AUSTRALIAN SPECIALISED ANIMAL PATHOLOGY

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Interpretation of the Leukogram

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White Blood Cells (Leukocytes)

The basic function of the white blood cell is to defend the body from infectious disease processes, as well as being involved in removal of dead cells and damaged tissue.

Evaluating the leukogram, including a total white cell count, a differential cell count, absolute numbers of specific leukocytes and examination of morphology on a blood smear, can help identify abnormalities that may suggest specific diseases such as a viral or bacterial infection or even a neoplastic process.

Interpretation however, relies on knowledge of the normal characteristics of the leukogram and on each of its components.

The leukocytes are comprised of five separate cell types, distinguished by:

- the nature of their cytoplasm
- the shape of their nucleus
- their staining characteristics

These vary in morphology somewhat between species, but can be divided into

Granulocytes (3 types):

- Neutrophils comprise a majority of leukocytes in the peripheral blood of dogs and cats.
    - Segmented neutrophils have an elongated nucleus with variable degrees of indentation and constriction (multi-lobed) and clumped chromatin. Cytoplasm contains numerous invisible to lightly stained granules.
- Eosinophils are usually present in only small numbers, but may increase as a response to parasitic infections and allergic/hypersensitivity disease.
    - Morphology varies between species, important characteristics however include a segmented nucleus similar to neutrophils, as well as prominent red-orange granules.
- Basophils are the least populous with only occasional cells identified, but when increased may also be associated with parasitic and allergic/hypersensitivity disease.
    - Nucleus is segmented, with cytoplasmic granules varying from small numbers of dark-violet granules in dogs, numerous faint purple-grey granules in cats and large numbers of dark-staining purple granules in large animals.

and

Non-granular leukocytes (2 types):

- Lymphocytes are generally the second most common leukocyte in the peripheral blood, representative of a diverse mixture of sub-populations, however, these are indistinguishable using standard laboratory techniques. Lymphocytes are responsible for production of immune-globulins, cell-mediated immunity and participate in modulation of inflammatory processes.
    - Lymphocytes are also relatively similar between species, but size may be variable, depending on the degree of activation.
        - The typical cell is small with a rounded nucleus, smooth chromatin and a scant amount of blue cytoplasm. Activation can lead to an increase in the number of enlarged cells. However the population almost always remains heterogenous.

- Activated lymphocytes may develop increased cytoplasmic volume and hence a lower N:C ratio. Cytoplasm may become more intensely basophilic. Nuclei may develop more open chromatin and very occasionally faint nucleoli.

- Monocytes are fewer in number. They participate in inflammatory and immune-responses. They may phagocytose bacteria, foreign material, damaged cells etc. They also play an important role in an immune-response by presenting antigen to lymphocytes.
    - The nucleus is highly variable in shape, however, chromatin is less condensed than neutrophils. Cytoplasm is lightly blue-grey and may contain vacuoles.

Means of Evaluating the White Cells

Determination of the white blood cell count (WCC) will give an appreciation of the total number of leukocytes. This can then be subdivided into the percentage and absolute count for each of the varying types of leukocytes.

The total number and proportion of leukocytes that comprise the white blood cell count will then aid in identifying a possible underlying disease process.

It is important to note that interpretation should mainly be based on the absolute cell numbers in preference to using the relative percentages of each cell type (differential).

The white blood cell count (WCC) is a measurement of the number of leukocytes in a litre of blood.

White cell differential is the proportion of each cell type expressed as a percentage. This is obtained from a combination of examination of a blood film and automated analysers counts. It is important to note that even with sophisticated analysers, the differential may be inaccurate and miss important morphological changes such as a left-shift. Hence a blood smear examination remains a strongly recommended component of a complete blood count.

Absolute counts are derived from the percentage of each cell type of the total white cell count.

Morphological assessment of each cell line is achieved through examination of the blood smear, and remains a vital part of interpretation. This may provide evidence of a left-shift and toxic change in the neutrophils, consistent with release of immature cells from the marrow. Allow assessment of lymphocyte morphology, especially important when interpreting the significance of an elevation in numbers.

Neutrophils and lymphocytes are the most numerous and well-studied leukocytes in the blood. Hence it is the pattern of change in these cell types that forms the basis of interpretation of the leukogram in general. However, a good understanding of neutrophils will aid significantly, both in understanding the patterns, but also when interpreting any less common changes.

Neutrophils in focus

Neutrophils participate in inflammatory responses, attracted to the site of inflammation/tissue damage by release of cellular and/or bacterial components, and by local production of chemical signals. In tissue they participate in phagocytosis of organisms and foreign material. Following phagocytosis, organisms and foreign material may be destroyed by cellular components, including the use of powerful oxidative and enzymatic processes.

They are produced predominantly in the bone marrow from the pluripotential stem cell, the level of production dependant on the presence of appropriate stimulation by inflammatory mediators, cytokines etc.

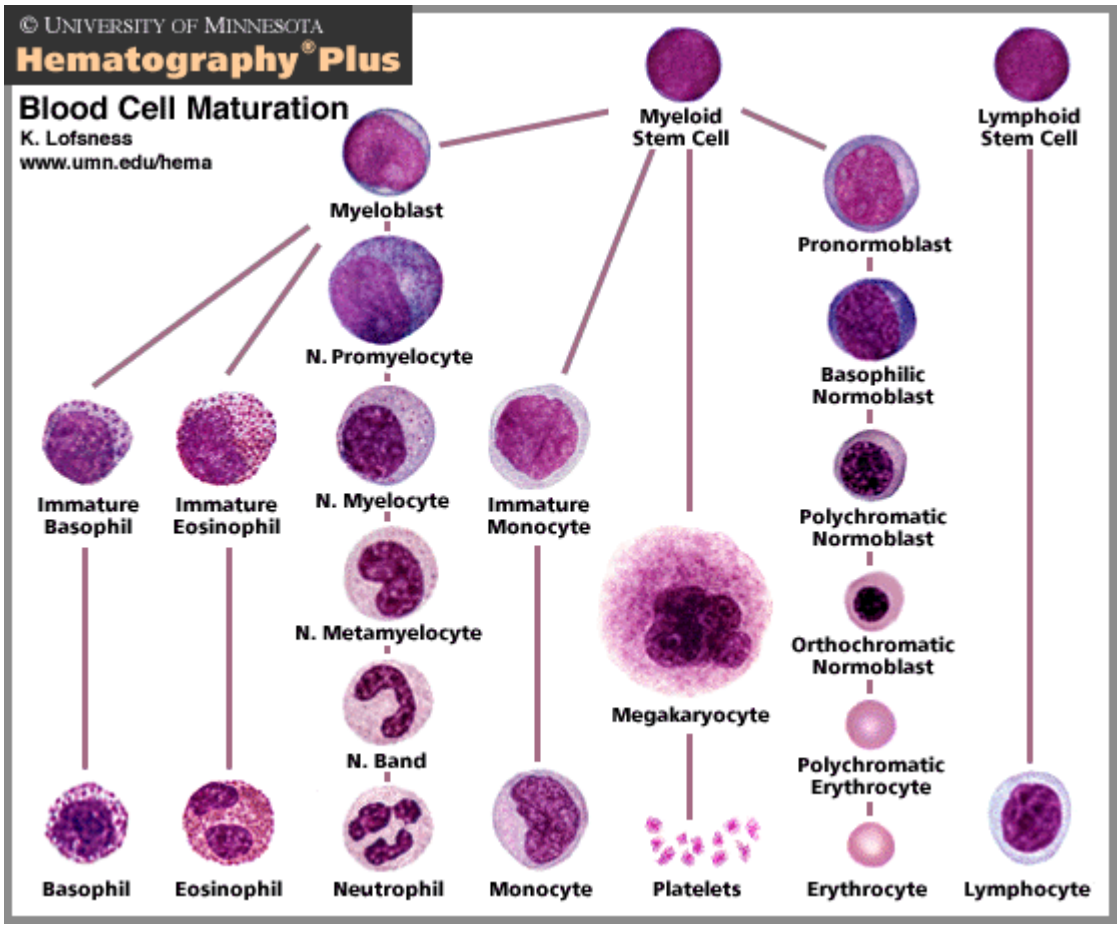


Figure 1.

The pluripotential stem cell, myeloblast, progranulocyte and myelocyte are capable of cell division and form the Proliferative pool within the bone marrow.
The metamyelocyte, band and segmented neutrophil are however not capable of division, but represent progressively more mature/differentiated neutrophils. These form the Maturation and storage pool within the bone marrow.

Neutrophil release from the bone marrow into the blood is ordered according to maturity. Under normal circumstances, only segmented neutrophils are released and hence there are very few immature cells (bands, metamyelocytes etc.) in the peripheral blood.

Note however that as the bone marrow storage of segmented neutrophils is depleted, progressively less mature cells will be released into the peripheral blood in order to meet tissue demand.

Immature cells will have progressively less segmented/constricted nuclei and progressive changes in cytoplasmic appearance, often referred to as Toxic change.

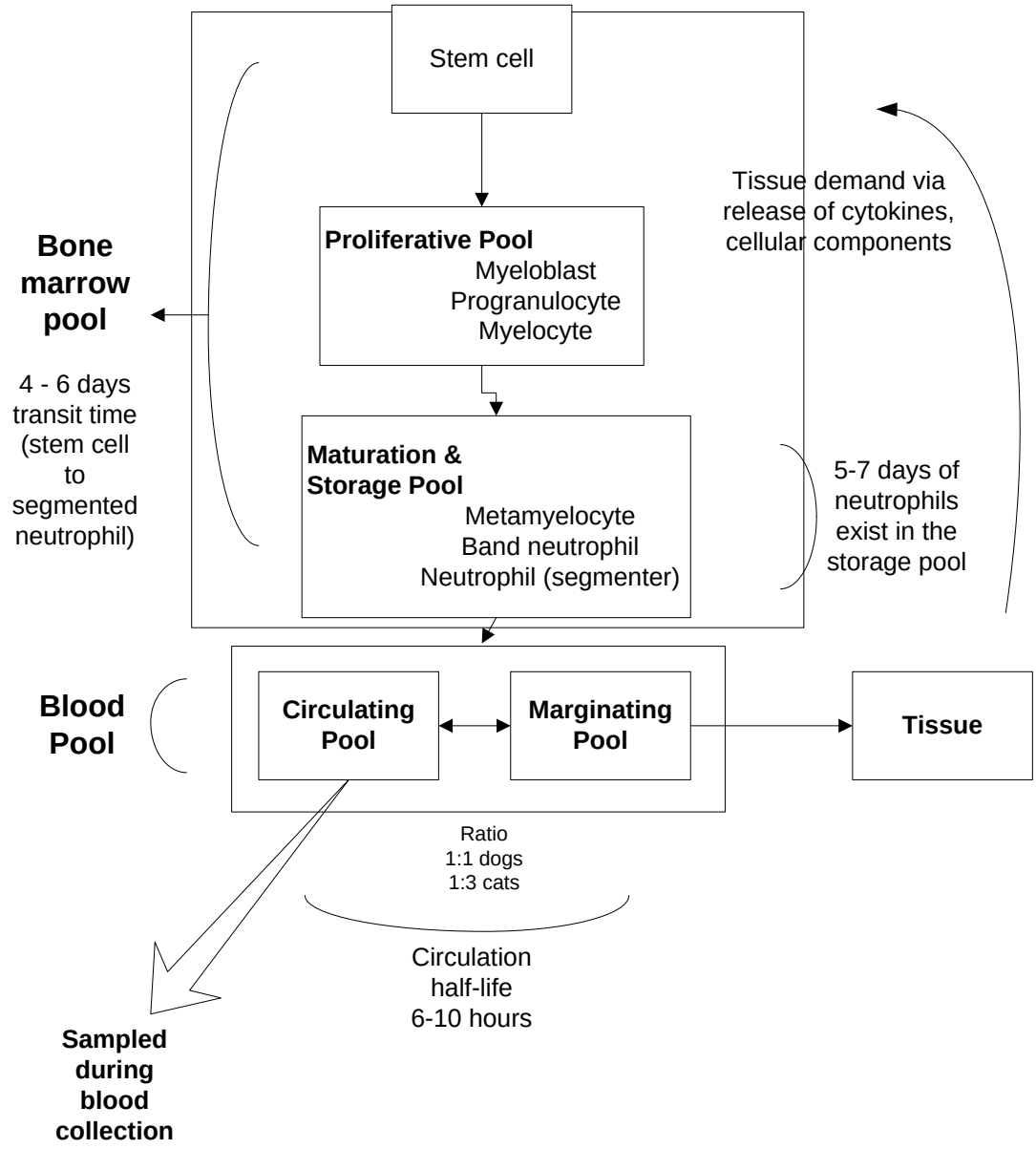


Figure 2.

Once within the peripheral blood, the neutrophils are distributed between the circulating and marginating pools, which they can freely move between.

It is the circulating pool that is directly assessed when performing the leukogram.

The marginal pool is comprised of neutrophils that interact with the endothelial lining of smaller blood vessels, which can be considered an intermediate step from which they may then migrate through the endothelium into tissues.
In dogs, circulating and marginal pools are approximately equal. In cats, the marginal pool is two to three times the size of the circulating pool.

Common Leukocyte Patterns

Inflammation

Neutrophils

The nature of the leukocyte response to inflammation is best understood in light of the neutrophil kinetic diagram above (figure 2).

Inflammation at its base level, is simply an increased demand for neutrophils and monocytes in the tissues of the body. Release of inflammatory mediators at the site of inflammation will lead to :-

Increased margination of circulating neutrophils and subsequent increased migration into tissue

Increased release of firstly segmented neutrophils (and progressively immature cells if stimulation is sufficient) from the bone marrow storage pool.

Stimulation of the bone marrow proliferative pool to increase neutrophil production

- Net result is the rate of marrow production, release into the blood, margination and migration into tissues is increased to provide the necessary neutrophils until the inflammatory lesion resolves.

The leukogram pattern will depend on the timing of sample collection and the balance between tissue consumption and bone marrow production. Hence the neutrophil numbers in inflammation can vary from severely depressed to markedly increased.

This is also partly dependant on the species differences. Dogs have a large marrow functional reserve and greatest ability to deliver cells to the blood. This is progressively less in cats, horses and least in ruminants.

Peracute, severe overwhelming inflammation

Tissue demand outstrips the ability of the marrow to deliver cells to the blood.

Very few cells in the circulating pool. Those present will usually be immature.

Pattern -

- Neutropaenia with a left shift and toxic change

Acute inflammation

Tissue demand is met or exceeded by increased marrow production and release of both segmented cells and immature cells from the storage pool

Pattern -

- Variable neutrophilia with varying degrees of toxic change and left-shift

Chronic inflammation

Tissue demand and marrow production may be balanced. Neutrophils numbers may be normal. Release of immature cells may not be required to meet demand.

Pattern -

- Neutrophils numbers may be normal or only marginally increased. Left-shift only mild or absent.

Lymphocytes

Absolute lymphocyte numbers are usually not affected by the presence of inflammation. The exception is in animals with a chronic process leading to persistent antigenic stimulation, however, this is an uncommon occurrence.
Note persistent immune-stimulation should also be associated with secondary morphological changes consistent with lymphocyte activation. Most notably this includes the presence of a heterogenous population of lymphocytes, including reactive lymphocytes.

Monocytes

A monocytosis may be seen in animals with an inflammatory process that have a high need for macrophages. This usually equates to a need for phagocytosis of necrotic tissue debris, foreign material, micro-organisms etc and may occur in both acute and chronic inflammatory processes.

Eosinophils (and basophils)

Most causes of inflammation have no influence on eosinophil or basophil numbers. These may however be increased when the inflammatory process involves a response to parasites or underlying hypersensitivity.

Stress Response

Cortisol release by the adrenal gland will occur subsequent to a wide range of processes, including systemic illness, metabolic disturbances, pain etc and will be mimicked by corticosteroid therapy (or in animals with hyperadrenocorticism/Cushing's disease). The most consistent change is the presence of a lymphopaenia.

Neutrophils

Corticosteroids cause a structural change in neutrophils, decreasing their ability to stick to the sides of blood vessels, effectively releasing cells from the marginated pool into the circulating pool. Bone marrow retention of mature cells is also decreased. The nett effect is an increase in segmented neutrophils in the leukogram. A left-shift (i.e presence of immature cells) should not occur.
Decreased margination can also slightly increase the overall retention time in the circulation, hence cells may continue to mature while circulating, leading to the presence of hypersegmented cells in the leukogram.

Lymphocytes

Corticosteroids lead to both lymphocyte apoptosis and also decrease the rate of recirculation from the lymphoid tissue and lymphatics. The nett effect is to lower the number of circulating lymphocytes in the blood.

Monocytes

The effect of corticosteroid release on monocyte numbers is variable, however a monocytosis can be seen, most especially in dogs.

Eosinophils

An eosinopaenia is also seen as a consequence of inhibition of release from the marrow and sequestration within tissues. However, given the number of eosinophils are generally low in most animals, changes in eosinophils may not necessarily be identified in a routine leukogram.

Stress Leukogram -

- Variable mature neutrophilia, sometimes with hypersegmentation, lymphopaenia, variable monocytosis, eosinopaenia

Excitement response (Adrenaline release)

Adrenaline leads to increased blood flow in small blood vessels, particularly muscle, which has the net effect of releasing neutrophils from the marginated pool into the circulating pool. Note that any increases are composed of only mature cells from the marginated pool.
Adrenaline mediated blockade of lymphocyte entry into the lymphoid tissue and increased mobilization from the thoracic duct, also leads to an increase in numbers of circulating lymphocytes.

Pattern -

- Variable neutrophilia and lymphocytosis, comprised of mature segmented cells and small, normal cells respectively

Less common leukocyte responses

Causes of Neutropaenia

Figure 2 provides an overview of possible influences on the numbers of neutrophils identified in the leukogram. Essentially the numbers of cells identified is a consequence of the balance between input from the bone marrow via the proliferative and storage pools, into the blood where cells can move between the circulating and marginal pool, before migrating into the tissues.

Hence a neutropaenia can result from a reduced release from the marrow, increased margination of neutrophils, or increased tissue migration. These are outlined in the table below, but notably will include any cause of marrow stem cell destruction, increased margination of neutrophils (e.g. endotoxaemia) or an increase in tissue demand.

Decreased marrow production

Pattern –

- Neutropenia with varying decreases in other cell lines (pancytopaenia if all cell lines affected)

Endotoxaemia

Pattern –

- Neutropaenia, sometimes with evidence of a left-shift and toxic change. Other cells lines not specifically affected.

Peracute inflammation Pattern previously discussed

Lymphocytosis

Lymphocyte numbers in the circulation are a consequence of the balance between migration of lymphocytes into and out of the lymphoid organs (lymph nodes, bone marrow, spleen, thymus etc) and tissues of the body via the lymphatics and thoracic duct, and to a lesser extent expansion or depletion of total body lymphocyte numbers.

Considerations will include

- Age related (higher numbers of circulating lymphocytes in young animals)

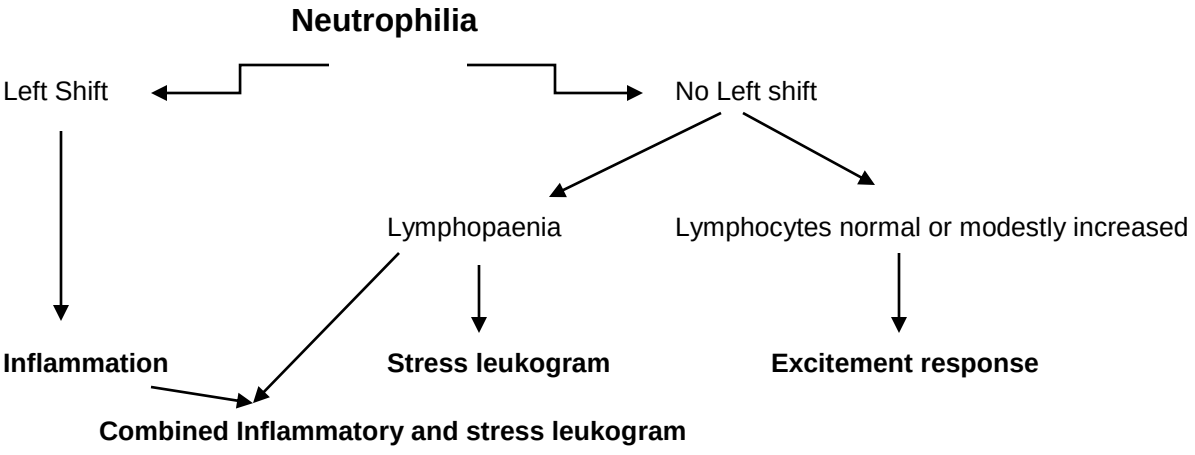
- Expansion of the total body lymphocyte numbers
  - Chronic immune-stimulation (uncommon)
  - Lymphoid neoplasia

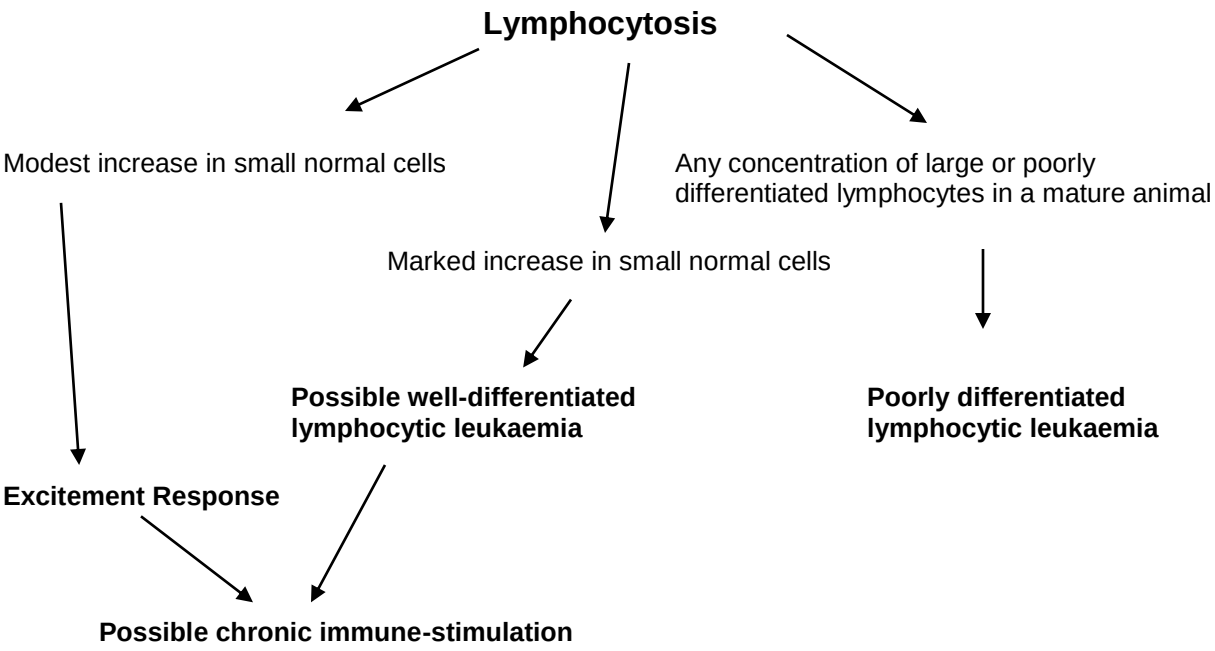
- Hypoadrenocorticism (lack of corticosteroids)

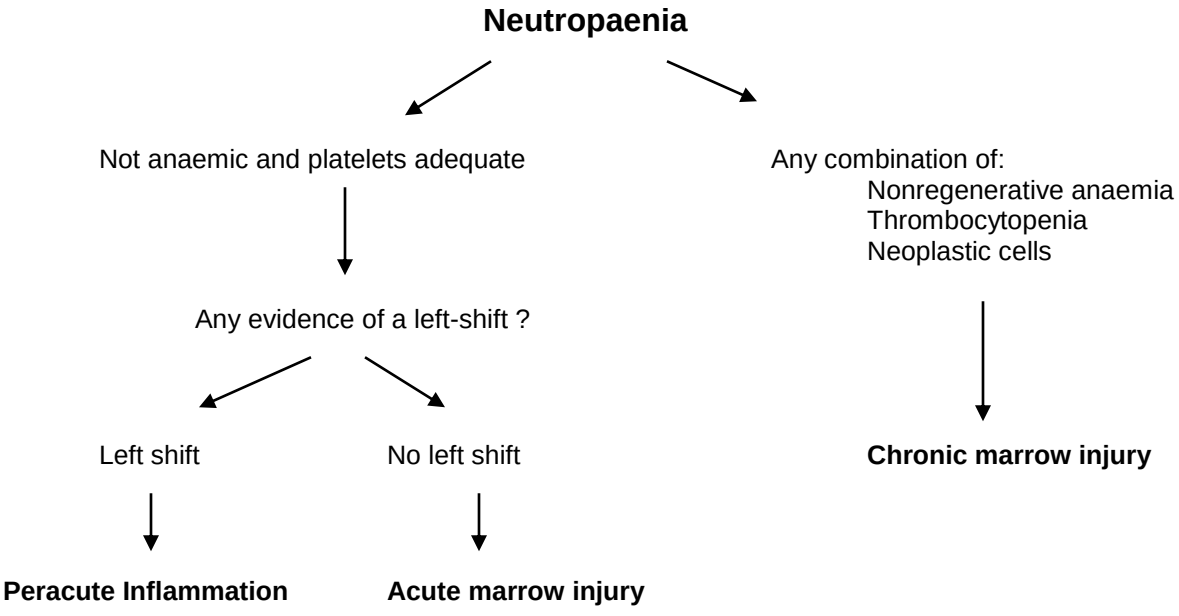
- Excitement response/adrenaline release

NOTE Examination of the lymphocyte morphology is a vital step in differentiating the possible causes of a lymphocytosis. Adequate differentiation is not possible without a blood smear examination.

Helpful Interpretive summaries




Lymphocytosis
Modest increase in small normal cells
Any concentration of large or poorly differentiated lymphocytes in a mature animal
Marked increase in small normal cells
Possible well-differentiated lymphocytic leukaemia
Poorly differentiated lymphocytic leukaemia
Excitement Response
Possible chronic immune-stimulation


Neutropaenia
Not anaemic and platelets adequate
Any combination of:
Nonregenerative anaemia
Thrombocytopenia
Neoplastic cells
Any evidence of a left-shift ?
Left shift
No left shift
Chronic marrow injury
Peracute Inflammation
Acute marrow injury

General Patterns of Leukocyte Responses

	WCC	Seg	Band	Lymph	Mono	Eos
Acute Inflammation	Increased	Increased	Increased	Decreased or no change	Variable	Variable
Chronic Inflammation	Increased or no change	Increased or no change	Increased or no change	Increased or no change	Increased	Variable
Overwhelming Inflammation	Decreased or no change	Decreased or no change	Increased	Decreased or no change	Variable	Variable
Hypersensitivity or Allergic reaction	Increased or no change	Increased or no change	No change	Decreased or no change	Variable	Increased
Excitement leukocytosis	Increased	Increased no change	No change	Mild to moderately increased	No change	No change
Stress leukogram	Increased	Increased	No change	Decreased	Increased or no change	Decreased or no change

RECOMMENDED BOOKS/REFERENCES

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