بنام خداوند بخشنده مهربان

NonMalignant Granulocyte & Monocyte Disorders

M.Farhadi
Tehran,1390

Overview

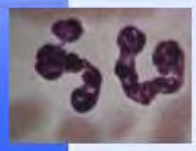
In this lecture we discuss " Benign Changes in Granulocytes & Monocytes as a response to various nonmalignant disease states and toxic challenges".

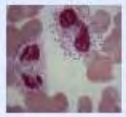
These changes include both "Quantitative & Qualitative variations that can be detected by Laboratory tests.

So it divided to changes in quantity & morphology of Neutophils,Eosinophils,Basophils & Monocytes in Acquired & Inherited states.

Peripheral White Blood Cells

- Two categories:
- 1. Granulocytes
  - Neutrophil: acute inflammation, first line of defense. (Band, segmented, hypersegmented)
  - Eosinophil: allergic response, parasitic
  - Basophil: severe allergic, heparin and histamine in granules





Introduction

* it well recognized that:
"Changes in leukocyte concentration & morphology are the Body's Normal Responses".
*most often ***One cell Type*** affected than others and provide important clue to Diagnosis.
*the cell type affected depends largely on it's Function(e.g. Bacterial infection----Neutrophils)
*thus absolute determination of cell type count aids in differential diagnosis ,especially when Total Leukocyte Count is Abnormal.

Leukocytosis

refer to a condition in which the total leukocyte count is more than 11.0x10^9/L in an adult.

* reference range vary significantly among different sources and laboratories.

**the absolute concentration of each cell type of leukocyte can be calculated from:

Absolute cells/L=Total Leukocyte Count/L x Percent of cell type from differential count

Leukopenia

Refers to a decrease in leukocytes below

4.5x10^9/L

usually due to decrease in Neutrophils but lymphocytes and other cell types rarely can be contribute.

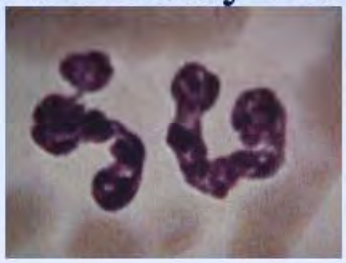
Morphologic or Qualitative variations of WBCs are noted by Stained Blood Smear Examination. Some of them affect cell Function while others-

NEUTROPHIL DISORDERS

Neutrophils are most numerous white blood cells within peripheral blood.
Neutrophilia is more common than neutropenia.
Qualitative changes requires careful microscopic examination.
Qualitative changes provide important diagnostic information.

Neutrophil

- Segmented neutrophils, most common WBC as well as most common stage of the neutrophil seen in peripheral blood.
- NOTE: granules are neutral staining, therefore not readily visible.



QUANTITATIVE DISORDERS

1- Caused by Malignant or Benign disorders.
2-Benign disorders are usually ACQUIRED and may cause an increase (*NEUTROPHILIA*) or decrease (*NEUTROPENIA*).
3- Neutrophilia is more common.
4-Three main mechanisms affecting neutrophil concentration in peripheral blood.

Factors Affecting Neutrophil Concentration in PB

*Bone marrow production & release

*Rate of Neutrophil egress to tissue or survival time in blood

*Rate of marginating to circulating pool

Neutrophilia

1-Neutrophil count varies with Age & Race
2-In adults exceeds 7.0x10^9 / L
3-Benign neutrophilia mostly as a result of reaction to physiologic or pathologic process and is called Reactive Neutrophilia & divided to:

Immediate , Acute , Chronic Neutrophilia

Neutrophil

- Band neutrophil: Immature peripheral neutrophil seen in Left Shift.
- (Maturation from bone marrow to peripheral blood: blast,progranulocyte, myelocyte, metamyelocyte, band, seg, hypersegmented).
- Nucleus is parallel borders. Looks like a sausage.

Immediate Neutrophilia

-Can Occure without Pathologic stimulus
-Probably a simple redistribution of marginated granulocyte pool(MGP) to circulating granulocyte pool(CGP).
- 50% of neutrophils are freely circulating & other 50% are loosely attached to vessels endothelial cells.Lab count only CGP.

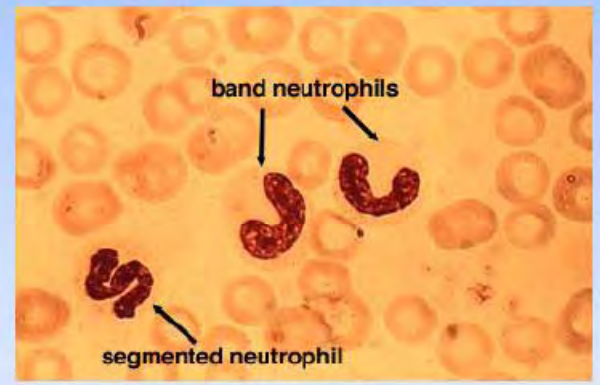
Immediate Neutrophilia

- Neutrophil increase is immediate but transient ,lasting about 20-30 minutes
- Appear to be independent of bone marrow input and tissue egress.
- Also called PSEUDONEUTROPHILIA or SHIFT NEUTROPHILIA, because no real change in number within vasculature occurs.
-Neutrophils are Mature &Normal.
-Due to Active Exercise,Epinephrine,Anxiety,Anesthesia

ACUTE NEUTROPHILIA

-Occurs within 4-5 hr of a pathologic stimulus
-Results from increase in the flow of neutrophils from BM storage pool to PB
-More pronounced than immediate neutrophilia
-The immature neutrophils may increase,more bands & if demand continues release of metamyelocyte & myelocyte seen.
-As BM production increase & storage pool replenished the WBC differential return Normal

Band Cells

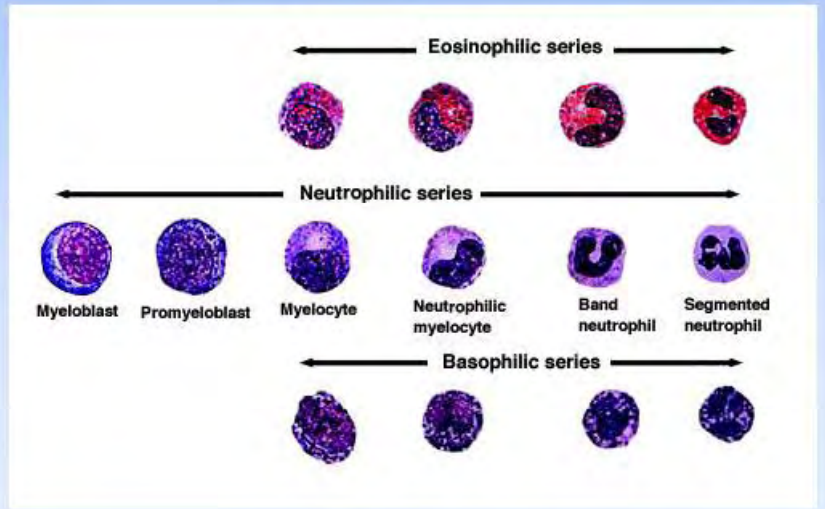


CHRONIC NEUTROPHILIA

-Follows Acute Neutrophilia
-If stimulus continues beyond a few days ,the storage pool depleted,& BM shows increaed numbers of early neutrophil precursors including Myeloblasts,Promyelocytes & Myelocytes.
-PB show increased Bands,Metamyelocyte,Myelocyte& rarely Promyelocyte so named=SHIFT TO THE LEFT

Leukocyte Morphology

Juvenile cells

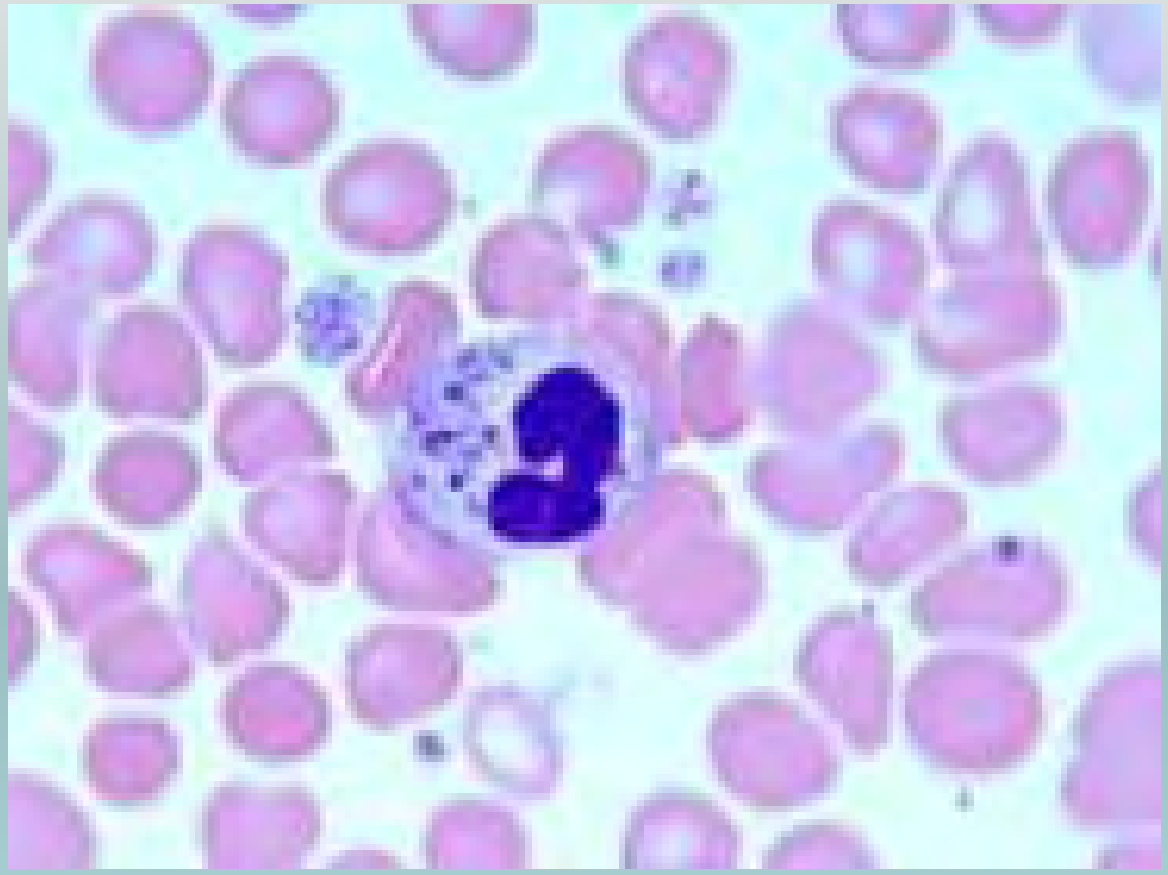


Conditions Associated with Neutrophilia

1- Acute Bacterial & Fungal Infections
2-Inflammatory Processes
3-Metabilic alterations;uremia,eclampsia,gout
4-Neoplasms
5-Acute Hemorrhage or Hemolysis
6-Rebound from BMT or CSF treatment
7-Certain chemicals,toxins,drugs
8-Chronic Myeloproliferative Disorders

Reactive Chronic Neutrophilia

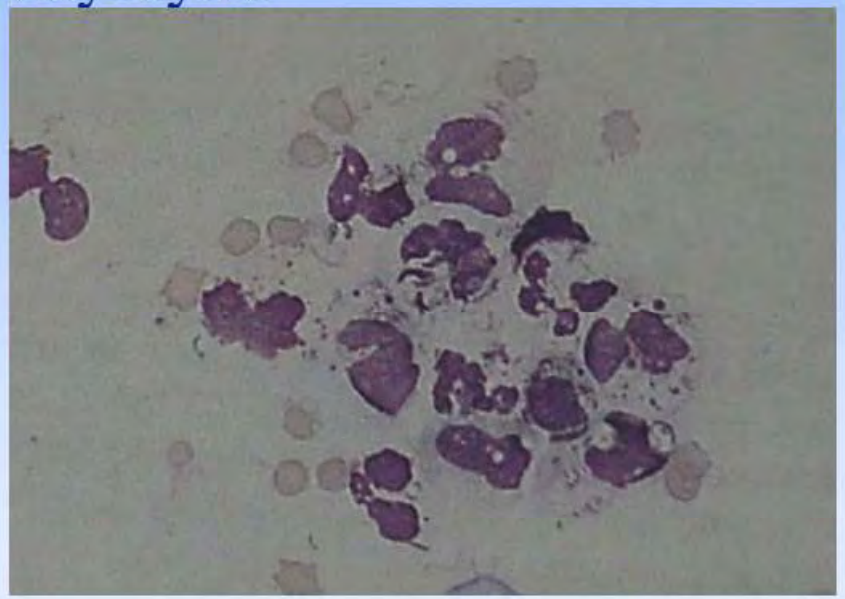
- caused by benign or toxic conditions
-total WBC count less than 50x10^9/L
-the immature cells seen in PB are Bands & Metamyelocytes
-toxic changes(toxic granulation,Dohle bodies & cytoplasmic vacuoles) seen even after WBC count return to normal.
-LAP score may elevated.



Bacterial Infection

-most common cause of neutrophilia(pyogenic ones as satph & strep)
-depend on bacterial;Virulence,Extend of infection & Response of host WBC count may range from 7.0x10^9/L to 70x10^9/L but usually it is around 10-25x10^9/L.
-in sever infections BM exhausted & Neutropenia developed with poor prognosis

Karyolysis



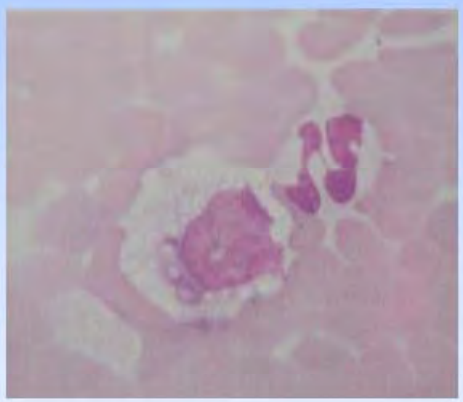
Other Considerations

* Chronic bacterial infection leads to chronic stimulation on BM & new steady state of increase Neutrophil production develop.
*Other organisms(Fungal,Rickettsia,Spirochete,Parasites
*Certain bacteria are with Neutropenia.
*Whooping cough is with Lymphocytosis.
*Early course of viral infections Neutrophilia.

Leukocyte Morphology

Blood Parasites

Histoplasma



Tissue Destruction/Injury,Inflammatory, Metabolic Disorders

Etiology;

By increasing Neutrophil input from BM in response to increased egress to the tissue.

Damaged tissue release cytokines act as chemotactin(WBC granules,O3-,crystalsUA,...

Disease States such as;Tissue necrosis & Infarction,Burns,Neoplasms,Trauma,Uremia,Rheumatoid arthritis,Gout,Drug intoxication,...

Leukemoid Reaction

Pathophysiology;extreme neutrophilic reactions to sever infections or necrotizing tissue.

Definition;a benign leukocyte proliferation by a total WBC count usually greater than 50,000/ql with many circulating immature WBC precursor

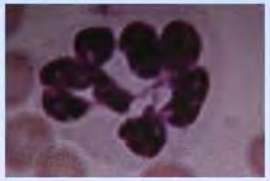
In Neutrophilic Leukomoid Rx, PB contain many Bands,Metamyelocytes,increased Myelocytes & Promyelocytes and rarely Blasts

Leukemoid Reaction...

* PB picture may indistinguishable from CML.
** D.Dx ; Genetic studies,Molecular analysis, Leukocyte Alkaline Phosphatase (LAP) score,...
***Contrary to Leukemia ,the leukemoid reaction is Transient,fade after inciting stimulus.
****Can be seen in;Chronic infections(TB), Ca of Lung,Stomach,Breast,Liver & others

Neutrophil

- Segmented
- Hypersegmented: 5 or more segments, ususally due to normal aging process, but can be seen in toxic situations.



Leukoerythroblastic Reaction

is characterized by the presence of nucleated erythrocytes & a neutrophilic shift to the left PB.

The Total Neutrophil count may; Increase,Decrease,or Normal.

The erythrocytes often exhibit poikilocytosiswith teardrop shapes & anisocytosis.Mostly often in Chronic Myeloproliferative states(myelofibrosis myelophthisis&sever hemolytic anemia(HDFN)

Stimulated Bone Marrow States

*when BM is stimulated to produce RBCs(in response to hemorrhage/hemolysis) neutrophils may also share in process lead to neutrophilia.
*Hematopoietic Growth Factors(GM-CSF) can rapidly increase total WBC count & it's precursor including Blasts(used in BMT,High dose Chemotherapy,Stem cell donate
*Drugs(Corticosteroid) I output, D migration

Physiologic Leukocytosis

- at birth & for first few days of life,with a slight shift to the left.

-Physiologic stress including ;
exposure to extreme temperture,emotional stimuli,exercise and labor during delivery could cause neutrophilia generally without a shift to the left.

NEUTROPENIA

Occurs when Neutrophil Count falls below; 1.5-2.0x10^9/L(varies with ethnic group).

Agranulocytosis, when neutrophil count is below 0.5x10^9/L.

mostly associated with high probablity of infection.Basophils & Eosinophils also commonly depleted in sever neutropenia.

Causes of Neutropenia

1-Decrease bone marrow production
2-Increase cell loss(due to immune destruction or increase neutrophil egress to tissue)
3-Pseudoneutropenia(increase neutrophil margination)
4-Spurious or False neutropenia result from neutrophil agglutination,disintegration&Lab instrumental problem or error

Decrease Bone Marrow Production

Bone marrow show myeloid hypoplasia,and M:E ratio is decreased.

With decrease production BM storage pool is depleted,neutrophil egress to tissue also decrease and both circulating & marginal pools decrease.

Immature cells may enter the PB in attempt to alleviate to neutrophil shortage.Cells younger than Band are less efficient in phagocytosis

Stem Cell Disorders

- Stem cell failure such as aplastic anemia, radiotherapy or chemotherapy or infiltration of hematopoietic tissue by malignant cells(myelophthisis).

-Of new leukemia cases 40% present with total leukocyte count less than 10x10^9/L,because normal precursor cells in BM replaced but malignant cells havenot egressed to PB yet.

Megaloblastic Anemia

-Neutropenia is a charactristic findings in megaloblastic anemia & myelodysplastic syndroms(MDS).
-Marrow is usually hyperplastic.
-Neutropenia result from abnormal myeloid cells being destroyed before release to peripheral blood(Ineffective granulopoiesis).

Congenital Neutropenia

Disorders of Production

cyclic neutropenia,familial neutropenia,Fnconi pancytopenia,Reticular dysgenesis,sever congenital neutropenia,Wiskott-Aldrich syndrom

Disorderd of RNA synthesis& processing

cartilage-hair hypoplasia,Dyskeratosis congenital,Shwachman-Diamond syndrome

Disorders of Metabolism*(Barth syndrome,Glycogen storage disease,type 1b)*

Disorders of vesicular transport*(Chediak-Higashi ,Cohen,Griscelli,Hermansky-pudlak*

Immune Neutropenia

*Antibodies directed against neutrophil-specific antigens(NA)may cause decrease in number .
*Leukocytes destroyed similar to RBCs(IHA)
*Immune mechanisms;1-Direct cell lysis or 2-Sensitization & subsequent sequestration in spleen.Two types present;Alloimmune and Autoimmune neutropenia

Alloimmune Neonatal Neutropenia

Occurs when transplacental transfer of maternal alloantibodies directed against infant's neutrophil antigens(infant Ags are paternal origin).
Affected infants may develop infections ,until neutropenia resolved usually in few weeks,same as Rh HDN except first child can affected.
Alloimmune Neutropenia can result : transfusion

Autoimmune Neutropenia

Two forms recognized:Primary & Secondary

Primary AIN ;antibody-coated neutrophils are sequestered&destroyed by spleen.Unknown etiology, Young children with fever & infection,spontaneous remission after 13-20 month.Secondary AIN in older patients with other autoimmune disorders(SLE,RA),antineutrophil Abs arenot only cause & actual target of Abs not known.

Hypersplenism

May result in a selective culling of neutrophils producing mild neutropenia.
The Bone Marrow exhibits neutrophilic hyperplasia.
Thrombocytopenia and occasionally Anemia may also accompany hypersplenism.

Spurious,or False Neutropenia

1- EDTA induced neutrophil adherence to RBCs
2-Disintegration of Neutrophils over time prior to testing
3-Disruption of abnormally fragil leukocytes during preparation of the blood for testing
4-Neutrophil aggregation(some paraproteins)
Psudoneutropeniashift of CGP to MGP in some endotoxin bacteria & hypersensitivity(transient)

Qualitative or Morphologic Neutrophil Abnormalities

Nuclear Abnormalities

Pelger-Huet ,Hypersegmentation,Pyknotic nuclei

Cytoplasmic Abnormalities

Dohle bodies,Toxic granules,Cytoplasmic Vacuoles,intracellular organism(fungi,bacteria)

Inherited Functional Abnormalities

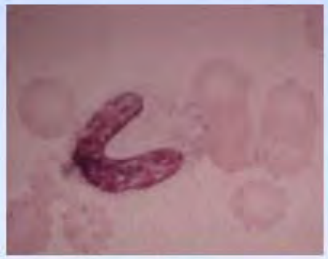
Alder-Reilly anomaly,Chediak-Higashi,May-Hegglin anomaly,Chronic Granulomatous Disease,Myeloperoxidase &AdhesionDeficiency,

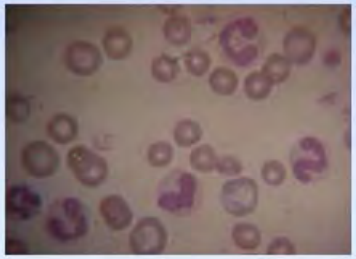
Pelger-Huet Anomaly

A benign anomaly,autosomal dominant fashion occuring 1 in 5000 individuals.
Nucleus doesnot segment beyond two-lobed stage & may appear round with no segmentation
Coarse chromatin clumping aid to DDx to Band.
"pince-nez" shape:dumbbell shape with two lobes connected by a thin strand of chromatin.
Acquired/pseudo Pelger-Huet in CMD/MDS

Band vs Seg

- When in doubt, throw it to the more mature side.
- Call it a seg, unless you' re sure its a band, since bands are seen in more severe inflammatory processes.



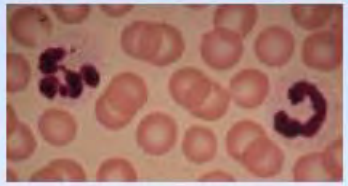


Hypersegmentation

larger than normal neutrophils with six or more nuclear segments are a common & early indicator of Megaloblastic Anemia.These cells
with pancytopenia & Macro ovalocytes typically accompany vit B12 defeciency.
Rarely hereditary hypersegmentation reported without clinical significance.

Neutrophil:

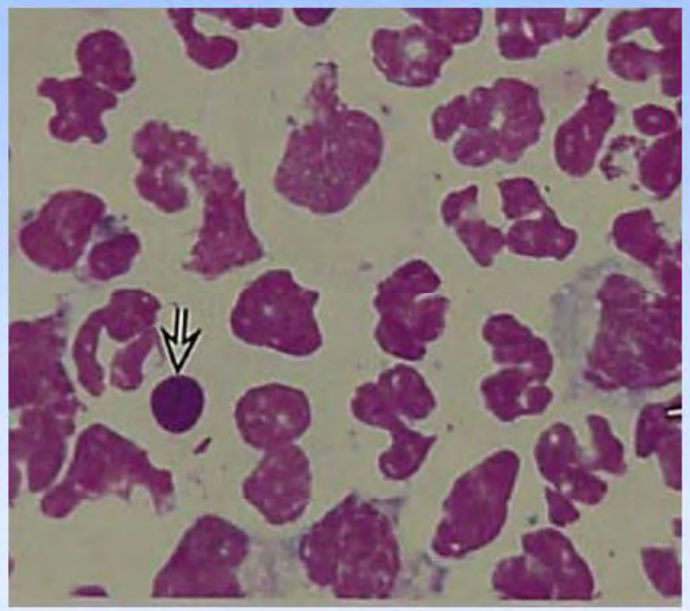
- Segmented; (able to find part of nucleus 1/2 width compared to another part)
- Barr Body: a sex linked chromosome seen in females.



Pyknotic Nucleus

Pyknotic or degenerating nuclei found in dying neutrophils in PB & other body fluids.
The nuclear chromatin condenses & the segments disappear becoming smooth,dark-staining spheres.
If nucleus round these necrotic cells may be confused with nucleated erythrocytes(nRBC).

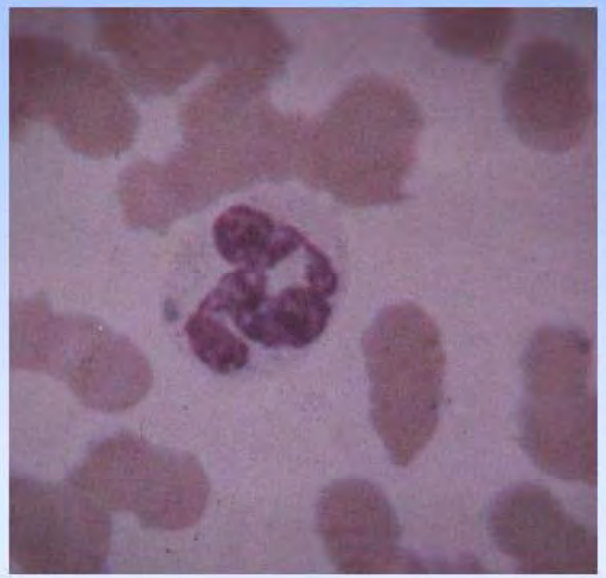
Pyknosis



Dohle Bodies

Dohle bodies are light gray-blue oval inclusions in cytoplasm of neutrophils & eosinophils.
Found near the periphery of cell.
Composed of aggregates of rough endoplasmic reticulum(RER).
May seen in sever infections,burns,cancer,drugs.
Looked for other toxic granulation & reactive morphologic changes.Same in May-Hegglin.

Dohle bodies



Toxic Granules

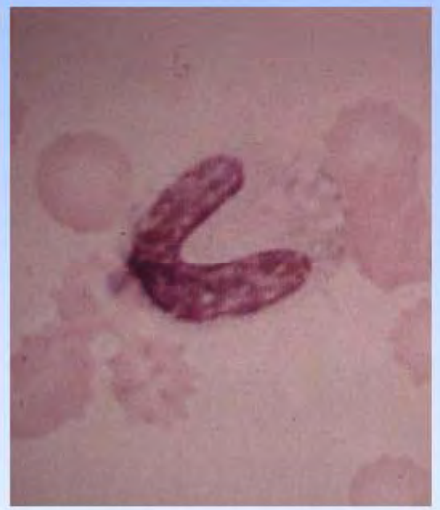
Are large,deep,blue-black primary granules in cytoplasm of segmented

Neutrophils & sometimes in bands &meta myelocytes.

Toxic granules retain their basophilia in the mature neutrophils

Toxic-like granules/inclusions can seen:
-as artifact with increased staining/low pH

Toxic Granulation



Cytoplasmic Vacuoles

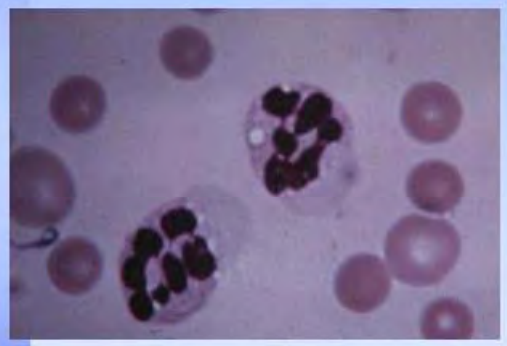
Appear as clear,unstained areas.

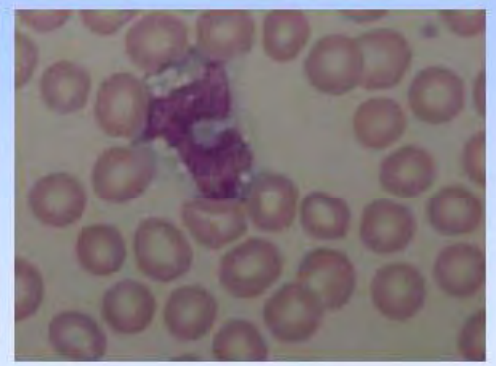
Represent the end stage of phagocytosis.

Seen in Same conditions of Toxic Granulation & Dohle bodies.

As artifact in stored EDTA blood but smaller &uniformly dispersed

Vacuolization

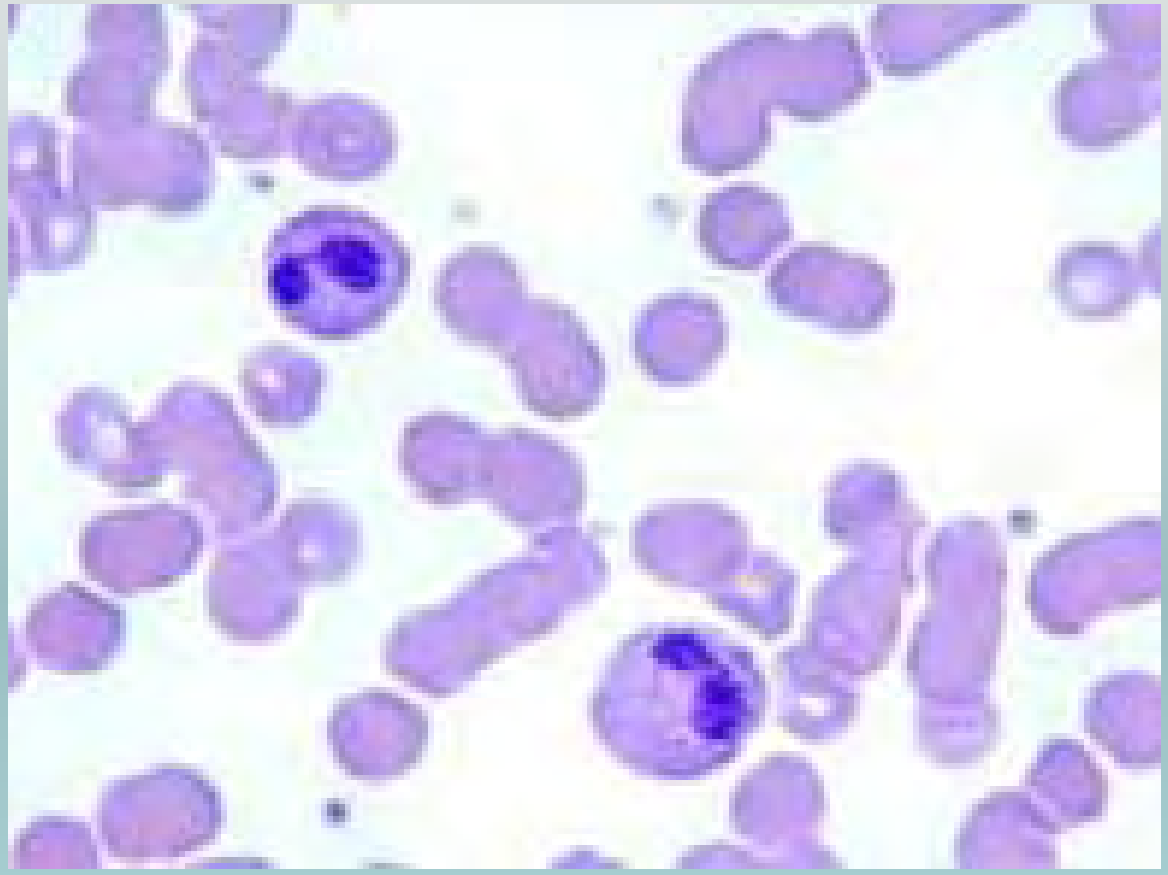




Inherited Functional Abnormality

Alder-Reilly Anomaly

-Inherited condition by presence of l arge purplish granules in all le ukocyte cytoplasm in lysosomal storage disorders(Hueler &Hunter s yndromes)mucopolysaccharide stained metachromatically with toluidine blue



Chediak-Higashi Syndrome

A rare autosomal recessive disorder,with death in infancy or childhood due to recurrent bacterial infections.

Giant gray-green peroxidase positive bodies &

Giant Lysosomes in WBC & other granule-contai ning cells of other tissues.

Result as fusion of primary & secondary neutrophilic granules.

Neutropenia & thrombocytopenia seen

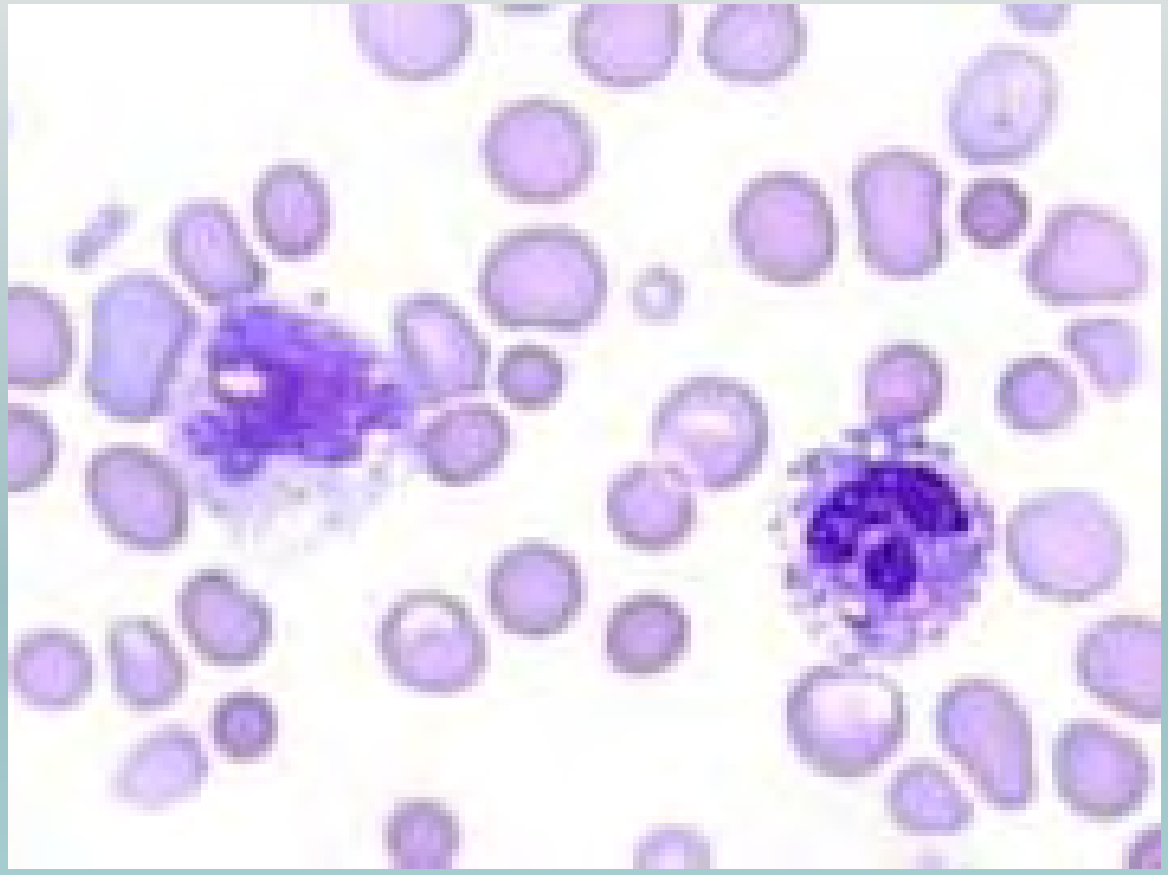
May-Hegglin Anomaly

Rare inherited autosomal dominant trait in which granulocytes contain inclusions similar

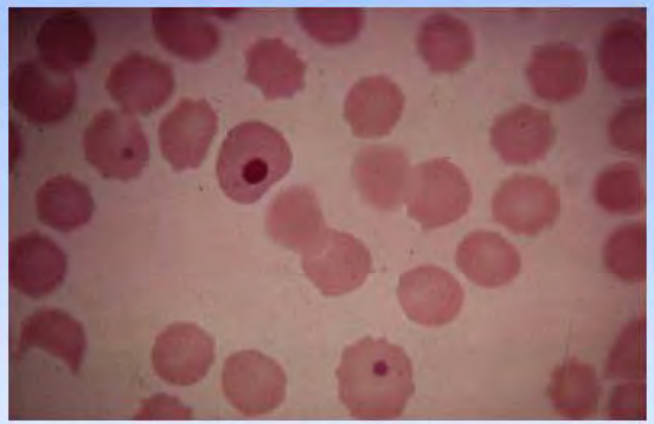
To Dohle bodies consisting mainly of RNA from rough endoplasmic reticulum.

Larger & more round vs Dohle bodies.

Variable thrombocytopenia & giant platlets may seen.Abnormal bleeding due to low platlets may exhibit.

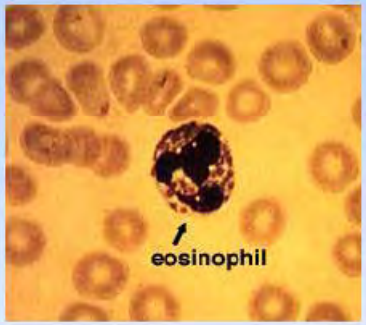


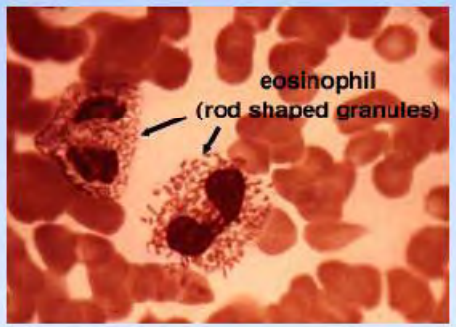
Distemper Inclusion Bodies



Leukocyte Morphology

'Normal' Eosinophil





EOSINOPHIL DISORDERS

Reactive(secondary)Eosinophilia

Primary Eosinophilia

Reactive (secondary)Eosinophilia

Increase above 0.4x10^9/L be induced due to T-lymphokines secretion:

1-Tissue invasive parasites

2-Allergic conditions

3-Respiratory tract disorders

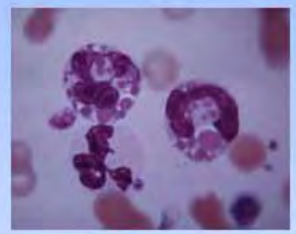
4-Gasterointestinal diseases

5-Skin & Connective tissue disorders

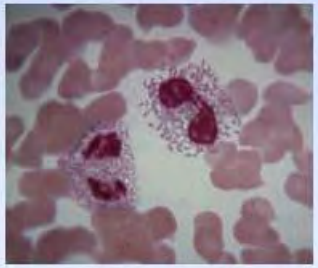
/

Eosinophil: allergic reaction, parasitology

- Canine: round granules



- Feline: rod-shaped granules



Primary Eosinophilia

Collectively known as **Hypereosinophilic syndrome(HES)** persistent blood eosinophils Above 1.5x10^9/L with tissue infiltration.

1-Idiopathic HES

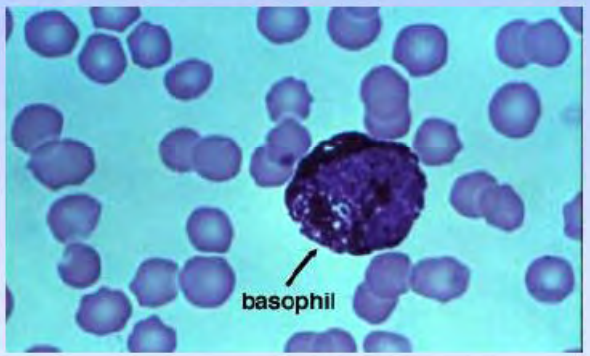
2-clonal eosinophilia:

-eosinophilic leukemia

-chronic eosinophilia

Leukocyte Morphology

'Normal' Basophil



Basophil & Mast cell Disorders

Both cells are important in inflammatory & allergic reactions ,release inflammatory mediators.Immune mechanisms maybe:

IgE-dependent &
IgE-independent

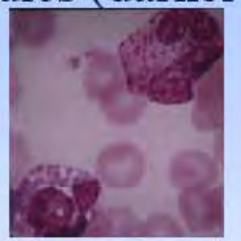
Basophilia=*increase above 0.2x10^9/L associated with immediate hypersensitivity R xs & chronic myeloproliferative disorder*

Basophilic Leukemia *very rare>80% basohi l*

Basopenia inflammation&immune Rxs

Basophil: severe allergic reaction

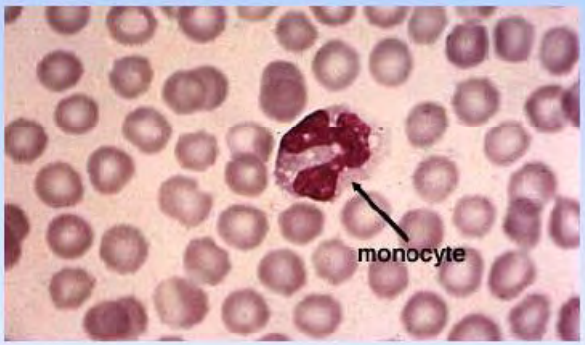
- Canine: dark blue granules (darker than the nucleus)



- Feline: Round lavender granules (suttle difference in color…look at shape of granule)

Leukocyte Morphology

'Normal' Monocytes



Quantitative Monocyte Changes

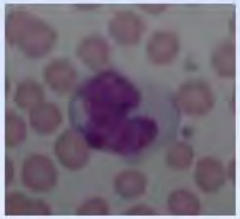
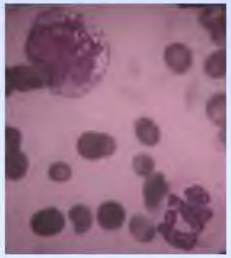
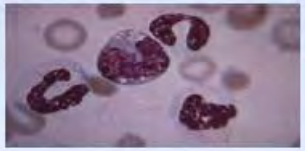
Neoplastic MDS/MPD
chronic myelomonocytic leukemia
juvenile myelomonocytic leukemia
chronic myelogenous leukemia
Acute monocytic,myelomonocytic&
myelocytic leukemia

Reactive inflammation/collagen/immune
disorders/certain infections

Monocytopenia stem cell disorders
such as aplastic anemia

Monocytes

- Largest cell, absent or present in low #'s
- Nuclei can be different shapes, oval, kidney-bean shaped, multiple indentations, amoeboid
- Moderate amount of cytoplasm, typically blue-gray, may have multiple, variably sized vacuoles.

Qualitative Monocyte Disorders

Lysosomal storage disorders

Gaucher Disease

Niemann-Pick Disease

Miscellaneous (Tay-sachs,Sandhoff & W olman disease)

Inherited defeciency in one or more enzymes in lipid metabolism

Sea-Blue Histiocytosis Syndrome

Peripheral WBC's

- 2. Agranulocytes
  - Lymphocyte-immune response
  - Monocyte-chronic inflammation phagocyte