

VETERINARY CLINICAL PATHOLOGY – HAEMATOLOGY

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HAEMATOLOGY – INTRODUCTORY NOTES

The pathophysiology of abnormal haematological patterns

Understanding erythrocyte kinetics gives an insight into the pathophysiological processes which give rise to diagnostic findings in haematological profiles.

Erythrocyte production

Rubriblast → prorubricyte → rubricyte → metarubricyte → reticulocyte → erythrocyte

During this process:

- ◆ Cells become smaller
- ◆ Nuclei become smaller and the chromatin more aggregated
- ◆ Cell division stops at the late rubricyte stage when a critical intracellular concentration of haemoglobin is reached
- ◆ The nucleus is extruded at the metarubricyte stage
- ◆ Cytoplasmic colour changes from blue to orange as haemoglobin is formed and RNA is lost
- ◆ Reticulocytes and erythrocytes migrate into the venous sinus of the bone marrow through transient apertures in endothelial cell cytoplasm.
- ◆ Reticulocytes remain in marrow 2-3 days before release and then mature in the blood or spleen.
- ◆ The maturation of erythrocyte precursors is a highly co-ordinated process of mutual cytoplasmic and nuclear maturation resulting in the self destruction of the nucleus and the restriction of cytoplasmic function and organelles to the minimum necessary for a haemoglobin transporting cell.
- ◆ The process is controlled by cytokines emanating primarily from the cells of the bone marrow stroma but also from outside the marrow. The cytokine interactions are extremely complex. Important cytokines include IL3, colony stimulating factors (granulocyte/monocyte = GM-CSF and Granulocyte = G-CSF)

which stimulate the BFU-E to differentiate into the CFU-E progenitor cell. BFU-E are then stimulated by erythropoietin in concert with other cytokines.

- ◆ The ability to self renew is lost at the rubriblast stage and the ability to divide is lost at the late rubricyte stage and is controlled by the cytoplasmic haemoglobin concentration
- ◆ The bone marrow has the capacity to increase erythropoiesis (> 7x in the dog), this is primarily due to increased stem cell input and to a lesser extent by shortened maturation time. It takes approximately 4-5 days for a stem cell to progress to a reticulocyte. Cells may be delivered faster by earlier reticulocyte release and skipped divisions but these are only of temporary benefit.
- ◆ Erythropoietin is produced by the peritubular cells of the kidney in response to hypoxia.

Actions

- ◆ Stimulation of the multiplication of CFU-E progenitor cells and differentiation into rubricytes.
- ◆ Stimulation of haemoglobin synthesis in dividing erythroid cells

Features of particular clinical relevance

- ◆ **Disruption of this process may be reflected morphologically as asynchronous cytoplasmic and nuclear maturation eg., retention of a nucleus in a fully haemoglobinised cell with expanded cytoplasm (megaloblastosis) is a subtle but recognisable and powerful diagnostic criterion for bone marrow dysplasia.**
- ◆ **Stromal diseases of the bone marrow give rise to an abnormal cytokine climate which causes dysmaturation or failure of maturation – the stroma no longer “nurtures” the developing cells. Many non-regenerative anaemias are likely to be “stromal” diseases.**
- ◆ **If haemoglobin is deficient eg., in iron deficiency, the erythrocytes put in an extra division thus becoming smaller – hence the microcytosis of iron deficiency.**
- ◆ **The response to an acute loss of RBC mass is maximal after 4-5 days. Acute anaemias frequently induce a response which involves the release of immature reticulocytes and nucleated RBCs into circulation**
- ◆ **Reduced renal mass which causes erythropoietin deficiency induces a non-regenerative anaemia which is a good model for the effect of removal of a growth factor. The recent trend for therapeutic administration of human recombinant EPO to dogs and cats with non-regenerative anaemia has led to the production of anti- EPO antibodies in some cases which has induced an even more profound non-regenerative anaemia. Non-regenerative anaemias due to EPO deficiency will be characterised by the absence or greatly reduced numbers of the entire erythrocyte series in the bone marrow from erythroblasts.**
- ◆ **Thyroid hormone and growth hormone increase tissue demand for oxygen thereby stimulating EPO production.**

Erythrocyte lifespan

The lifespan in dogs is approximately 110 days, in cats 70 days.

Variation in lifespan may be involved in the interbreed differences in canine RBC parameters eg., greyhounds and sight hounds tend to have higher RBC counts than other breeds, these dogs also have a shorter RBC lifespan of 70 days.

Clinical relevance of lifespan

Blood loss (haemorrhage or haemolysis) anaemias can be viewed as disorders which reduce RBC lifespan thereby creating an imbalance between RBC production and destruction which reduces the RBC count at any particular time and requires an increase in production to restore the balance. This concept is very useful in understanding the pathogenesis and also the means of treating immune mediated haemolytic anaemias. Haemolytic anaemias which are poorly regenerative (often because precursors are also destroyed) respond more slowly to treatment than regenerative cases partly because the modes of treating IMHA (eg., corticosteroids) primarily rely on reducing the rate of peripheral RBC destruction rather than increasing production. Treatments which reduce destruction but at the same time suppress production (eg., chemotherapeutic agents such as cyclophosphamide) are less likely to be successful than treatments which reduce destruction without suppressing production.

Destruction of erythrocytes

During the process of differentiation RBCs lose many of the more general functions of cells in order to become a highly specialised cell for haemoglobin transport. This includes such fundamental changes as loss of the nucleus and the mitochondria (no oxidative metabolism). The metabolic processes of the RBC are therefore very simple and it is the progressive failure of these basic pathways which leads to RBC senescence. The senescent RBC is unable to survive in the rigorous climate of the circulation and is removed and destroyed by macrophages in the liver and spleen.

RBC Senescence

- ◆ Reduced deformability (membrane)
- ◆ Reduced maintenance of cell shape
- ◆ Abnormal internal fluidity of haemoglobin
- ◆ Reduced membrane viscoelastic properties
- ◆ Reduced energy generation
- ◆ Inability to maintain membrane integrity and ionic gradients
- ◆ Inability to repair oxidative damage
- ◆ Loss of membrane surface charge

- ◆ Changes in membrane permeability
- ◆ Increased osmotic fragility

Osmotic fragility is proportional to the distensibility of the RBC which is dependent upon the amount of membrane present. Therefore the greater the MCV the greater the osmotic resistance. Hence species differences in osmotic fragility and also the fragility of abnormal RBCs such as spherocytes.
- ◆ Expression of age-specific antigen on the surface of RBCs leads to binding of an anti-erythrocyte IgG which is then recognised by phagocytic macrophages and leads to phagocytic removal of the senescent RBC.

Clinical relevance

- ◆ **In immune mediated haemolytic anaemias RBCs are not only removed from circulation by phagocytes but the cells which are damaged by partial phagocytosis (spherocytes) are more fragile and more likely to lyse in circulation.**
- ◆ **Activated neutrophils and oxidative damage may alter the antigenic properties of the RBC membrane leading to erroneous expression of antigens which result in haemolytic destruction. This is the rationale for defining immune mediated haemolytic anaemia as a “reactive disease” induced by factors such as infections or immunostimulation rather than as a “primary autoimmune disease”.**

Metabolic pathways of the mature erythrocyte

- ◆ Embden-Meyerhof pathway – this replaces oxidative metabolism as the pathway for generation of ATP and NADH. The ATP is essential for the maintenance of membrane function whereas NADH is used to reduce methaemoglobin (oxidised haemoglobin).

Clinical relevance

Progressive failure of this pathway due to loss of enzymes leads to energy deficiency, loss of membrane integrity and RBC senescence. The enzymes cannot be maintained or resynthesised because the protein synthesis pathways of the RBC have been removed during maturation. Enzyme deficiencies in this pathway can lead to membrane failure and haemolytic anaemia

- ◆ **Pyruvate kinase deficiency – Basenji, beagle, westie, cairn. Energy deficient RBCs have reduced lifespan. Reduced exercise tolerance from 6 months, pallor, and splenomegaly. Moderate to marked anaemia with persistent marked reticulocytosis. Spherocytes absent. Death by 4 years of age from myelofibrosis or osteosclerosis (bone marrow stromal disease) with hepatic haemosiderosis.**
- ◆ **Phosphofructokinase deficiency – English Springer spaniel. Energy deficient RBCs have reduced lifespan. Persistent compensated haemolytic anaemia – low/normal RBC parameters with**

persistent reticulocytosis. Haemolytic crises induced by episodes of alkalosis. Alkaline fragility of RBCs due to reduced 2,3 DPG which makes the RBC susceptible to increases in intracellular pH. Aged animals develop a severe progressive myopathy and abnormal polysaccharide deposits.

- ◆ **Hexose –monophosphate pathway – maintains reduced glutathione. Reduced glutathione neutralises oxidants before they can denature haemoglobin..**

Clinical relevance – deficiencies in this pathway lead to oxidative susceptibility, formation of heinz bodies and anaemia.

- ◆ **Methaemoglobin reductase pathway – maintains haemoglobin in a reduced state necessary for oxygen transport. Enzyme deficiencies lead to methaemoglobin accumulation and cyanosis.**
- ◆ **Luebering-Rapoport pathway – formation of 2,3 DPG. Increased DPG favours oxygen release to tissues by lowering oxygen affinity of haemoglobin. Anaemic animals usually have increased 2,3 DPG.**

Pathways of RBC destruction

Two routes:

- ◆ **Phagocytosis by macrophages (major route). In the phagosome haemoglobin is released and broken down into haem and globin. Iron is released and stored as ferritin or haemosiderin within the macrophage. Globin is broken down into constituent amino acids and recycled. Haem is cleaved to biliverdin and carbon monoxide. Biliverdin is reduced to bilirubin and excreted from the cell into blood where it binds albumin and is transported to the liver.**
- ◆ **Intravascular lysis with release of haemoglobin into plasma (minor route). Haemoglobin released into plasma binds to haptoglobin (an alpha 2 globulin). Complex is removed by the liver preventing entry of haemoglobin into urine.**

Clinical relevance

Haptoglobin is usually sufficient to bind 150 mg/dl of haemoglobin. Discolouration of plasma occurs when haemoglobin is 50–100 mg/dl, therefore discolouration of plasma occurs before haemoglobinuria. If haptoglobin becomes saturated the free haemoglobin splits into dimers which pass the glomerular filter. It is then reabsorbed by the proximal tubules and metabolised. Unabsorbed haemoglobin passes into the urine causing haemoglobinuria. In haemolytic anaemia the same routes of removal are used but one or the other will predominate.

- ◆ **Fate of bilirubin – Unconjugated bilirubin leaves the macrophage and returns to the liver bound to albumin – in the bound state it cannot pass the glomerular filter. In the liver bilirubin is conjugated.**

Clinical relevance

Conjugated bilirubin released into the blood instead of the bile in situations of cholestasis can pass the glomerular filter and in dogs there is a low renal threshold, hence cholestasis sufficient to cause bilirubinuria can be present without hyperbilirubinaemia. But in haemolysis the source of conjugated bilirubin is unconjugated bilirubin within the plasma, therefore hyperbilirubinuria without concurrent hyperbilirubinaemia is most unlikely.

A diagnostic concept of erythrocytes:

Simple membrane bound pods which originate in bone marrow and during their lifetime visit every tissue and capillary bed in the body. Morphological features of the erythrocytes either acquired during differentiation or during circulating life inform the diagnostician of the status of the tissues, circulation and bone marrow.

Haematological patterns

Cell counts

- ◆ The kinetics of entry and loss of cells from circulation

Morphology

- ◆ The status of individual cells which is a direct reflection of the health of the bone marrow, the circulation and the tissues

Red blood cell morphology

Clinically important abnormalities

- ◆ **Hypochromic RBCs** – flatten more than normochromic cells in a film. Show a thin rim of haemoglobin around an increased pale central concavity. Are deficient in haemoglobin and therefore put in an extra division during maturation becoming smaller cells (microcytes). In iron deficiency cells become microcytic before becoming hypochromic.
- ◆ **Stomatocytes and target cells** – RBCs with increased membrane in comparison to content and therefore adopt different folded conformations appearing mouth-like or like targets when viewed in 2 dimensions. All reticulocytes have excessive surface membrane and can appear like polychromatic stomatocytes or target cells and this finding is irrelevant. When mature RBCs adopt these shapes it usually indicates that the membrane lipids have altered in response to eg., chronic inflammation or liver disease.
- ◆ **Reticulocytes** – large, folded polychromatic cells. These are the primary indicators of RBC regeneration in cats and dogs.

- ◆ **Echinocytes or Burr cells** – these are crenated cells with uniform circumferential spiculation. They occur in renal disease but the most common cause is RBC dehydration/EDTA artefact in aged blood. Rattle snake envenomation causes all the RBCs in circulation to become echinocytes for 24-36 hours. The effect is dose dependent and due to phospholipase A2 in the venom.
- ◆ **Acanthocytes** – shrunken RBCs with uneven, assymetrical spiculation. This is a special form of RBC fragmentation. Acanthocytes are important early markers of vascular anomalies such as haemangiosarcoma or microvascular angiopathies. They also accompany chronic liver disease.
- ◆ **Spherocytes** – these are RBCs which have had pieces of membrane removed by macrophages, the biconcave shape is lost and the cells become small dark spheres with no central pallor. Spherocytes are the hallmark of immune mediated damage to RBCs. They are a more powerful diagnostic criterion than a positive coombs test. Incomplete sphere formation occurs with partial loss of the biconcave shape. Such cells have reduced central pallor when viewed in 2 dimensions.
- ◆ **Keratocytes** – these cells have developed a membranous blister on one side which ruptures leaving claw-like spikes. This is a form of fragmentation in response to chemical and physical injury.
- ◆ **Eccentrocytes** – these cells have undergone oxidative damage causing adhesion of opposing membranes. This squeezes out the haemoglobin from one side of the cell. Such cells appear clear at one end and dark at the other when viewed in 2 dimensions. This is a good marker for oxidative anaemias in dogs. Where cats form Heinz bodies, dogs form eccentrocytes. The most common cause of eccentrocyte formation in dogs is onion poisoning.
- ◆ **Heinz bodies** – these are non-staining (with Romanowsky stains) particles of denatured haemoglobin. They stain basophilic with new methylene blue. They are common in cats, which are particularly susceptible to oxidative damage to RBCs.
- ◆ **Howell Jolly bodies** – these are nuclear remnants which result from incomplete extrusion of the nucleus from maturing reticulocytes. They are present in normal blood films but increased numbers tend to be seen in splenectomised patients.
- ◆ **Nucleated RBCs. Metarubricytes and rubricytes** frequently appear in regenerative anaemias. The presence of nucleated RBCs in the absence of polychromasia is a strong indicator of bone marrow dysfunction. It may also occur in patients with splenic diseases or splenectomy. Normal dogs sometimes have occasional metarubricytes in circulation and a low level of polychromasia. Identifying megaloblastic RBCs or abnormalities in nuclear cytoplasmic maturation in nRBCs in the peripheral circulation is a very useful indicator of bone marrow dysfunction/dysplasia. It may be termed erythremic myelosis. This is of particular relevance in cats with FELV. The presence of nRBCs +/- basophilic stippling without polychromasia is a feature of severe lead poisoning.
- ◆ **Erythroblasts and prorubricytes** are usually markers of myelodysplasia or myeloproliferative disease/erythroid leukaemia.
- ◆ **Basophilic stippling** – this is quite a common finding in regenerative anaemias in cats and can be interpreted as a feature of RBC regeneration in this species.

- ◆ **Parasites – these are rare in the UK. The most common entity is Haemobartonella Felis which has a patchy distribution and is a very difficult microscopic diagnosis. Microscopy is an insensitive way of detecting the parasite. The organisms fall off the RBCs in anticoagulants.**
- ◆ **Inclusion bodies – distemper inclusions – a rare finding in severe distemper cases.**

A clinical approach to leukocyte kinetics

Granulocytes

A short-lived cell which leaves the bone marrow on a one way journey to the tissues

Bone marrow production

- ◆ proliferation/mitotic compartment

Pluripotential stem cell gives rise to myeloid stem cell which forms the granulocyte/monocyte colony forming units (progenitors) which form myeloblasts under the influence of GM-CSF and G-CSF. Myeloblasts differentiate in a continuous sequence through named (recognisable) stages – promyelocyte and myelocyte. Three divisions occur in the myelocyte stage, therefore most of the increase in cell numbers occurs at this stage. Proliferation and maturation run at the same time in this compartment. Transit time is 2.5 days. Approximately 20% of neutrophils are in this compartment at any one time in healthy mammals. In healthy dogs up to 20% of granulopoiesis is ineffective with premature death of myelocytes.

- ◆ Storage/maturation compartment

Metamyelocytes, bands and mature segmented neutrophils are non-replicating stages which make up this compartment. All can function as effector cells of the immune system. Approximately 80% of neutrophils in bone marrow are in this compartment, this represents a 5 day supply. Release is orderly and age-dependent in health. Premature release of bands in inflammation results in a peripheral left shift.

- ◆ Release from marrow

Mediated by IL1 and tumour necrosis factor.

Increased release from storage is responsible for rapid neutrophilia (earlier than 2 days)

This causes a shift to the left and a relative increase in the proliferative pool as the storage pool becomes depleted.

With intense acute peripheral neutrophil demand the left shift will intensify and metamyelocytes or even earlier stages may appear in circulation. A left shift indicates decreased granulocyte reserve.

Increased recruitment of stem cells into the proliferative compartment occurs at the earliest demand for neutrophils but 3-5 days are needed for the increased stem cell recruitment to influence the number of circulating neutrophils.

In response to demand the proliferative department may undergo extra divisions and reduced myelocyte attrition (ineffective granulopoiesis). The effect of increased divisions and reduced ineffective granulopoiesis is realised in the peripheral circulation within 2-3 days.

Neutrophil kinetics in the circulation

- ◆ Adhesion molecules (see section on drivers and controllers of neutrophil kinetics) on neutrophils and endothelial cells cause neutrophils in the post capillary venules to move sluggishly in the periphery of the axial flow of blood. Consequently the concentration of neutrophils in the post capillary venules is higher than that in the large vessels. Neutrophils which are partially adherent to the endothelium are the marginal neutrophil pool. These do not appear in the neutrophil count in blood taken from a large vessel.
- ◆ Neutrophils moving as fast as erythrocytes and plasma within arteries and veins are the circulating neutrophil pool. The circulating pool approximately equals the marginal pool in dogs but in cats the marginal pool has 3x as many neutrophils as the circulating pool.

Clinical relevance

Release of neutrophils from the marginal pool by eg., increased blood flow due to catecholamines will increase the neutrophil count without any evidence of a left shift.

The average transit time for a neutrophil through the blood in health is approximately 10 hours, all blood neutrophils are replaced 2.5 x each day.

Neutrophils migrate unidirectionally into tissues in response to “chemokines”

In health aged neutrophils are removed by macrophages of spleen, liver and bone marrow. Some are lost in secretions and across mucus membranes.

Models of neutrophilia and neutropenia.

Three factors govern blood neutrophil concentration:

- ◆ Rate of release from bone marrow
- ◆ Distribution between marginal and circulating pools
- ◆ Rate of emigration into tissues

Clinical relevance

- ◆ **Corticosteroid administration increases bone marrow release rate of neutrophils by 3 – 7.5 x and reduces neutrophil adherence shifting neutrophils from the marginal pool into the circulating pool.**

- ◆ **Early gram positive bacterial infections are accompanied by accelerated release of neutrophils from the bone marrow, expanded total neutrophil and marginal neutrophil pools, decreased circulating half life but with a normal circulating neutrophil pool = masked neutrophilia.**
- ◆ **Neutropenia in infections**
 - ◆ **Endotoxin causes a rapidly developing and transient neutropenia due to reduced circulating half life, increased margination and enhanced emigration. Margination and tissue demand outstrip production leading to neutropenia.**
 - ◆ **Infections involving organs with very large surface area such as peritoneal and pleural membranes, lungs, GI tract – extravasation occurs over a wide front and leads to massively increased demand and neutropenia.**

Drivers and controllers of neutrophil kinetics

- ◆ Controllers – chemokines and cytokines
- ◆ Drivers – adhesion molecules - selectins, integrins and their receptors

Chemokines

Chemokines control precisely the attraction of leukocytes to tissue in inflammation. They are chemotactic cytokines. Forty have been identified to date eg., Interleukin 8, macrophage inflammatory protein (MIP), Lymphotactin, Rantes. Alpha chemokines attract neutrophils and lymphocytes. Beta chemokines attract monocytes, basophils, eosinophils and lymphocytes.

- ◆ Chemokines provide the directional cues for the movement of leukocytes in development, homeostasis and inflammation
- ◆ Control the continuous circulation of lymphocytes through blood, tissues and lymphatics
- ◆ Control normal movement of macrophages, mast cells and eosinophils into tissues
- ◆ Selectively recruit leukocytes into inflamed tissues. Nearly all cells can secrete chemokines given the appropriate stimulus and thereby recruit leukocytes into the area eg., the concentration of a specific chemokine in broncho-alveolar lavage fluid from a patient with sarcoidosis correlated directly with the number of activated T-lymphocytes recruited into the fluid.
- ◆ Chemokines acting on eosinophils are found in increased concentrations in atopic dermatitis, allergic rhinitis and asthma. They are the molecular link between antigen-specific immune activation and the migration of eosinophils into tissues.
- ◆ Chemokine “patterns” or “climate” control the inflammatory patterns of leukocytes in tissue and therefore in circulation.

Cytokines

- ◆ Principally produced by activated lymphocytes and macrophages
- ◆ Modulate the function of other cell types

- ◆ Cytokine mediators of inflammation eg., IL1, Tumour necrosis factor (TNF)
 - ◆ secreted by activated macrophages
 - ◆ secretion stimulated by endotoxin, immune complexes and physical injury
 - ◆ effects on endothelium, leukocytes, fibroblasts and induction of systemic acute phase reactions
 - ◆ Endothelium - endothelial activation – synthesis of endothelial adhesion molecules, growth factors, eicosanoids and nitric oxide

- ◆ Cytokines control haematopoiesis eg., colony stimulating factors, IL2, IL3, IL4 and IL5. Production and differentiation of eosinophils is controlled by IL4 and IL5.

Adhesion molecules

Integrins and selectins

- ◆ Selectins form loose attachments to circulating leukocytes allowing them to marginate and roll along the endothelium
 - ◆ Early leukocyte rolling is mediated by rapid redistribution of P-selectin from Weibel-Pallade bodies in endothelial cells.
 - ◆ Later selectin production is induced by cytokines eg., expression of e-selectin by endothelium and L-selectin expression by leukocytes.

- ◆ Integrins – expression and activation is under the control of chemokines
 - ◆ Mediate firm adhesion of leukocytes to endothelium
 - ◆ Examples – mac1 = CD11/CD18 and ICAM1 (receptor)

Sequence of events

- ◆ Rapid and relatively loose adhesion causes rolling and is mediated by selectins
- ◆ Leukocyte activation by endothelial mediators and chemokines increases the avidity of integrin binding and leads to firm adhesion of the leukocytes
- ◆ Leukocytes undergo stable binding to the endothelium through beta 2 integrins and ICAM, then undergo pavementing (flatten against endothelium) and then transmigrate between endothelial cells.
- ◆ **Leukocyte adhesion deficiency (LAD) – Irish Setters. This is due to lack of Mac 1 integrins. Affected dogs develop extreme neutrophilic leukocytosis (> 100,000)**

Monocyte kinetics

Maturation sequence – CFU-GM - monoblast – promonocyte – monocyte.

- ◆ At least 3 divisions occur during the sequence.
- ◆ Maturation time is rapid (24-36 hours)
- ◆ Released straight into blood – no marrow storage pool
- ◆ Blood transit time approx 24-36 hours.

Clinical relevance

Monocytes are released into circulation early in acute inflammation as well as being a cardinal feature of chronic inflammation. When the neutrophil storage pool is rapidly and severely depleted in massive neutrophil consumption, monocytes may be the first cell to respond to the challenge with increased peripheral blood cell counts. Monocytes effectively replace neutrophils in situations of extreme neutrophil consumption. Monocytopenia is of little interpretive significance in blood profiles

Monocytes circulate and enter tissues where they are transformed into macrophages.

- ◆ Tissue macrophages are long-lived and have the ability to replicate, differentiate into eg., kupffer cells, or be activated to form activated macrophages, epithelioid cells or giant cells. The activated macrophage is a “dangerous beast”
 - ◆ Increase in cell size
 - ◆ Increased lysosomal enzymes
 - ◆ More active metabolism
 - ◆ Increased ability to phagocytose and kill microbes
- ◆ Numerous tissues contain cells of monocyte origin bearing different names
 - ◆ Macrophages of exudates
 - ◆ Alveolar macrophages
 - ◆ Connective tissue histiocytes
 - ◆ Macrophages of spleen, bone marrow, lymph nodes (dendritic cells)
 - ◆ Kupffer cells of liver
 - ◆ Langerhans cells, dendritic cells (skin)
 - ◆ Osteoclasts of bone
 - ◆ Microglia of the CNS

Tissue macrophages are the “prima donnas” of inflammation. They orchestrate the sequence of responses to tissue invasion by a combination of:

- ◆ a vast arsenal of chemical mediators for the control of other cells
 - ◆ cytokines
 - ◆ chemokines
 - ◆ growth factors

- ◆ angiogenesis factors
- ◆ fibrosis factors
- ◆ direct weaponry for the destruction of tissues and pathogens
 - ◆ toxic O₂ metabolites
 - ◆ proteases
 - ◆ coagulation factors
 - ◆ arachidonic acid metabolites
 - ◆ nitric oxide
- ◆ central role as antigen presenting cells for immune recognition
- ◆ phagocytic activity
 - ◆ effective phagocytes of debris, old blood and effete cells
 - ◆ less effective than neutrophils in phagocytosis of pathogens

Clinical relevance

Chronic inflammation is frequently associated with marked tissue damage. Some pathogens survive and replicate in macrophages leading to disseminated infection and unbridled chronic inflammation eg., leishmaniasis, ehrlichiosis. Transformation of monocytes to macrophages in circulation is a sign of a severe and well established chronic inflammatory process eg., endocarditis. The presence of increased erythrophagia in bone marrow or even in circulation is a sign of severe immune mediated haemolytic anaemia. Characteristic features of cells in malignant histiocytosis include vacuolation and evidence for phagocytic activity.

Eosinophil kinetics

Maturation sequence – GM-CFU – myeloblast – promyelocyte – eosinophilic myelocyte – eosinophilic metamyelocytes – eosinophilic bands – mature eosinophils. Eosinophils cannot be identified before the myelocyte stage.

Production and maturation parallel neutrophils

- ◆ there is a storage pool of eosinophils and eosinophilic left shifts do occur but are quite infrequently recognised.
- ◆ IL5 is the major cytokine controlling eosinophil production, GM-CSF and IL3 also have roles.
- ◆ Transit time in peripheral blood is short (T_{1/2} 30 mins)
- ◆ There is a marginal pool
- ◆ The destination of eosinophils is subepithelial sites in skin, lung and GI tract.
- ◆ Eosinophils occasionally recirculate

- ◆ Active in defence against parasites and hypersensitivity reactions. Are capable of phagocytosis but less effective killers of bacteria than neutrophils. Are recruited by various neoplasms - mast cell tumour, lymphoma, leukaemias.
- ◆ Corticosteroids cause sequestration of eosinophils into tissues and inhibit release of eosinophils from bone marrow.

Basophil kinetics

Quantitatively of little significance in blood profiles.

Maturation parallels other granulocytes. Unrecognisable before the myelocyte stage.

Bone marrow storage is minimal.

- ◆ Although similar in function there is no evidence to suggest that basophils are the precursors of tissue mast cells
- ◆ No proof of a common mast cell/basophil progenitor
- ◆ Basophilia is rare but may accompany parasitisms. It can also be a marker for myeloproliferative disease (sometimes in combination with thrombocytosis).

Lymphocyte kinetics

Pluripotential stem cell gives rise to the myeloid stem cell and the lymphoid stem cell. The myeloid stem cells gives rise to CFO-GM. Lymphoid stem cells give rise to T and B lymphocytes.

T-lymphocytes are derived from bone marrow and mature in the thymus and function in cell mediated immunity

B-lymphocytes are derived from the bone marrow and function in humoral immunity. B and T-cells are distinguished by surface markers.

Kinetics of B-lymphocytes

- ◆ Bone marrow
- ◆ Lymphoid stem cell – pre-B cell – immature B-cell – mature B-cell (stages identified by rearrangement of surface markers)
- ◆ Mature B-cell homes in on secondary lymphoid tissues eg., lymph node follicles, splenic white pulp, tonsils, peyers patches etc.
- ◆ Mature B-cell undergoes antigen-induced transformation which is a combination of proliferation and differentiation
 1. Small lymphocyte
 2. Small cleaved cell

3. Large cleaved cell
 4. Small non-cleaved cell
 5. Large non-cleaved cell
 6. Immunoblast
 7. Plasma cell/memory B-cell
- ◆ Untransformed B-cells and memory B-cells may recirculate (in the minority compared to recirculating T-cells)
 1. Efferent lymph
 2. Thoracic duct
 3. Blood
 4. Postcapillary venules of the cortex of lymph nodes
 5. Lymphoid parenchyma
 6. Efferent lymph

Kinetics of T-lymphocytes

- ◆ Bone marrow
 - ◆ Lymphoid stem cell – pre-Tcell
- ◆ Pre-Tcell migrates to the thymus – combination of maturation and proliferation occurs
 - ◆ Early precursor cell
 - ◆ Intermediate precursor cell
 - ◆ Immediate precursor cell
 - ◆ Mature T-cell
- ◆ Many T-cells are wasted in the thymus, the remainder leave the thymus and home in on secondary lymphoid tissues eg., paracortical areas of lymph nodes, periarteriolar sheaths of spleen.
- ◆ T-cells which encounter their pre-programmed cell bound antigen transform through a similar process to B-cells involving proliferation and differentiation.
- ◆ T-cells form the majority (60-70%) of the recirculating lymphocyte population.

Functions

- ◆ CD4+ T-cells (T-helper 1 and 2) regulate virtually all the other cells of the immune system including cytotoxic T-cells (CD8+ cells), B-cells, macrophages and NK cells
- ◆ CD8+ T-cells are primarily cytotoxic cells involved in cell-mediated immunity and graft rejection.

Clinical relevance

Quite simple interpretive criteria are applied to numerical changes in peripheral lymphocyte counts despite the complexity of the underlying kinetics. Understanding the kinetics of lymphocytes is important in the complex area of lymphoproliferative diseases.

Briefly:

- ◆ Lymphocyte leukaemias are neoplasms of the bone marrow stem cells
- ◆ Lymphomas are neoplasms of the secondary lymphoid organs
- ◆ Lymphomas are therefore, neoplasms of lymphocytes undergoing transformation and present with widely varying morphology eg: small cleaved cell type, immunoblastic type, large non-cleaved type, myeloma
- ◆ This accounts for the diverse morphology of neoplastic lymphocytes. Cells classified as lymphoblasts make up only a small percentage of the morphological variation to be found in lymphomas.
- ◆ The cytological diagnosis of lymphoma requires the identification of a monomorphic population of lymphoid cells apparently ousting the normal populations of the lymph node. If the the only criterion for diagnosing lymphoma is the identification of a certain percentage of lymphoblasts (as quoted in many cytology texts), the diagnosis will be missed in a significant proportion of cases.

Neutrophil Toxicity

This is a group of morphological abnormalities useful for discriminating between inflammatory and neoplastic processes. In severe toxæmic states granulopoiesis becomes suppressed and neutrophil morphology is altered by both maturation defects and the direct effects of toxins and lysosomal enzymes. These are termed toxic changes- in order of increasing toxicity –

- ◆ Dohle bodies – remnants of rough ER resulting from defective cytoplasmic maturation. Angular, poorly defined basophilic bodies in outer regions of the cytoplasm. Small numbers of neutrophils containing dohle bodies are normal especially in cats.
- ◆ Foaminess (muddy coloured) of the cytoplasm. Intracytoplasmic release of lysosomal enzymes and restricted cytolysis.
- ◆ Increased cytoplasmic basophilia. Increased cytoplasmic ribosomal RNA.
- ◆ Cytoplasmic vacuolation (note that this is a common sample ageing change in normal neutrophils). Due to lysosomal cytolysis.
- ◆ Inappropriate appearance of intracytoplasmic azurophilic granules. These are primary granules which are retained instead of being lost during maturation.
- ◆ Other maturation defects – nucleus undergoes maturation without cell division. Formation of giant bands, mature neutrophils with bizarre twisted/ribbon-like nuclei, neutrophils with ring form/doughnut nuclei.

CASE REPORTS IN CLINICAL PATHOLOGY

Haematology

A jaundiced dog

◇ Species Canine
◇ Breed Cocker
◇ Age 11 Years

Haematology

◇ RBC	1.51	Lo	5.0 -8.5
◇ Hb	6.0	Lo	12.0-18.0
◇ HCT	9.6	Lo	37.0-55.0
◇ MCV	63.0		60.0-80.0
◇ MCH	39.6	Hi	19.0-23.0
◇ MCHC	62.7	Hi	31.0-34.0
◇ Platelets	182	Lo	200 -500
◇ WBC	20.0	Hi	6.0 -15.0
◇ Neutrophils	16.3	Hi	3.0-11.5
◇ Bands	0.18		0.0-0.3
◇ Lymphocytes	0.71	Lo	1.0-4.8
◇ Monocytes	0.18		0.0-1.3
◇ Eosinophils	0.36		0.0-1.25
◇ nRBC	3.7		0.0-4.0

◇ Comment - Red cells are a mixture of hypochromic polychromatic cells and spherocytes. NRBC's ++. Platelet count appears normal in the film. Haemolysed RBCs ++. Occasional monocytes contain phagocytosed RBCs. Reactive lymphocytes +. Agglutination +

A 12 year old female retriever with anaemia, weakness and spondylosis

◇ RBC	2.8	Lo	5.0 -8.5
◇ Hb	7.0	Lo	2.0-18.0
◇ HCT	20	Lo	37.0-55.0
◇ MCV	71.0		60.0-80.0
◇ MCH	24.6		19.0-26.0
◇ MCHC	34.4		31.0-37.0
◇ Platelets	29	Lo	200-500

◇ WBC	4.2	Lo	6.0-15.0
◇ Neutrophils	0.13	Lo	3.0-11.5
◇ Bands	0.08		0.0-1.3
◇ Lymphocytes	3.47		1.0-4.8
◇ Monocytes	0.0		0.0-1.3
◇ Eosinophils	0.0		0.0-1.25
◇ Other cells	0.55		
◇ Retics% 0.4			
◇ Retic count	11.3		
◇ Coombs test	Pos 1/512		
◇ Comment - Film confirms platelet count, blast cells occ, atypical lymphocytes +, scanty polychromatic cells, anisocytosis ++, spherocytes +			

An emaciated rescue dog with very severe flea infestation

- ◇ Owner RSPCA
- ◇ Species Canine
- ◇ Breed GSDX
- ◇ Age 8 Years

Haematology

◇ RBC	5.32		5.0-8.5
◇ Hb	5.0	Lo	12.0 -18.0
◇ HCT	17.5	Lo	37.0 -55.0
◇ MCV	33.0	Lo	60.0 -80.0
◇ MCH	9.3	Lo	19.0-23.0
◇ MCHC	28.3	Lo	31.0-34.0
◇ Platelets	4119	Hi	200-500
◇ WBC	18.21	Hi	6.0-15.0
◇ Neutrophils	14.9	Hi	3.0 -11.5
◇ Lymphocytes	2.19		1.0-4.8
◇ Monocytes	1.09		0.0-1.3
◇ Eosinophils	0.00		0.0-1.25
◇ Haematologist Comment Markedly hypochromic. Schistocytes/fragments +++ Platelets increased, many giant forms present.			

Haematological monitoring following iron replacement

◇ RBC	6.70		5.0-8.5
◇ Hb	7.2	Lo	12.0-18.0
◇ HCT	22.9	Lo	37.0-55.0
◇ MCV	34.0	Lo	60.0-80.0
◇ MCH	10.7	Lo	19.0-23.0
◇ MCHC	31.3		31.0-34.0
◇ Platelets 4235	Hi		200-500
◇ WBC	12.92		6.0-15.0
◇ Neutrophils	6.86		3.0-11.5
◇ Bands	0.40	Hi	0.0-0.3
◇ Lymphocytes	4.44		1.0-4.8
◇ Monocytes	1.21		0.0-1.3
◇ Eosinophils	0.00		0.0-1.25

◇ Haematologist Comment Massive red cell fragmentation schistocytes. Platelets greatly increased with large forms. Pleomorphic lymphocytes. Microcytosis and hypochromia

◇ RBC	8.72	Hi	5.0-8.5
◇ Hb	11.7	Lo	12.0-18.0
◇ HCT	35.8	Lo	37.0-55.0
◇ MCV	41.0	Lo	60.0-80.0
◇ MCH	13.4	Lo	19.0-23.0
◇ MCHC	32.6		31.0-34.0
◇ Platelets 2087	Hi		200-500
◇ WBC	14.34		6.0-15.0
◇ Neutrophils	9.79		3.0-11.5
◇ Bands	0.68	Hi	0.0-0.3
◇ Lymphocytes	3.64		1.0-4.8
◇ Monocytes	0.23		0.0-1.3
◇ Eosinophils	0.00		0.0-1.25

◇ Haematologist Comment - Fragments/schistocytes still present ++. Polychromasia +. Platelet count appears increased in the film.

An elderly dog with episodes of unexplained pallor and reduced exercise tolerance

- ◇ Species Canine
- ◇ Breed Cocker Spaniel
- ◇ Age 10 years
- ◇ Sex Female

- ◇ Bouts of pallor and weakness
- ◇ Quite rapid resolution followed by normality
- ◇ No apparent trigger factors for bouts

Haematology

◇ RBC	4.89	Lo	5 – 8.5
◇ Hb	11.5	Lo	12 – 18
◇ HCT	35.0	Lo	37 – 55
◇ MCV	72		60 – 80
◇ MCH	24.3		19 – 26
◇ MCHC	33.9		31.5 – 37
◇ Platelets	202		160 – 500
◇ WBC	7.8		6.0-15.0
◇ Neutrophils	6.33		3 – 11.5
◇ Bands	0		<0.3
◇ Lymphocytes	1.03		1.0 – 4.8
◇ Monocytes	0		0 – 1.3
◇ Eosinophils	0.44		0 – 1.25
◇ nRBCS	0.28		0 – 4.0

◇ Comment Polychromasia +, Anisocytosis +, Occ Burr cell, Eccentrocytes +, Platelet count appears normal in the film

Test Patterns

These two haematological profiles appear very similar but actually reflect two completely different pathological processes. The underlying disease process can be discerned by careful interpretation of the profiles.

In one case the granulocyte series has undergone malignant transformation – genetic damage, release of oncogenes or dysfunction of cancer suppressor or apoptosis genes, multistep carcinogenesis.

In the other case the granulocyte series is responding to an intense inflammatory “chemokine climate “ by increased synthesis, margination and tissue infiltration.

Pattern 1

RBC	7.0 x 10 ⁹ /μl
HCT	45 l/l
MCV	64μl
MCHC	31
HB	14g/l
Platelets	250 x10 ⁶ /μl

Pattern 2

RBC	7.0 x 10 ⁹ /μl
HCT	45 l/l
MCV	64μl
MCHC	31
HB	14g/l
Platelets	160 x10 ⁶ /μl

WBC 80 x10⁶/μl
 Neuts 63 x10⁶/μl
 Bands 10 x10⁶/μl
 Lymphs 2 x10⁶/μl
 Monos 1.0 x10⁶/μl
 Eos 0.7 x10⁶/μl
 Metamyel 2 x10⁶/μl
 Myelos 1 x10⁶/μl
 Blasts 0.5 x10⁶/μl

WBC 80 x10⁶/μl
 Neuts 64 x10⁶/μl
 Bands 10 x10⁶/μl
 Lymphs 0.8 x10⁶/μl
 Monos 1.7 x10⁶/μl
 Eos 0 x10⁶/μl
 Metamyel 2 x10⁶/μl
 Myelos 1 x10⁶/μl
 Blasts 0.5 x10⁶/μl

Morphology

Occasional eosinophilic metamyelocytes

Morphology

>30% dohle bodies

A young dog with acute lameness, conjunctival haemorrhages, neck pain and rapid deterioration in mentation

◇ Species Canine

◇ Breed Boxer

◇ Age 1 year

◇ Intense neck and head pain responsive to dex

◇ Conjunctival haemorrhages

◇ Haematoma between scapulae

◇ Joint pain

◇ Large right cerebral haemorrhage on MRI

◇ Increased buccal mucosal bleeding time

Biochemistry and haematology

◇ Total protein 75 54-77

◇ Albumin 31 25-37

◇Globulin		44		25-52
◇Total calcium	2.6			2.0-3.0
◇Phosphate		2.3	Hi	0.8-1.6
◇Urea	8.3			2.0-9.0
◇Creatinine		92		40-106
◇Alk Phos		109	Hi	0-50.0
◇ALT	58	Hi		0-25.0
◇Total bili		17		0.0-20
◇Bile acids		48		0.0-50
◇Glucose		5.6		2.0-5.7
◇CK		140	Hi	0.0-90
◇Cholesterol		5.1		3.8-7.0
◇RBC	4.9	Lo		5.0-8.5
◇Hb		10.8	Lo	12.0-18.0
◇HCT	33	Lo		37.0-55.0
◇MCV	68.0			60.0-80.0
◇MCH	22.1	Hi		19.0-23.0
◇MCHC		32.6	Hi	31.0-34.0
◇Platelets		200		200-500
◇WBC	29.3	Hi		6.0-15.0
◇Neutrophils		26.0	Hi	3.0-11.5
◇Lymphocytes	1.17			1.0-4.8
◇Monocytes		1.76	Hi	0.0-1.3
◇Eosinophils		0.0		0.0-1.25
◇Basophils		0.29		0.0-0.2
◇Platelet count appears normal in the film . Slight polychromasia. Poikilocytes +				
◇PT		6.4		8.0-12.0
◇APTT	10.3			12-20
◇Bleeding time corrected following desmopressin				
◇VWF	45	Lo		>50

DIAGNOSIS – Angiostrongylus vasorum infection

A young Arab mare with marked pyrexia, weakness, tremor and tachycardia following intravenous injection

- ◇ Breed Arab
- ◇ Age 1 year
- ◇ Pyrexia 41C
- ◇ Tremor and weakness
- ◇ 15 minutes after IV injection
- ◇ Heart rate 70
- ◇ Resp rate 15

Haematology

◇RBC	6.11		5.5-9.0
◇Hb	7.5	Lo	8.0 -14.0
◇HCT	22.9	Lo	24.0-44.0
◇MCV	39.0		39.0-52.0
◇MCH	12.3		15.0-26.0
◇MCHC	32.9		30.0-37.0

◇Platelets See comment

◇WBC	4.0	Lo	6.0-10.0
◇Neutrophils	2.19	Lo	2.7 -6.0
◇Bands	0.23		<0.3
◇Lymphocytes	1.51		1.5-5.0
◇Monocytes	0.08		0.0-0.7
◇Eosinophils	0.0		0.0-0.9

◇Platelets clumped in film –actual count appears normal. Toxic degenerate neutrophils

Diagnosis – Streptococcal septicaemia

Haematology

after treatment with ceftiofur 2g IM daily for 10 days – full clinical recovery

◇RBC	9.62		5.5-9.0
◇Hb		12.9	8.0 -14.0
◇HCT	37.6		24.0-44.0
◇MCV	39.0		39.0-52.0
◇MCH	13.4		15.0-26.0
◇MCHC		34.4	30.0-37.0
◇Platelets		258	90-400
◇WBC	11.2	Hi	6.0-10.0
◇Neutrophils		5.8	2.7 -6.0
◇Lymphocytes	5.0		1.5-5.0
◇Monocytes		0.11	0.0-0.7
◇Eosinophils		0.22	0.0-0.9
◇Platelet count appears normal in the film			

A middle aged flat coat retriever with anaemia and depression

◇ Species canine

◇ Age 7 years

◇ Sex Male

◇ Depressed for 3 days

◇ Pale

◇ IMHA suspected

Haematology

◇RBC	2.72	Lo	5.0–8.5
◇Hb	7.1	Lo	12.0–18.0
◇HCT	19.7	Lo	37.0–55.0
◇MCV	72.0		60.0–80.0
◇MCH	26.1		19.0-26.0
◇MCHC	36.1		31.5-37.0

◇Platelets See comment

◇WBC	17.56	Hi	6.0-15.0
◇Neutrophils	11.76	Hi	3.0-11.5
◇Bands	1.4	Hi	>0.3
◇Lymphocytes	3.51		1.0-4.8
◇Monocytes	0.7		0.0-1.3
◇Eosinophils	0.0		0.0-1.25
◇nRBCs	1.4		
◇Other cells	0.18		

◇Occ atypical lymphocyte, occ mast cell, 1 blast cell, occ toxic neutrophil, polychromasia ++, anisocytosis++, giant platelets actual count appears slightly reduced (100-120), histiocytes +, erythrophagia noted

Diagnosis – systemic circulating malignant histiocytosis

A YOUNG CAT WITH INAPPETANCE, POOR WEIGHT GAIN AND PERSISTENT PYREXIA

◇Species – Feline

◇Breed – DLH

◇Age – 2 years

◇Sex – male neutered

◇Failure to thrive

◇Persistent pyrexia

◇Swollen abdomen

◇ Inappetance

Biochemistry and Haematology

◇ Total protein	108	Hi		54-80
◇ Albumin		18	Lo	21-39
◇ Globulin		90	Hi	15-57
◇ Sodium		164	Hi	125-158
◇ Potassium		5.5		3.6-6.0
◇ Chloride		127		117-140
◇ Total Ca		2.28		2.0-3.0
◇ Phosphate		1.43		1.2-2.6
◇ Urea	5.9			4.0-12.0
◇ Creatinine		153		80-180
◇ Alk Phos		20		0.0-50.0
◇ ALT	69	Hi		0.0-40.0
◇ Total bili		61	Hi	0.0-10.0
◇ Bile acids		71	Hi	0.0-50.0
◇ Glucose		5.8		3.5-6.6
◇ CK		129		0.0-152
◇ RBC	4.5	Lo		5.5-10.0
◇ Hb		7.0	Lo	9.0-17.0
◇ HCT	21	Lo		27-50
◇ MCV	46			40-55
◇ MCH	15.3			13.0-17.0
◇ MCHC		33.0		31.0-36.0
◇ Platelets		350		170-650
◇ WBC	21.7	Hi		4.0-15.0
◇ Neutrophils		18.7	Hi	3.0-12.5
◇ Lymphocytes	1.0	Lo		1.5-7.0
◇ Monocytes		1.83	Hi	0.0-0.8
◇ Eosinophils		0.22		0.0-1.5
◇ Platelets clumped in film, actual count appears normal. Rouleaux ++				
◇ FELV	Negative			
◇ FIV	Negative			
◇ Coronavirus	Positive >10240			

Diagnosis - FIP