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Leukocytic Disorders

- Non-Neoplastic Disorders
  - Granulocytic and Monocytic Disorders
  - Lymphocytic and Plasmacytic Disorders
- Neoplastic Disorders
  - Acute Myeloid Leukemias (AML)
  - Chronic Myeloproliferative Disorders (MPN)
  - Myelodysplastic syndrom (MDS)
  - Precursor (Lymphoblastic) B and T cell Neoplasms (ALL)
  - Mature B Cell Neoplasms
  - Mature T Cell and Natural Killer Cell Neoplasms
  - Histiocytic and Dendritic Cell Neoplasms
  - Hodgkin Lymphoma

The concentration of all the white cells

- also known as
  - the total leukocyte count or
  - white blood cell (WBC) count,
- relative and absolute
- *leukocytosis,* which is an increase in the total WBC (white blood cell) count above the upper limit of normal for age and sex, and
- *leukopenia,* which is a decrease in the total WBC count below the lower limit of normal for age and sex.

- Increase in any cell type may be clinically important,
- but decrease is usually significant only for neutrophils.
- Usually an isolated monocytopenia, eosinopenia or basopenia identified in a CBC is not considered a significant abnormality.

purpose of the examination of leukocytes

- Establishing a diagnosis.
  - specific diagnosis – for example, in leukemia.
  - More frequently, this examination is more helpful diagnostically when interpreted with other clinical or laboratory data – for example, in acute appendicitis, infectious mononucleosis,
- Another purpose is to help in establishing a prognosis.
  - For example, leukopenia in acute appendicitis or pneumonia is considered prognostically unfavorable.
- Following the course of disease.
  - For example, toxic effects of radiotherapy and chemotherapy may be recognized, and recovery monitored, by examination of leukocytes.

Neutrophilia

- Neutrophilic leukocytosis or neutrophilia refers to an absolute concentration of neutrophils in the blood above normal for age.
- The normal reference interval (established for each laboratory separately) is approximately
  - 1.8-7.0 x 10^3 for adults
  - 1.0-8.5 x 10^3 in young children.
- neutrophils in the
  - marginal granulocyte pool (MGP) : cells adhering to vessel walls
  - circulating granulocyte pool (CGP): nonadhering cells

Mechanisms:

- (1) the rate of release of the cells from BM
  - infection, stress, endotoxin (high dose), steroid administration
- (2) MGP to CGP
  - Severe exercise, hypoxia, stress, or the injection of epinephrine , steroid administration and other stressful situations (e.g., trauma, severe pain):
  - Neutrophilia is short lived (minutes to hours in duration, not days).
  - Interleukin-6
- (3) the rate of consumption of the cells in the tissues (ie, cell loss).
  - steroid administration

Neutrophilia (N > 7500)

- Physiologic (pseudoneutrophilia)
  - Severe exercise, hypoxia, stress, or the injection of epinephrine
  - smoking-pregnancy
- Pathologic
  - Acute infection (bacterial and other infections)
  - Acute inflammatory - collagen vascular, vasculitis
  - Tissue Injury – Inflammation- necrosis
    - MI-Burn - trauma, RBC hemolysis
  - Neoplastic - carcinomas, sarcomas, MPD
    - Myelocytic Leukemia-Myeloid metaplasia -Polycythemia Vera
  - Miscellaneous causes
    - Acute hemorrhage -Splenectomy - ARF -Eclampsia-DKA
  - Medications
    - Corticosteroids - Pb -Hg - GM CSF - Lithium-Beta agonists

In acute infection

- increased margination and outflow from blood to tissues would lead to neutropenia
- Because the latter overcompensates, the result is a neutrophilia.
- increased CGP (neutrophilia) and MGP in the face of the increased flow of neutrophils from the blood into the inflammatory site.
- An increase in immature peripheral blood granulocytes is usually present, often termed 'shift to the left:

- marrow will show granulocytic hyperplasia (increased myeloid to erythroid) M: E ratio and increased cellularity, with maturation intact.

- If the demand for neutrophils is extremely great (severe infection) there may be depletion of the marrow pool and a decreased MGP &CGP (neutropenia)

- In these instances, the marrow will show
  - increased numbers of, early neut. precursors, through the myelocyte stage,
  - but decreased numbers of metamyelocytes, bands, and neutrophils.

Neutropenia

- Neutropenia is a reduction of the absolute neutrophil count (ANC)
  - Below 1.5-2 x 10^9/L for white people and
  - below 1.2-1.3 x 10^9/L for black
- The term agranulocytosis has been used for severe neutropenia,
  - usually <0.5 x 10^9/L
  - associated with depletion of eosinophils and basophils as well.
- The term severe chronic neutropenia (SCN) refers to patients with
  - ANC < 0.5 x 10^9/L for months or years,
- If the neutrophil count is less than1 x 10^9/L, the risk of infection is considerably increased over normal. and
  - if less than 0.5 x 10^9/L, the risk of infection is greater still.

Causes of Neutropenia

- Drugs
  - cancer chemotherapy,chloramphenicol, sulfas/other antibiotics,
  - phenothiazines, benzodiazepine, antithyroids,anticonvulsants,quinine,
  - quinidine, indometacin, procainamide, thiazides
- Radiation
- Toxins- alcohol, benzene compounds
- Intrinsic defects : Fanconi's,Kostmann's,cyclic neutropenia, Chediak-Higashi
- Immune·mediated : collagen vasculardisorders, RA,AIDS
- Hematologic : MA, MDS-,marrow failure,marrow replacement
- Infectious :any overwhelming infection
- ineffective granulocytopoiesis :MA – MDS-antifolate drugs
- Others : starvation,hypersplenism

Mechanism

- (1) decreased flow of neut from marrow into blood as a result of either
  - lack of production or
  - ineffective production (i.e., a proliferation or maturation defect);
- (2) increased removal of neutrophils from the blood (survival defect)
- (3) altered distribution between CCP and MCP
- (4)combinations of these mechanisms.
  - drugs induce neutropenia through several mechanisms

infections

- Transient neutropenia may occur early in some infections, followed by leukocytosis once the marrow production catches up with the demand.
- As previously noted, in severe, extensive bacterial infection, neutropenia with a shift to the left may be due to inability of marrow production to keep up with the peripheral utilization.

- Some bacterial infections, notably brucellosis and *Salmonella* infections, are prone to be associated with neutropenia;
  - they may have some depressing effect on the marrow as well.
- Patients with viral infections such as measles and rubella have neutropenia for several days after appearance of the rash;
  - this is probably due in part to increased utilization.
- Other acute viral infections, such as hepatitis, IM, and influenza, may also cause acute neutropenia.
  - Lymphocytosis is present and persists after the neutropenia subsides.

Radiation

- destroys BM progenitor cells as well as marrow stromal elements.
- Radiation type, dose. and duration are all factors that determine the extent of bone marrow damage, such as aplasia or hypoplasia.
- Radiation affects and alters or damages a number of molecular targets, including DNA structure, gene translation and transcription, and apoptotic and other signaling pathways both within and between cells.
- Lymphocytes are most sensitive and are directly killed by exposure.
- The lymphocyte count correlates with, and has been used to assess dose and s everity of exposure
- other hematopoietic precursors undergoing mitosis are very sensitive to injury and death.

constitutional disorders

- usually present at birth or early infancy and are rare.
- Those due to myeloid hypoplasia or a proliferation defect include
  - Fanconi's anemia,
  - Kostmann's syndrome,
  - Schwachman-Diamond syndrome, and
  - cyclic neutropenia.

- Those that are due to a maturation defect include
  - myelokathexis and
  - Chediak-Higashi syndrome.

congenital neutropenia

- the two most common causes of congenital neutropenia are
  1. neutropenia of pregnancy-induced hypertension (PIH - most common) and
  2. overwhelming bacterial infection.
     - Typical signs of infection including a granulocytic left shift, toxic granulation, and Dohle bodies, while these changes are not seen in PIH.
     - Neither usually continues beyond the first week of life, and other causes should be searched for in infants with persistent neutropenia

- primary immunodeficiency diseases
  - Males with X-linked agammaglobulinemia (XLA)are often neutropenic,
    - This is due to various mutations in the gene *Btk* (Bruton's or B lymphocyte tyrosine Kinase) on the long arm of the X chromosome (q22).
  - congenital syndromes having a prominent association with neutropenia include
    - selective IgA deficiency,
    - common variable immunodeficiency
    - hyper IgM syndrome

Kostmann's syndrome
(Severe Congenital Neutropenia)

- **severe congenital neutropenia, autosomal recessive type 3** (SCN3)
- The disorder was discovered in 1956 by Swedish doctor Kostmann.
- appearing in early infancy.
  - originally termed 'infantile genetic agranulocytosis:
- AR pattern of inheritance, (SCN3)
  - both sporadic and AD cases also occur. (SCN1)

- **Diagnosis**
- CBC&PBS: An absolute neutrophil count (ANC) chronically less than 500/mm3 is the main sign of Kostmann's.
  - A standard bone marrow test can give correct diagnosis.
- BMA: usually shows the presence of early granulocytes (promyelocyte/myelocyte arrest) but few maturing forms are seen;
  - neutrophil survival is normal.

Pathophysiology

- SCN3 : mutations in the HAX1 gene are associated with Kostmann disease.
- In autosomal dominant form of SCN1, although the underlying genetic defect in myeloid precursor cells is not entirely elucidated, mutations in the gene (ELA2) encoding neutrophil elastase appear to be present in most patients.
- These mutations may be responsible for the untimely initiation of apoptosis in myelocytes, producing their premature destruction, and interrupting the normal cycle of maturation.
- Regular administration of exogenous granulocyte colony-stimulating factor clinically improves neutrophil counts and immune function and is the current mainstay of therapy, although this may increase risk for myelofibrosis and acute myeloid leukemia in the long term.

Fanconi's anemia

- FA is a genetic disease with an incidence of 1 per 350,000 births, and a higher frequency in Ashkenazi Jews and Afrikaners in South Africa.
- FA is the result of a genetic defect in a cluster of proteins responsible for DNA repair.
- As a result, 20% or more of FA patients develop cancer, most often AML
  - Older patients are extremely likely to develop MDS , head and neck, esophageal, GI cancers
- 90% develop bone marrow failure by age 40.
- 60-75% have congenital defects, commonly short stature, abnormalities of the skin, arms, head, eyes, kidneys, and ears, and developmental disabilities.
- Median age of death was 30 years

- Because of the genetic defect in DNA repair, cells from people with FA are sensitive to drugs that treat cancer by DNA cross-linking, such as mitomycin C.
- The disease is named after the Swiss pediatrician who originally described this disorder, Guido Fanconi
- It should not be confused with Fanconi syndrome, a kidney disorder also named after Fanconi.

Fanconi's anemia

- usually occurs in childhood and rarely presents in adulthood.
- Diagnosis is made by cytogenetic analysis looking for chromosome breakage after exposure to either diepoxybutane or mitomycin C.
- One gene, *FANCD1,* is actually *BRCA2,* which, like *BRCA1,* is a DNA repair gene that appears to be a putative breast and ovarian cancer susceptibility gene identified in certain families with an inherited predisposition to breast and ovarian cancer.

cyclic neutropenia

- is a form of neutropenia which tends to occur every 3 w and lasting 3-6 days at a time due to changing rates of cell production by the bone marrow
- Typically, oscillations of neut. and monocyte levels (between near normal levels and very low levels) occur over an approximately 21-day period.
- Patients typically present with recurrent episodes of symptomatic infection (fatigue, mouth ulcers, cervical lymphadenopathy, fever)
- The disease usually presents in childhood, but may present in adulthood.

- It is often present among several members of the same family.
- Treatment includes G-CSF and usually improves after puberty.

- Cyclic neutropenia is the result of autosomal dominantly inherited mutations in ELA2, the gene encoding neutrophil elastase

Chronic familial neutropenia

- or benign familial neutropenia
- a lower than 'normal' neutrophil count found in some ethnic populations.
- It is an incidental and clinically stable finding with
- no predisposition to infection,
- and is considered a genetic variation.

autoimmune diseases

- rheumatoid arthritis (RA)
- systemic lupus erythematosus (SLE)
- Primary autoimmune myelofibrosis

- Felty's syndrome (FS) :
  - The combination of chronic neutropenia and RA
  - patients will develop symptoms due to both RA (e.g., subcutaneous nodules, contractures, erythema, warmth, tenderness, symmetric involvement of large and small joints, musculoskeletal pain) and chronic neutropenia (symptoms of recurrent bacterial and fungal infections).
  - Many of these cases are associated with large granular lymphocyte (LCL) leukemia.

starvation

- In starvation, cellularity tends to be decreased,
- BMB: serous fat atrophy or gelatinous transformation of the bone marrow

- This change shows a loss of hematopoietic cells within the marrow stroma replaced by small or shrunken fat cells expanded by an intercellular, homogeneous, eosinophilic material.
- Bone marrow hypocellularity is typically seen with advanced disease.
- Neutropenia with bone marrow hypoplasia can be associated with a decrease in plasma or serum lysozyme (muramidase).

Pseudoneutropenia

- may be caused by increased margination of neutrophils in some individuals, without a decrease in the total granulocyte count. Rather than showing an equal distribution between MGP and CGP, an increased proportion of neutophils appears to be present in the MGP.
- Small doses of endotoxin will cause a shift of neutrophils into the MGP from the CCP, giving an apparent neutropenia, prior to causing a leukocytosis.
- In animals, anesthetic agents such as ether will cause the same kind of pseudoneuuopenia.

Morphologic change in NEUT

- Toxic change
- Dohl inclusion body
- May Hegglin anomaly
- Alder Reilly anomaly
- Pelger Huet anomaly
- Chediak Higashi syn.

Toxic change

- Toxic granulation
- Irregular basophilia of cytoplasm
- Cytoplasmic vacuole
- Several spicule extending out from the nucleous

- However, toxic change in neutrophils do not reflect a "toxic effect" of bacteria on neutrophils but are morphologic abnormalities acquired during maturation under conditions that **intensely stimulate neutrophil production and shorten the maturation time** in marrow.
- This accelerated maturation occurs secondary to cytokine stimulation, which is usually in reponse to inflammation.

Toxic granulation

- dark blue to purple cytoplasmic granules in the meta, band, or neut. stage.

- Normally, neutrophil granules are tan to pink in color in neut, meta, bands,
- Even the nonspecific or azurophil granules that are dark blue in the promyelocyte stage normally lose their basophilia in the mature neutrophil,

- Toxic granules are azurophil granules that have retained their basophilic staining reaction by lack of maturation, or that have developed increased basophilia in the mature neutrophil.
- In addition, perhaps a greater proportion of the granules being of the azurophil type.

- simulating toxic granules may occur in normal cells with
  - prolonged staining time or
  - decreased pH of the staining reaction.

- They are peroxidase-positive and may be numerous or few in number;
- there may be less peroxidase activity in toxic than in normal neutrophils.
- Toxic granulation is found in
  - infections or
  - other toxic conditions, but may also be seen in
  - noninfectious reactive conditions

Dohle inclusion body

- are single or multiple blue cytoplasmic inclusions.
- They represent remnants of free ribosome or rough endoplasmic reticulum
- Scarlet fever-burns-AA

May Hegglin anomaly

- AD - non muscle myosin heavy chain 9 gene *(MYH9)* on chromosome 22q
- mutations appear to alter the assembly and stability of myosin
- Characterized by large pale basophilic inclusions resembling Dohle bodies
- EM : altered RNA and ribosomes
- Associated with Giant platelets, and sometimes thrombocytopenia
  - The anomaly is usually benign but may be associated with bleeding.
- More prominent than Dohle bodies
- Occasionally in other cells
- Abolished with ribonuclease

- Granulocyte function is normal.

- mutations in *MYH9* are, at least partially, responsible for a phenotypic spectrum of illness.
- This spectrum appears to include
  - May-Hegglin anomaly, and
  - Sebastian,
  - Fechtner, and
  - Epstein platelet syndromes
- This spectrum may be phenotypically modified by aberrant fibulin-I gene expression, which also may be responsible for the clinical variability of disease Fibulin-I is a gene encoding for a secreted glycoprotein present in the basal membrane of many organs.

Alder Reilly anomaly

- Dense, prominent, larger than normal azurophilic granulation in all WBC was described by Alder in 1939
- Most characteristic of these disorders are the metachromatic granules surrounded by a clear zone seen in lymphocytes.
- is associated with the genetic mucopoly- saccharidoses. (Hurlers syn)
  - lack the lysozymal enzymes necessary to break down mucopolysaccharides.

Pelger Huet anomaly

- AD
- The trait is benign and occurs in 1 in 6,000 people.
- In the homozygote state the nucleus is round.
- In heterozygotes : bilobed nuclei ("pince-nez" cells) resembling bands.
- Fewer than 10% of cells contain 3 lobes
- Cell function is normal.

- pseudo-Pelger-Huet
  - MDS-MPD
  - Granulocytic leukemia
  - following drug therapy
  - certain infections.

  - Round-ring shape nucleous
  - Hypogranular cytoplasm

- underlying abnormality is mutation of the lamin B receptor gene

Hypersegmentation

- 5 lobes > 5% or a single cell with 6 lobes
- is one of the first hematologic abnormalities seen in MA.
- Normal 3 - 5 lobes.
- Eosinophils have fewer than 4 lobes
- basophils have fewer than 3 lobes.
- is sometimes referred to as a myeloid "right shift".
- may accompany other disorders such as IDA.

ChediaK Higashi syn.

- AR
- abnormally fusion of lysozymes.
- This disorder may affect granulocytes, LYM, and monocytes.
- Leukocyte functional abnormalities exist
- Chemotaxis and phagocytosis is defective.
- Platelets lack dense granules and platelet function is abnormal.
- Giant melanosomes in occular and skin tissues result in hypopigmentation.

- Partial orulocutaneous albinism,
- photophobia,
- immune deficiency,
- abnormally large granules in leukocytes
- and other granule-containing cells, neurologic defeas,
- frequent pyogenic infections

- An accelerated lymphoma-like phase ocrurs, with LAP, HSM, and pancytopenia; lymphoid infiltrates are widespread,
- death ensues at an early age.

- Granulocytes, mono, and lym contain giant granules
- which appear to be abnormal lysosomes.
- The pathogenesis of this disorder is linked to an abnormality of granule maturation, causing enlargement and apparent fusion of granules and vesicles (such as lysosomes, melanosomes, and platelet dense granules) in all cell types.

- Leukocyte functional abnormalities exist

- Mutation in the gene *CHS/beige* coding for the CHS1 or Lyst protein.
- The LYST (lysosomal trafficking regulator) gene on chromosome 1
- appears to be involved in regulation of vesicular size, trafficking, and intracellular movement such that vesicular migration and release are abnormal

symptoms

- Albinism -- silvery sheen to the hair, light-colored eyes
- Increased infections in the lungs, skin, and mucous membranes
- Jerky eye movements (nystagmus)
- Infection of affected children with certain viruses, such as EBV, can cause a deadly illness resembling the blood cancer lymphoma.
- Decreased vision
- Mental retardation
- Muscle weakness
- Nerve problems in the limbs (peripheral neuropathy)
- Nosebleeds or easy bruising
- Numbness
- Tremor
- Seizures
- Sensitivity to bright light (photophobia)
- Unsteady walking (ataxia)

Myelokathexis

- 'Myelokathexis' applies to peripheral neutropenia with the presence of bone marrow neutrophils.
- WHIM (warts, hypogammaglobulinemia, infection, and myelokathexis) syndrome is a rare, autosomal dominant disease of leukocyte trafficking involving chromosome 2q21.
- The disease appears to be due to
  - mutations in the chemokine receptor gene *CXCR4* and
  - altered leukocyte response to its funaional ligand CXCLI2.
- MIC: severe peripheral neutropenia with lymphopenia
  - , but with granulocytic hyperplasia of the bone marrow

Functional Disorders of *Neutrophils*

- display both altered morphology and function.
  - Chediak-Higashi syndrome (CHS)
  - specific granule deficiency (SGD),

- functional disorders with essentially normal morphology
  - chronic granulomatous disease (CGD),
  - myeloperoxidase deficiency (MYD),
  - leukocyte adhesion deficiency (LAD) exhibit