (HV-1) [44,45]. Other causes to consider in the differential diagnosis of lymphopenia are immunodeficiency such as combined immunodeficiency syndrome in the Arabian foal [2,46].

2.3-Morphological Characteristics and Physiology of Eosinophils

EOS are cells slightly larger than neutrophils that contain large, reddish-orange granules in the cytoplasm, often obscuring the nuclei and giving a raspberry-like appearance, with a pale blue cytoplasm. The lobulated nucleus seldom shows fine filamentation. Degranulated EOSs are vacuolated and are rarely seen in health [2,4]. The amount of EOS in peripheral blood is low, because most of these cells migrate into tissues, such as the bronchial mucosa, gastrointestinal tract. The half-life of circulating EOS is about 2 to 12 hours [4,47].

2.3.1. Quantitative Alterations of Eosinophils

2.3.1.1. Eosinophilia

Eosinophilia, defined as the increase in the number of EOS over 800 cells/mm³, suggests an antigen-antibody interaction in tissues in which there is a large number of mastocytes, such as skin and lung, or a parasitic problem that produces sensitization. Foals do not have circulating EOSs and display a substantial increase by the age of 3 months. It is assumed that this fact results from exposure to parasites or other antigens. The chance of eosinophilia becomes more likely and its intensity is more marked in cases of nematodes, due to the necessity of tissue migration to complete its life cycle [4].

Furthermore, the inflammation in the skin, gastrointestinal and genitourinary tracts may cause eosinophilia secondary to mastocyte cell degranulation [48]. However, it seems paradoxical that many of these diseases may appear with a severe eosinophilic tissue infiltration without concomitant presence of peripheral eosinophilia [49]. The increased presence of these cells in the damaged tissue is associated with the degranulation of BAS or mast cells that release chemotactic factors derived from complement, with the synthesis of vasoactive amines and cytokines and immune complex deposition within tissues [11].

Peripheral eosinophilia most commonly results from allergic reactions, parasitic infections, including habronemosis, strongylosis and pediculosis [50,51]. Eosinophilic myeloproliferative leukemia is uncommon, although it has been reported in a horse [52]. A marked eosinophilia also has been seen in horses with lymphoma and transitional cell carcinoma [11]. Eosinophilia is also found in the equine multisystemic eosinophilic epitheliotropic disease [53,54].

2.3.1.2. Eosinopenia

Eosinopenia or reduction in the number of circulating EOSs, is difficult to evaluate in horses because leukograms of clinically normal animals contain small amounts of this type of cell. Nevertheless, eosinopenia has been described in association with active infectious or inflammatory processes as well as in response to increasing concentrations of endogenous or exogenous corticosteroid and stress [2,15].

2.4. Morphological Characteristics and Physiology of Monocytes

MON are the largest WBC in circulation, with a large, broad, variable in shape nuclei (oval, bilobed, horseshoe) with lacy chromatin and gray-blue cytoplasm with small azurophilic granules. The cytoplasm can also have a few clear vacuoles of variable size, located in the cell periphery and with a foamy appearance [12,55]. After their production in the bone marrow, MON are released into the bloodstream. In circulation, they distributed between the circulating and marginal pools, with a ratio of 1/3.5 between them. This ratio remains constant in different physiological states and in response to disease. The mean circulating MON life is about 8.4 hours, and there is not exchange at the tissue level and blood. In the tissues, the MONs matures into macrophages, a transformation that is accompanied by changes in ultrastructure, in the appearance of cellular receptors or by metabolic

changes. The half-life of macrophages ranges from several days to months [55]. Prolonged storage of blood has resulted in artifact increases in the proportion of mononuclear cells.

2.4.1. Quantitative Alterations of Monocytes

Monocytosis or increased peripheral MON concentration appears with intense phagocytosis processes as tissue necrosis, intravascular hemolysis or chronic suppurative diseases [10,56]. Monocytosis is often seen in the post-acute or recovery phase

following a viral infection [57], in myelomonocytic and granulocytic myeloproliferative disorders and other types of leukemias [27, 58]. The monocytopenia or decrease in the number of circulating MONs has no clinical relevance [2,11].

2.5. Morphological Characteristics and Physiology of Basophils

BAS are cells slightly larger than the NEUs, with a lobulated nucleus, although to a lesser extent than NEUs, a cytoplasm from blue to gray, with large

Table 2: Differential Diagnosis of the Quantitative Alterations of the Different Subtypes of Leukocytes in Horses

White blood cell subtype	Disorder	Differential diagnoses	Comments
Neutrophils	Neutrophilia (>6000 cells/mm ³)	Spleen contraction	Acute stress, exercise
		Inflammation- infection	Look for toxic, degenerative changes or/and left-right shift
		Neoplasia	Less common
	Neutropenia (< 3000 cells/mm ³)	Bone marrow injury	Ionizing radiation Chemotherapy, drugs Leukemia Severe sepsis-endotoxemia Myelofibrosis Osteopetrosis
		Increased tissue demand	Septicemia and endotoxemia Viral infections
		Others	Hyperadrenocorticism Corticoid administration Chronic stress
Lymphocytes	Lymphocytosis (> 5000 cells/mm ³)	Acute stress	Release from spleen
		Chronic infection- inflammation	Prolonged antigenic stimulation
		Lymphoid neoplasia	Lymphoma, lymphocytic leukemia, multiple myeloma, plasmacytoma
	Lymphopenia (< 2000 cells/mm ³)	Increased glucocorticoids	Chronic stress, glucocorticoid administration, hyperadrenocorticism
		Early phase of viral infection	Mainly respiratory virus
		Immunodeficiency syndromes	
Eosinophils	Eosinophilia (> 800 cells/mm ³)	Immune reactions Parasitic infections Eosinophilic myeloproliferative leukemia Equine multisystemic eosinophilic epitheliotropic disease	
Monocytes	Monocytosis (> 1000 cells/mm ³)	Necrosis Intravascular hemolysis Chronic suppurative disease Recovery from a virus infection Leukemia	

amounts of granules distributed irregularly, and with an intense purple stain that vary in size and shape and can mask the nucleus [12]. Cytoplasm varies from clear to light blue [2].

2.5.1. Quantitative Alterations of Basophils

The increased number of blood BAS, basophilia, is difficult to assess, since these cells are few times in the circulation. However, the most common causes of basophilia are hypersensitivity reactions, hyperlipemia, allergic dermatitis, and inflammatory or neoplastic diseases. Basophilia has been reported in a horse with basophilic enterocolitis [59].

A summary of the main differential diagnoses of quantitative alterations of the equine white blood cells are presented in Table 2.

2.6. Hematopoietic Neoplasm in Horses

Leukemia is the neoplasia of one or more cell lines of the bone marrow, with distorted proliferation and development of leukocytes and their precursors. It differs from lymphoma in that leukemias arise from the bone marrow and lymphoma from the lymph nodes. Leukemia includes myeloproliferative and lymphoproliferative disorders and can be characterized according to the cell of origin (lymphoid or myeloid), the evolution of the clinical signs (acute or chronic) and the presence or absence of abnormal cells in peripheral blood circulation (leukemic, subleukemic and aleukemic leukemia). Myeloproliferative disorders are defined as a primary bone marrow dysplasia or neoplasia in which one or more blood cells of non-lymphoid lines increase in number. The main myeloproliferative disorders in horses are malignant histiocytosis and myeloid leukemia, the latter being classified as monocytic and myelomonocytic, granulocytic, primary erythrocytosis or polycythemia and megakaryocytic leukemia. Lymphoproliferative disorders indicate all the neoplastic and dysplastic conditions arising from lymphoid cells. The most common lymphoproliferative disorders in horses are lymphoid leukemia, plasma cell or multiple myeloma and lymphoma. Lymphoma is the most common hematopoietic neoplasia in horses and usually involves lymphoid organs, without leukemia, although bone marrow may be affected after metastasis. Lymphoma could be classified according to the organs involved and four main clinical categories have been established: generalized-multicentric, alimentary-gastrointestinal, mediastinal-thymic-thoracic and cutaneous. The clinical signs, hematological and

clinical pathological findings, results of bone marrow aspirates, involvement of other organs, prognosis and treatment, if applicable, are presented for each type of neoplasia (revised by Muñoz *et al.* [27]).

Myelodysplastic disorders refer to cells that are not truly neoplastic, but there is a suspicion of neoplasia. Myelophthistic disorders refer to the replacement of normal bone marrow by neoplastic or inflammatory tissue, with a loss of the normal architecture of bone marrow, although this condition has been uncommonly reported in horses [60].

3. CONCLUSIONS

The understanding of normal WBC numbers, parameters and morphology is necessary to identify abnormalities in the leukon in horses. This article summarized the normal hematological characteristics of leukocyte parameters, the common causes of alterations in neutrophils, lymphocytes, eosinophils, monocytes and basophils as well as the laboratory basis for its interpretation.

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