

WBC

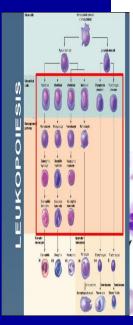
Nonmalignant
Disorders

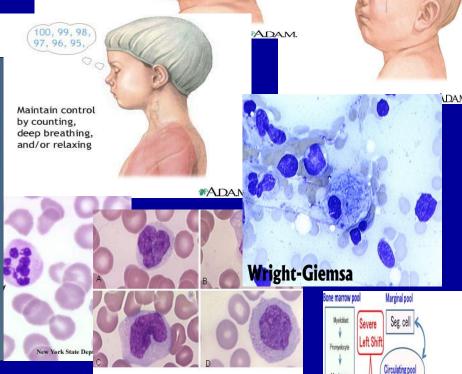
S.Hosseini

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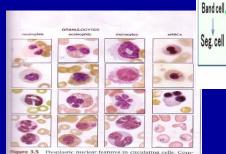
nt will probably regardless of or procedure

Seg. cell

12-24 hours after the onset

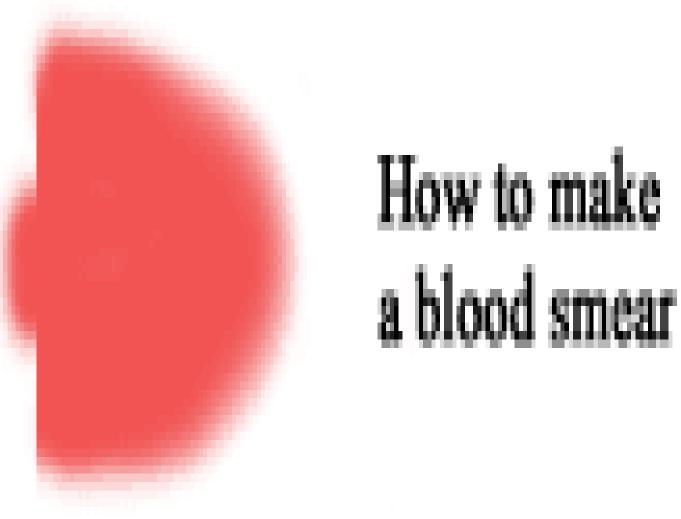
Source: Lichtman MA, Shafer MS, Felgar RE, Wang N:
Lichtman's Aldas of Hematology: http://www.accessmedicine.com
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Toddler will probably cry regardless of test or procedure



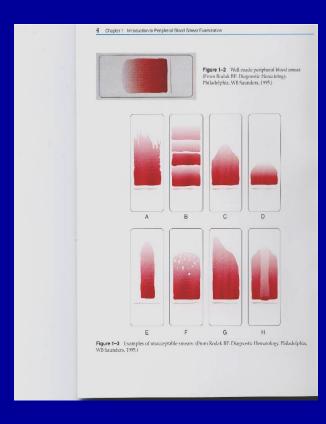
Dysplastic nuclear features in circulating cells. Com the image taken from several cases of myelodysplastic syndroms wing dysplastic nuclear features seen in circulating granuloes and nucleated RBCs. The right lower figure shows numerous

Blood smear



Prepration of blood smears for microscopic evaluation

Is an art that is Perfected By Experince.



Good Peripheral Blood Smear



Prepare blood films within 4(3) h of the blood collection in K EDTA.

Stain the film within one hour of preparation with a Romanowsky stain, containing fixatives; or fix within one hour with "water-free" (i.e., <3% water) methanol for later staining.

Non-Malignant Changes of WBC

Quantitative (changes in numbers)

Qualitative (morphologic alterations)

WBC differential count Leukocytic formula

% correlation between different forms of WBC

Eosino phils	Baso phils	Neutrophils				8 8	
		myelo cyte	juvenil e	band	segme nted	Lymph	Mono cytes
2-5	0-1	0	0	2-5	50-70	20-40	3-10

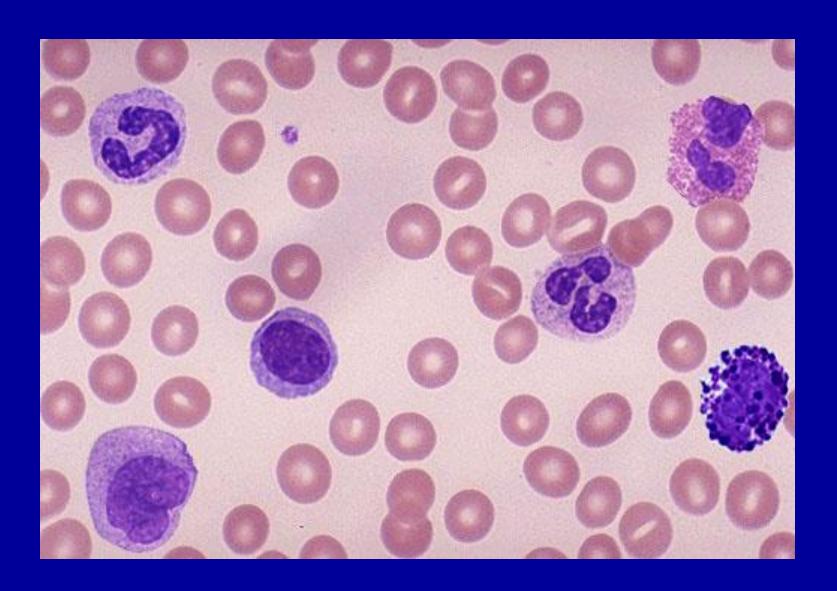
Absolute value = % of WBC type * total WBC count 100

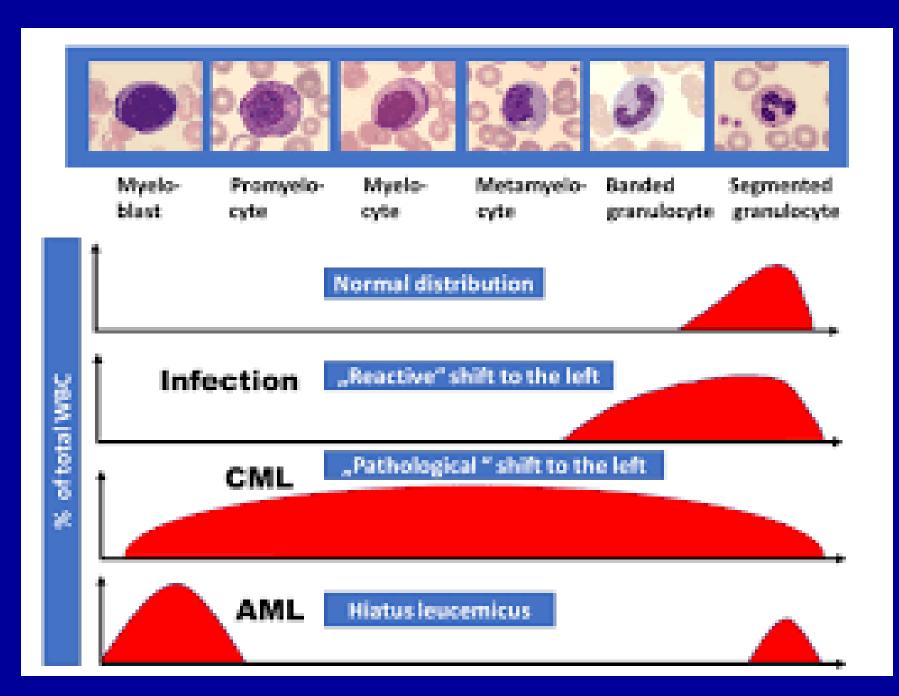
Myeloid maturation

neutrophil myeloblast promyelocyte myelocyte metamyelocyte band

MATURATION

Normal white blood cells

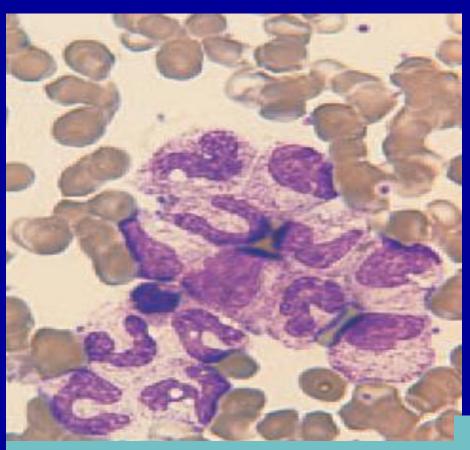




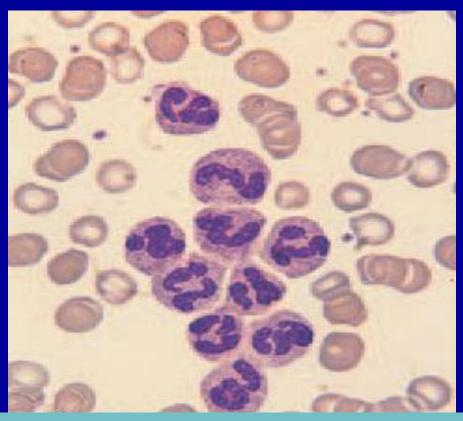
Some Alternations & Abnormalities That May Be Present In Neutrophile Nucleoli

- Left shift
- **\Delta** Hyper segmentation
- **Hyposegmentation**
- ***** Increased nuclear projections
- Ring nucleoli
- ***** Botryoid nucleus
- Dense chromatin clumping
- Detached nuclear fragments

WBC Aggregation

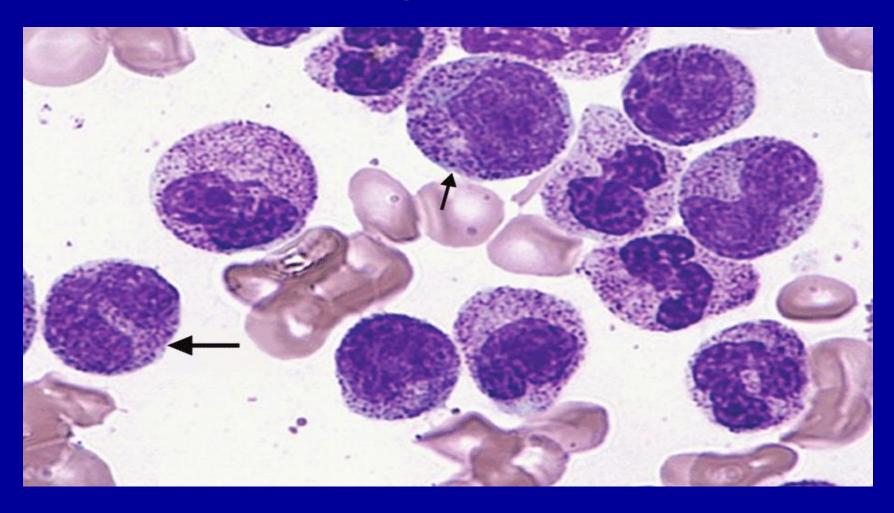


patient with overwhelming sepsis



Patient with rheumatoid arthritis showing neutrophil aggregation caused by a cold Ab.

Shift to the left Toxic granulation



Nuclear morphology of neutrophils

- The depends on different endogenous and exogenous factors
- lead to hypo- or hypersegmentation
- Normally 2-4 segmented nucleus.
- Could be induced, by colchicine treatment
- The range of this phenotypic variation is known:
- As "norm of reaction," which can be of major relevance for clinical diagnosis and therapeutic intervention
- A hereditary cause of hyposegmentation is the Pelger-Huët-Anomaly (PHA), an autosomal dominant trait, which is caused by mutations in the *LBR*-gene

Neutrophil Nuclear Iobulation

Counting the number of nuclear segments.

- (Rule of threads) segment is defined by lobes which are connected only via a thin chromatin thread
- (Rule of thirds) by a chromatin bridge whose width is <1/3 of the width of the respective lobes

Hypersegmentation Report

Hypersegmentation is associated with vitamin B12, folic acid, and iron deficiency and cases of myelodysplasia

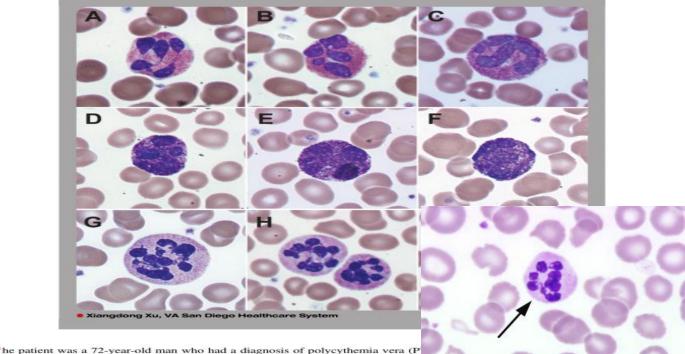
In normal blood average lobe count is 2.5-3.3

- Hypersegmentation is reported if >3% of neut have 5 lobes or more.
- More sensetive index in bandemia.
- A better right shift index is:

 no.of neut. With 5 lobes or more × 100 >16.9 abnormal
 no. of neut. With 4
 lobes



Nuclear hypersegmentation of neutrophils, eosinophils, and basophils due to hydroxycarbamide (hydroxyurea)



Hydroxycarbamide (hydroxyurea [HU]) was started at 500 mg and gradually in blood count showed the following: white blood cell (WBC) count, $11.5 \times 10^9/L$; re 13 g/dL; hematocrit, 37.8%; mean corpuscular volume, 106.5 fL; platelets, 423 × 1 identified 5% circulating blasts (not shown), macrocytosis, and marked megaloblastic changes in WBCs. Interestingly, nuclear

New York State Dept. of Health

hypersegmentation was identified in eosinophils (panels A-C), basophils (panels D-F), and neutrophils (panels G-I).

HU inhibits DNA synthesis by reducing the enzymatic activity of ribonucleoside reductase and is the mainstream cytoreductive agent for PV. Even though hypersegmented neutrophils are often observed with HU treatment, eosinophilic or basophilic hypersegmentation is an unusual observation.



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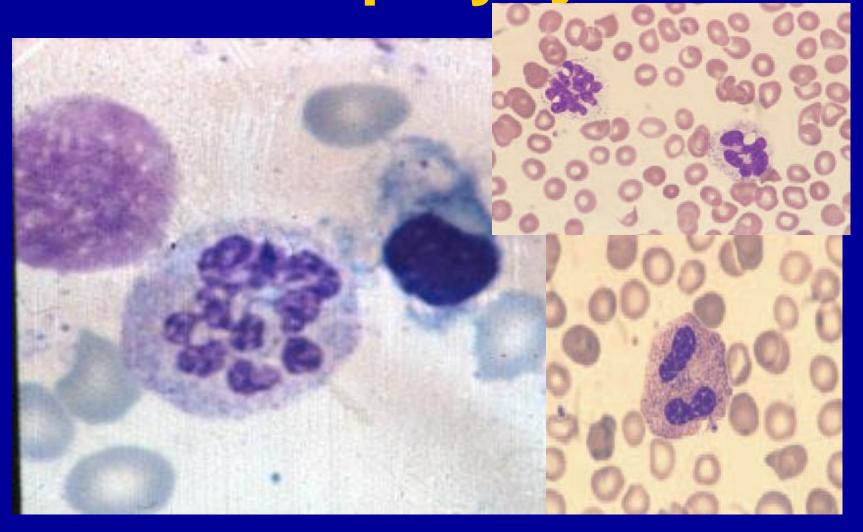
Macropolycytes

- ♣ Is about twice the size of a normal neutrophil ,15–25 µm
- Analysis of its DNA content shows that it is tetraploid rather than diploid, the number of lobes present being increased proportionately.
- Some macropolycytes are binucleated.
- Occasionally seen in the blood of healthy subjects.
- ♣ Increased numbers are seen in an inherited (autosomal dominant) condition in which 1–2% of neutrophils are giant with 6-10-lobed nuclei, or with twin mirror-image nuclei.
- Increased numbers, together with rather non-specific dysplastic features, have been described in DiGeorge's syndrome

Macropolycytes

- Macropolycytes, including binucleated cells, have been observed following the administration of G-CSF and are present in increased numbers in megaloblastic anaemia.
- In megaloblastic anaemia they have a DNA content varying between diploid and tetraploid
- In contrast to hypersegmented neutrophils, they are derived from giant metamyelocytes.
- They have also been reported in chronic infection, CNL and other myeloproliferative disorders, and following the administration of cytotoxic drugs and antimetabolites.

Macropolycytes



Hyposegmentation

A transient phenomenon in inflammatory processes and myelodysplastic syndromes

Moreover, it can be due to side effects of drugs, such as ibuprofen or valproate

Intravenous application of colchicine to

Nuclear Projections in Neutrophils for Supporting the Diagnosis of Trisomy 13

Trisomi 13 Tanısını Desteklemede Nötrofillerdeki Nükleer Cıkıntılar

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To the Editor

Trisomy 13 is a rare genetic disorder characterized by severe multiple congenital anomalies. Structural anomalies of neutrophils may be supportive for the diagnosis of trisomy 13.

A newborn was born by vaginal delivery after 29 weeks of pregnancy. Physical examination revealed symmetric growth restriction, low-set hypoplastic ears, aplasia cutis congenita areata on the vertex, postaxial polydactyly of the foot, bilateral microphthalmia, an umbilical cord cyst, and heart murmurs. Echocardiography showed truncus arteriosus type I. Review of the peripheral blood smear revealed two or more small threadlike pedunculated projections attached to the surface of the nuclei in more than 60% of the neutrophils (Figure 1). The diagnosis of trisomy 13 was made by chromosomal analysis. The infant died at 2 days of life because of massive pulmonary hemorrhage.

The presence of threadlike pedunculated projections attached to the surface of the nuclei of neutrophils was described in trisomy of the D group of chromosomes (13, 14, and 15) and also in trisomy 18 [1,2]. Two or more nuclear projections detected in more than 15% of neutrophils may be highly suggestive of these trisomies [3]. We suggest that identification of characteristic structural anomalies of neutrophils on a blood smear may be used for supporting the diagnosis of these trisomies.

Keywords: Trisomy 13, Blood smear, Neutrophilic nuclear projections

Anahtar Sözcükler: Trisomi 13, Periferik yayma, Nötrofilik nükleer projeksiyon

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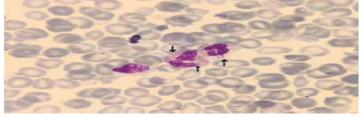
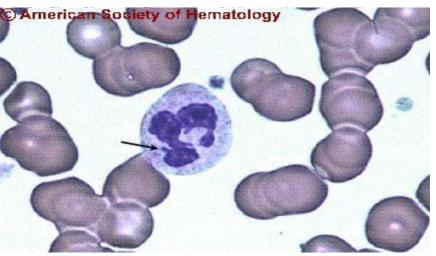
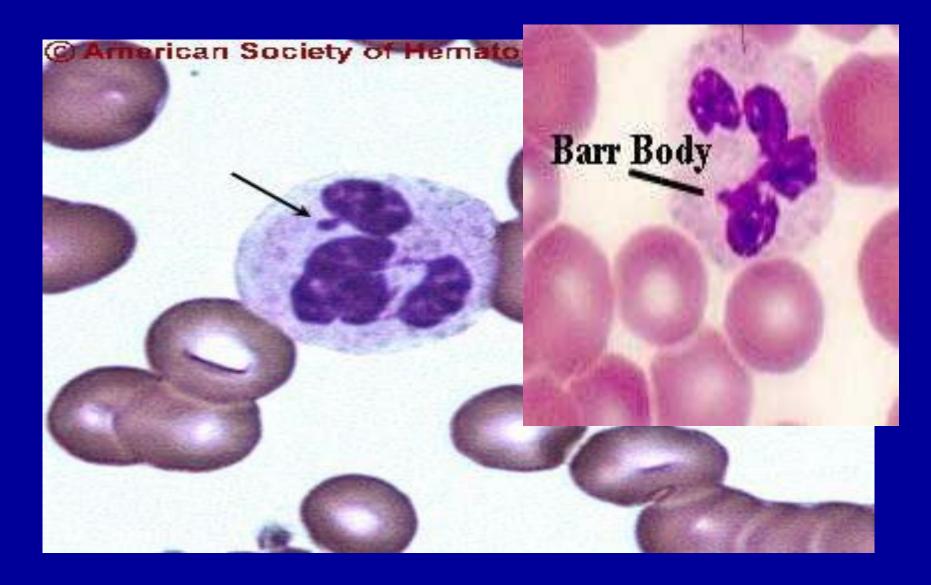


Figure 1. Peripheral blood smear showing threadlike pedunculated projections attached to the surface of the nuclei of neutrophils.

Informed Consent: Our patient's parent gave consent.



Drum Stick

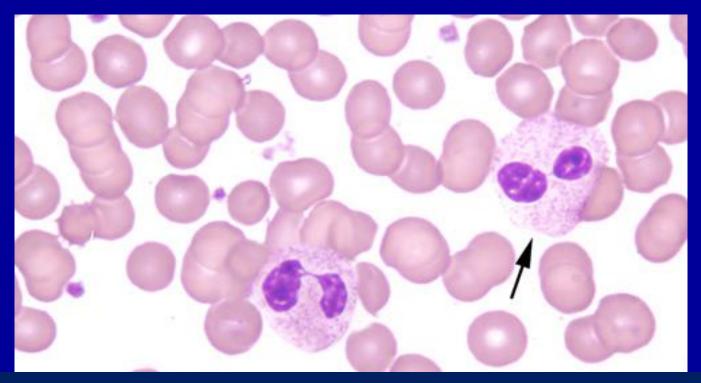


Hereditary Nuclear Morphologic Abnormalities of WBC

Pelger-Huit:

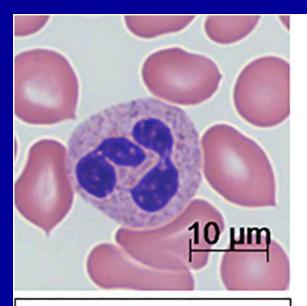
- ✓ Hereditary Hyposegmentation
- ✓ Autosomal dominant 1/5000
- ✓ pince-nez segmentation
- Distinguish between shift to left
- Very rare
- Benign
- Distinguish between myelody./ mylopro. Pelger -Huit "Pseudopelger huit "(hypogranular ,round nucleus rather than dumbell shape)

What is your diagnosis? How do you report?

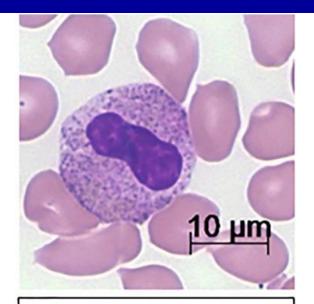


The majority of neutrophils are in band forms or have bi-lobed nuclei, with coarsely clumped chromatin, which is highly suggestive of *Pelger Huet Anomlay*

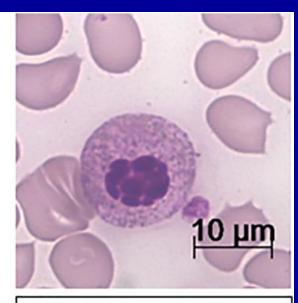
Classification of granulocyte nuclear segmentation of an individual homozygous for the wild type LBR (lamin B receptor) gene (a), heterozygous (Pelger anomaly, b) and homozygous for the mutant LBR gene (c) according to two optical classifications, rule of threads, rule of thirds, and a morphometric analysis, the circularity index.



Rule of Threads: 2 segments Rule of Thirds: 4 segments Circularity index: 4.42



Rule of Threads: 1 segment Rule of Thirds: 1 segment Circularity index: 1.52



Rule of Threads: 1 segment Rule of Thirds: 1 segment Circularity index: 1.21

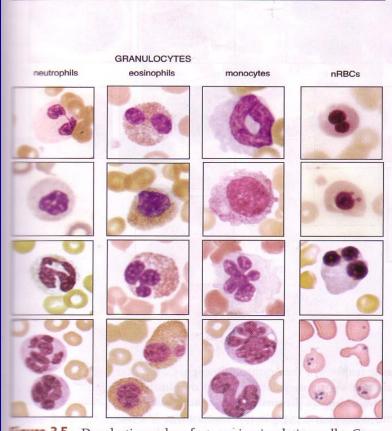
Α

В

C

MDS dysplastic features Pseudo-Pelger Huet Anomaly





The state of the several cases of myelodysplastic syndrome several cases

Ring Nucleoli

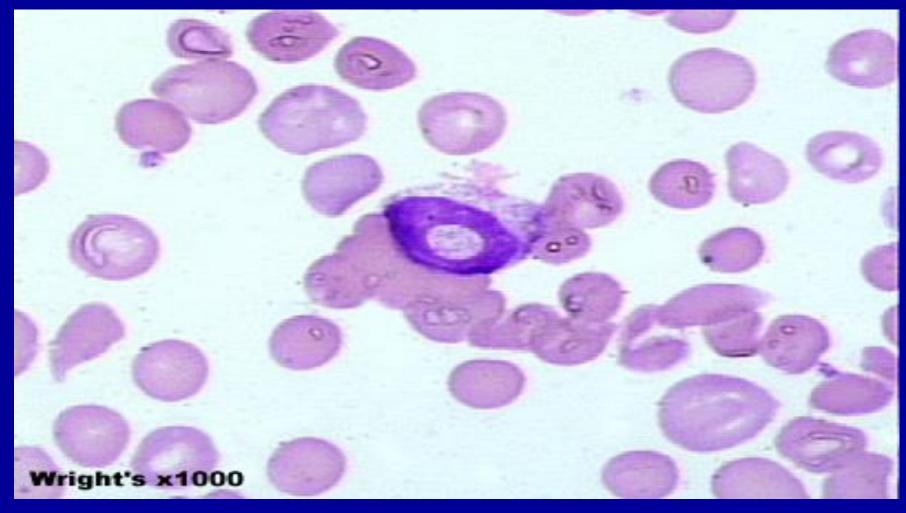
CML

AML

CNL

❖ MEGALOBLASTIC ANEMIA

Neutrophil with Ring Nucleus Doghnaut Shaped

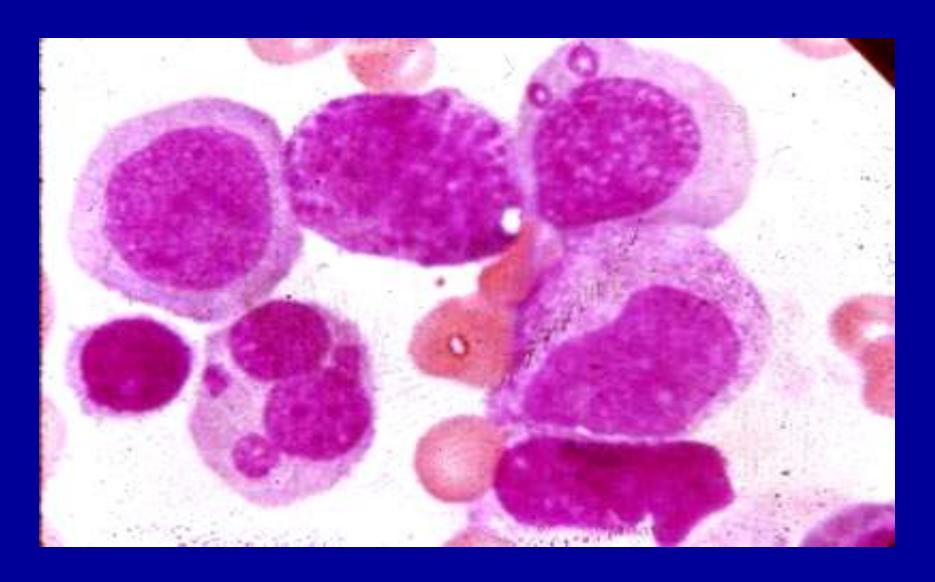


DETACHED NUCLEAR FRAGMENTS

- DYSPLASTIC GRANULOPOESIS DUE TO HIV
- ADMINISTRATION OF DRUGS INTERFERING WITH DNA SYNTHESIS
- CHLORAMBUCILE
- MYELOPHENOLAT

- **♦** MOFTILE
- TACROLIMUS

Detached Nuclear Fragments



BOTROYID NUCLEOUS

***** HEAT STROKE

+ HYPERTHERMIA

***** BURNES

Botryoid Nucleus



Degenerative Changes

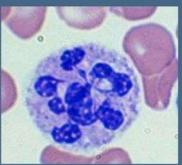
Apoptosis

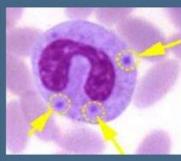
- Controlled process of cell death
- Occures when cytokines and growth factors regulating cell growth and survival are depleted
- More frequent in leukemia and infection
- May develop in stored blood after 12-18 hr
- Even at 4c

Degenerative forms of leukocytes

Neutrophils with hypersegmented nucleus

- ↑ level of glucocorticoids
- •B12 deficiency.



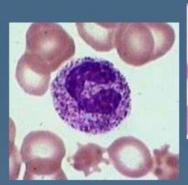


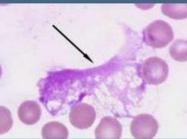
Leukocytes with **Döhle bodies**

- infections
- poisoning
- burns

Leukocytes with toxic granulation

- severe inflammation
- tumor necrosis



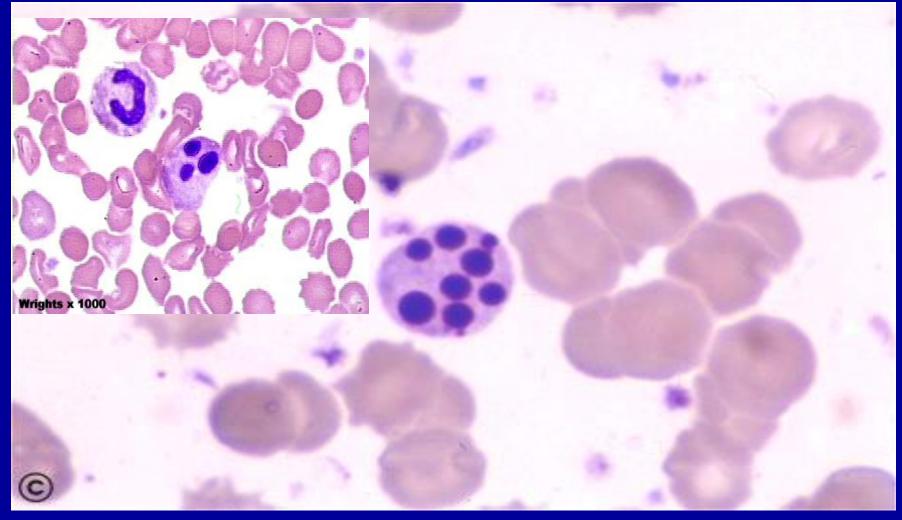


Gumprecht's cells (shadows)

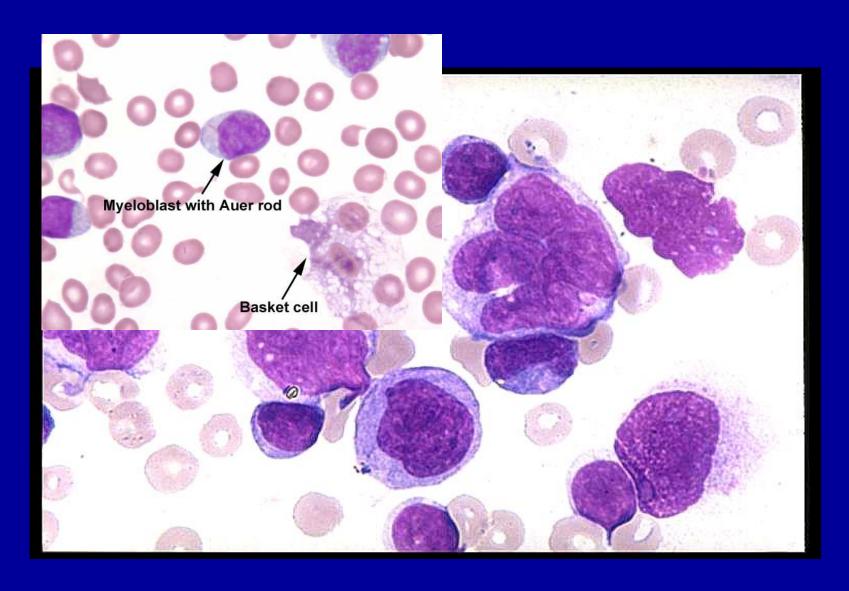
smudge cells

 cell's partial breakdown during preparation of a smear (CLL)

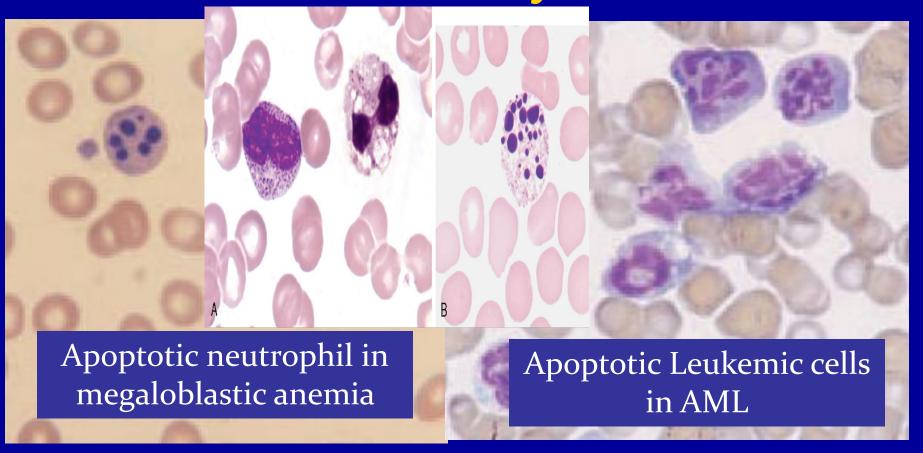
Necrobiotic Change (Apoptotic Neutrophile)



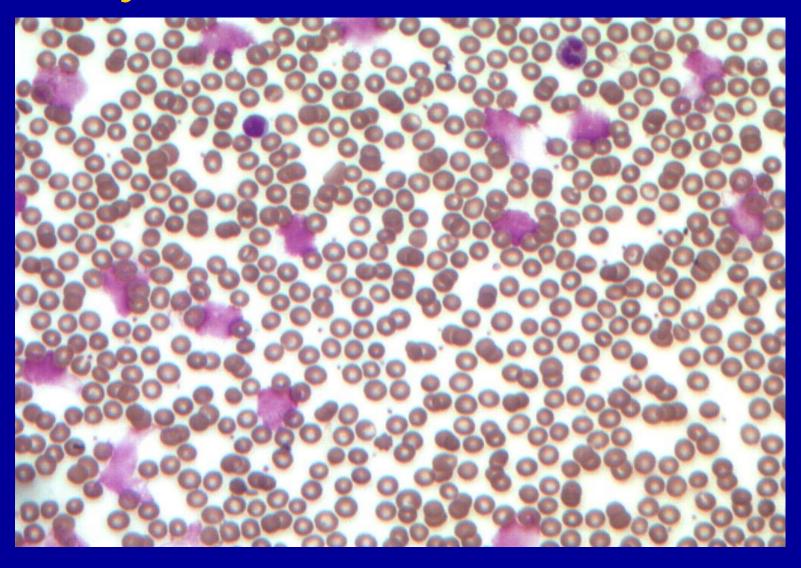
Smear ,Smudge,Basket cells



Necrobiotic (apoptotic) neutrophils and other myeloid



PBS of a patient with Dx of CLL, What do you do for diff. count?



Less than 2% of the leukocytes may be smudged, except in some lymphoproliferative disorders.

Only if the disrupted cell is still clearly identifiable (e.g., an eosinophil) should it be included in the differential count

Adding one drop of 22% human albumin to five drops of blood markedly reduces smudge formation. Make the blood film from the albumin-blood mixture.

SOME ALTERNATIONS & ABNOMALITIES OF NEUT. CYTOPLASM

- REDUCED GRANULATION
 - MDS SYN.
 - CONGENITAL LACTOFERRIN DEFFICIENCY
- INCREASED TOXIC GRANULATION
 - PREGNANCY, INFECTION, INFLAMMATION
 - G-CSF,GM-CSF THERAPY
 - ALDER –REILY ANO.
 - ► CNL
 - APLASTIC ANEMIA
 - ► MDS
 - ► HES

ABNORMAL GRANULATION

- CHEDIAC HIGASHI SYN.& RELATED ANOMALIES
- ALDER- REILY ANOMALY

AML

MDS

INHERATED CONDITIONS WITH ABNORMAL GRANULES OR INCLUSIONS

CHEDIAK- HIGACHI ANOMALY.

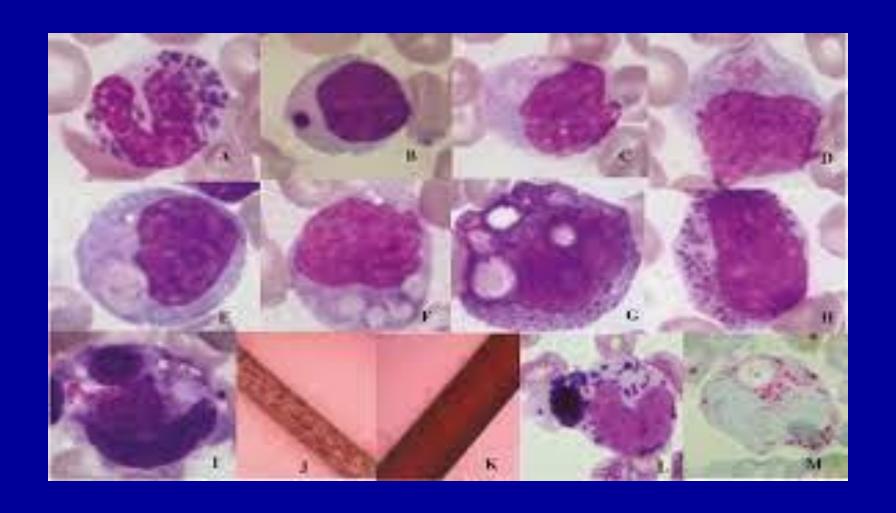
ALDER- REILY ANOMALY.

MAY- HEGGLIN ANOMALY.

Chediak-Higashi syndrome

- Genetic rare, autosomal recessive condition.
- characterized by infection, albinism, (hypopigmentation, silvery hair, photophobia) lymphadenopathy, hepathosplenomegaly, neuropathy and cytopenias
- Large granules in granulocytes, monocytes, occasionally lymphocytes.melanocytes (fusion of primary & secondary granules)
- Cells engulf but do not kill microorganisms.
- Serious often fatal repeated pyogenic conditions

Chediak- Higachi Anomaly



Disorders affecting Multiple Cell Lines

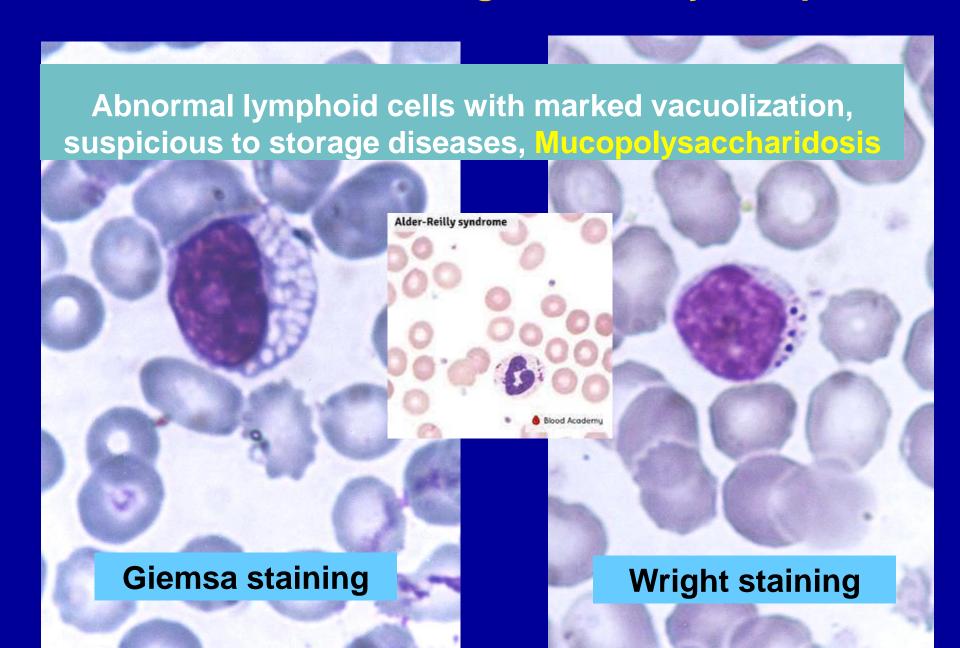
Alder-Reilly Anomaly:

- Associated with "storage diseases"
- mucopolysaccarides accumulate in cytoplasm of tissues and blood cells deficiencies in specific enzymes to degrade mucopolysaccharides (Hurlersyndrome Hunter syndrome: gargoylism)
 - Large coarse dark granules in all cell lines looks similar to toxic granulation)

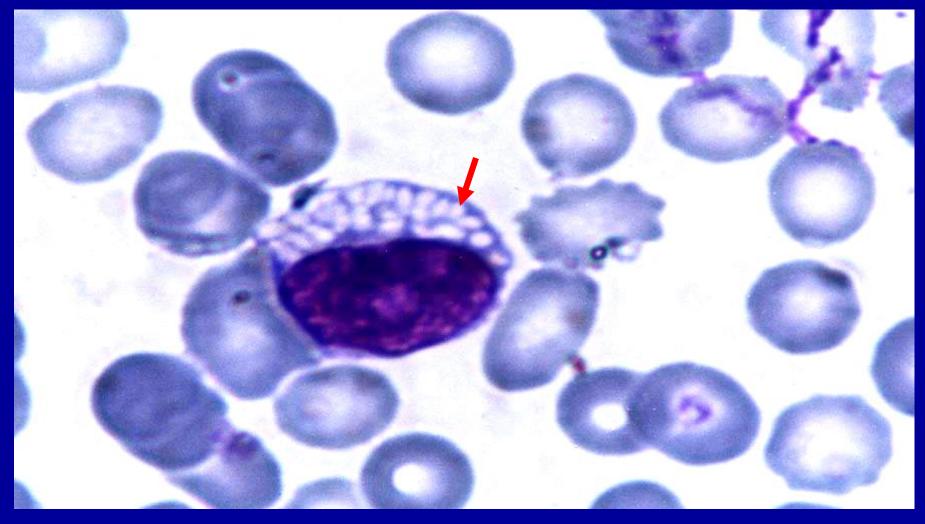
Alder-reilly Anomaly

- Dark red & purple inclusions
- May resemble toxic granulation
- Inclusions or vacuoles in lymphocytes
- Nature of the granules are mucoplysacharid or other abnormal carbohydrates
- IN Neut, eos, baso, lymph, mono (rarely)

What is the main finding? What do you report?



Mucupolysaccharidosis vacoules in lymphocytes (gimsa stain)



Mucupolysaccharidosis, Wright Stain



MAY - HEGGLIN ANOMALY

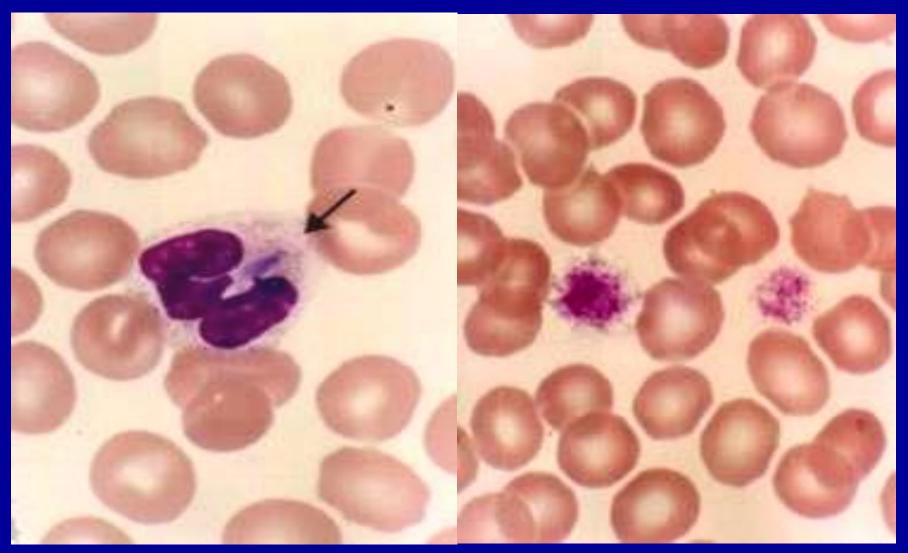
- Autosomal dominant inheritance
- THROMBOCYTOPENIA
- GIANT PLTS
- RESEMBLE DOHOLE BODIES
- ❖ AMORPHOSE AREA OF CYTOPLASM CONTAINIG
 STRUCTURES RELATED TO RIBOSOME

May-Hegglin

- Gray-blue spindles shaped inclusions in granulocytes and monocytes.
- Dense fibrils of RNA
- Leukopenia, thrombocytopenia, giant platelets
- Asymptomatic to mild hemorrhagic problems

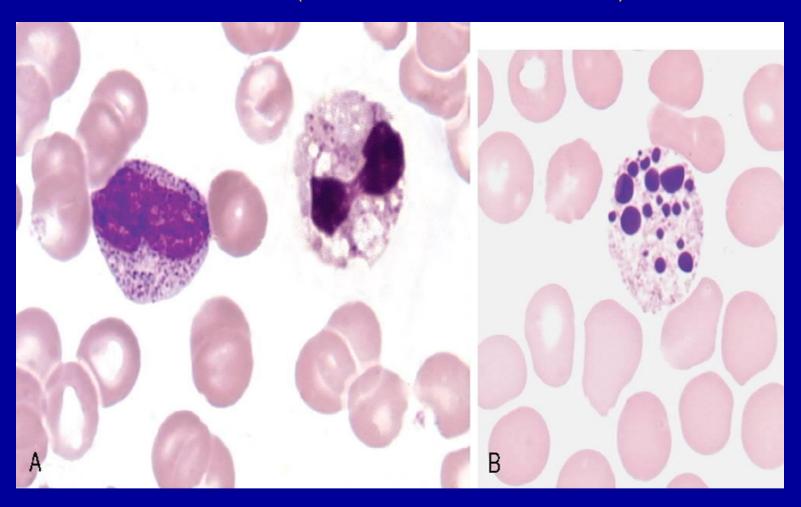
May- Hegglin Anomaly

large, elongated, bluish inclusion in the neutrophil cytoplasm.



A, pyknotic cell. The cell is also highly vacuolated.

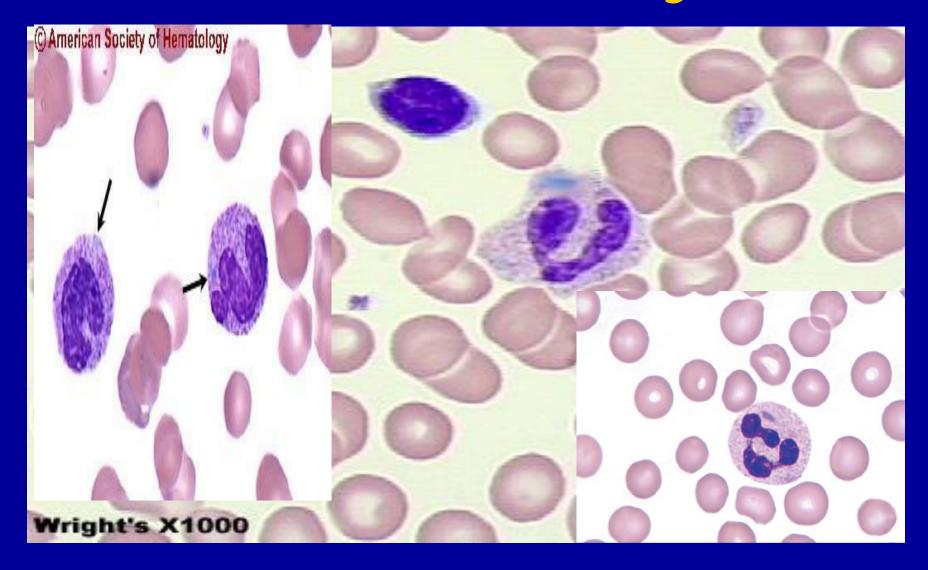
B, Neutrophil that has died nucleus has disintegrated into numerous rounded spheres of DNA with no filaments(necrotic or necrobiotic cell.)



DOHLE BODIES & SIMILAR INCLUSIONS

- INFECTION, INFLAMATION, BURNES
- PREGNANCY,G-CSF THERAPY
- ♦ MDS
- AML
- MAY-HEGGLIN ANOMALY
- FETCHNER SYN.&RELATED ANOMALIES
- KAWSHIAKOR

Dohle Body

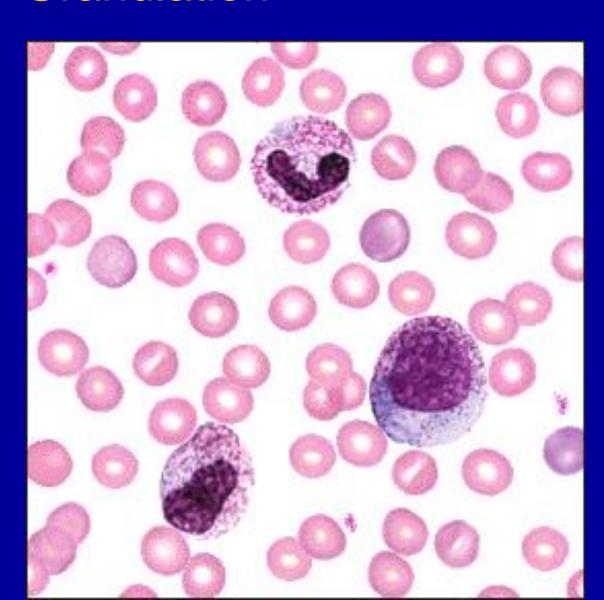


Toxic Granulation

Shift to the left & reactive changes in neutrophils

Response to G-Cr GM-CSF therapy

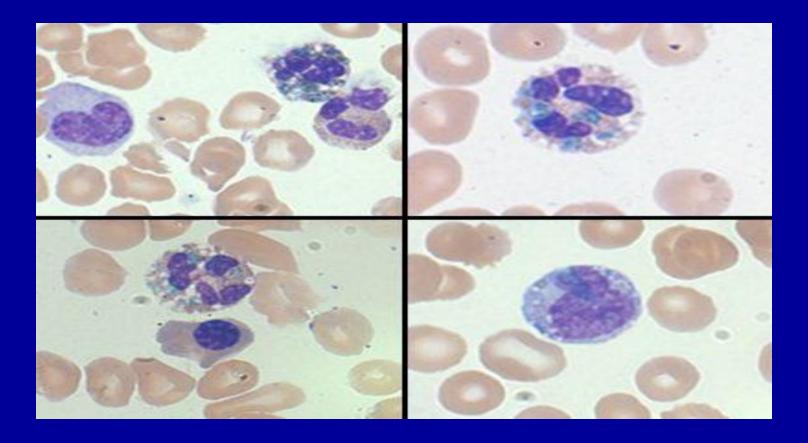
Bacterial infections



Hypogranular Band

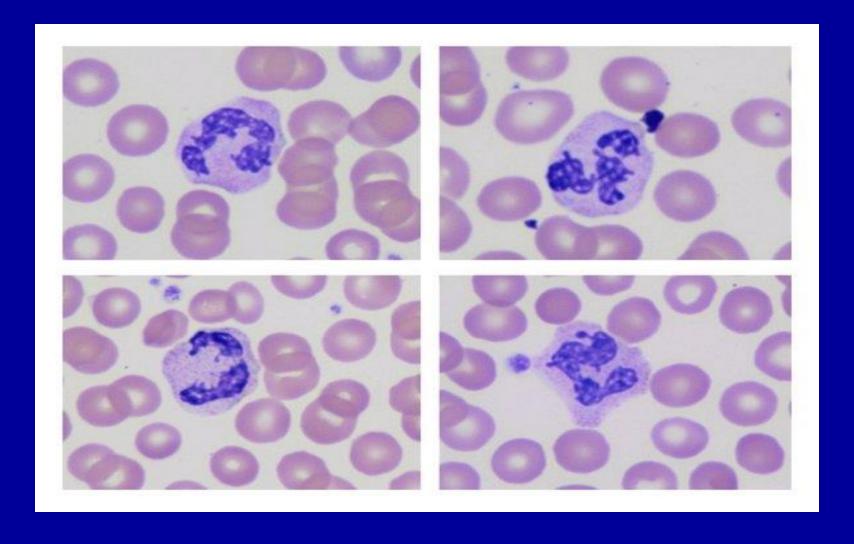


"Death crystal"



The appearance of these blue-green inclusions in the neutrophils and monocytes and the association with a high mortality rate give credence to the importance of reporting these inclusions particularly in the context of elevated liver enzymes and lactic acidosis. Further study of these blue-green inclusions is necessary to evaluate the nature of their appearance and the outcome for the patient

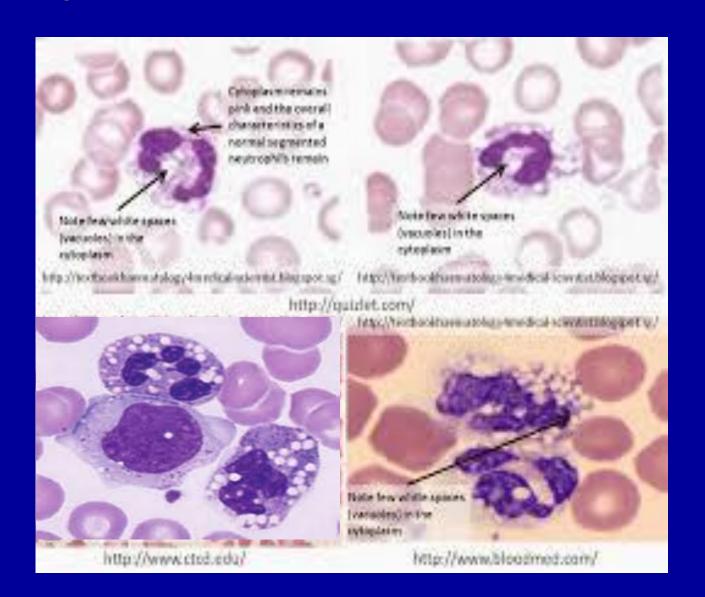
Neutrophils with "barbed-wire" aberrant nuclear projections (Wright Giemsa stain).



Cytoplasmic Vacoulation

- **♣ INFECTION, INFLAMATION G-CSF, GM-CSF**
- ACUTE ALCOHOL POISONING
- JORDANS'ANOMALY
- CARNITINE DIFFICIENCY
- KAWSHIAKOR
- MYELOKATHEXIS(SOME FAMILIES)

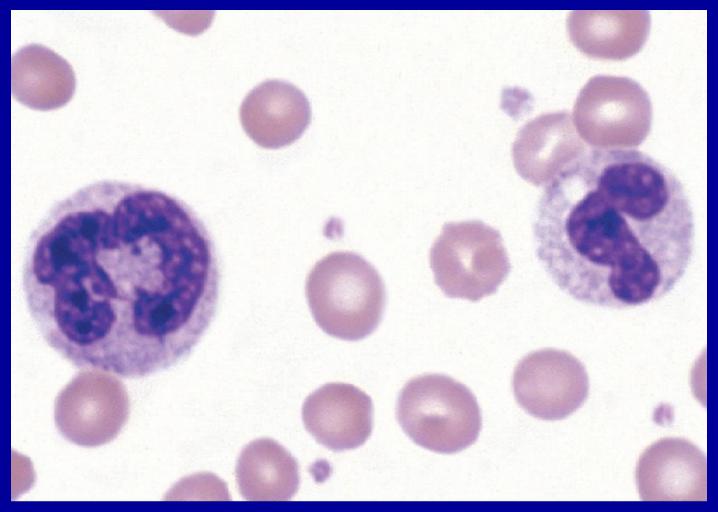
Cytoplasmic Vacoulization



Neutrophil anisocytosis.

The neutrophil to the left is larger than the other neutrophil. This is

often caused by cytoplasmic swelling.

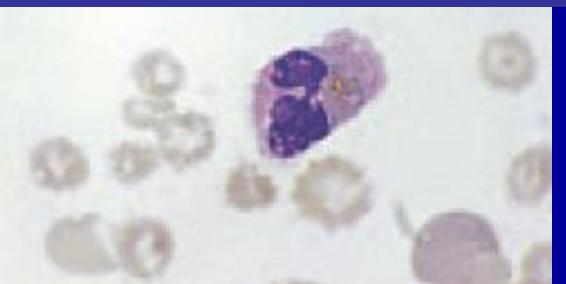


Phagocytized Material in Neutrophiles

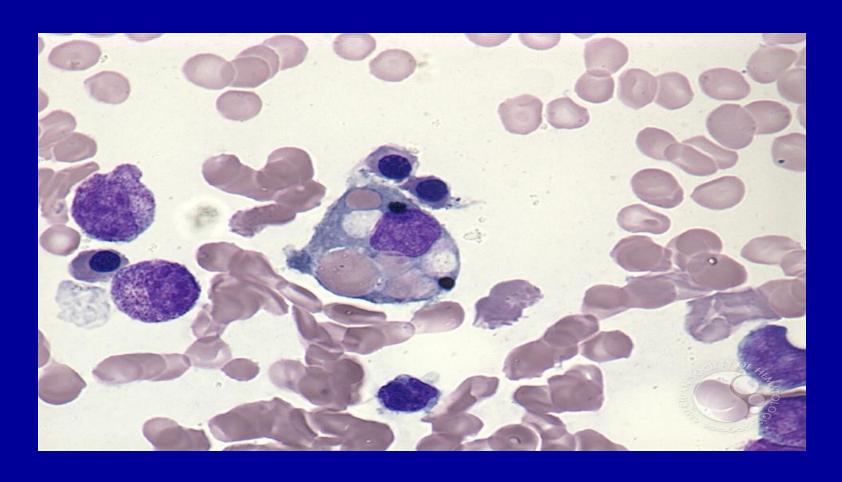
- Bacteri & fungi
- Parasites
- Cryoglobulin
- Mucopolysacharide
- Nucleoprotein(SLE)
- Melanin
- Billirubin
- Cystein crystals
- erythrocytes

A Neutrophil Containing Refractile Bilirubin Crystals.

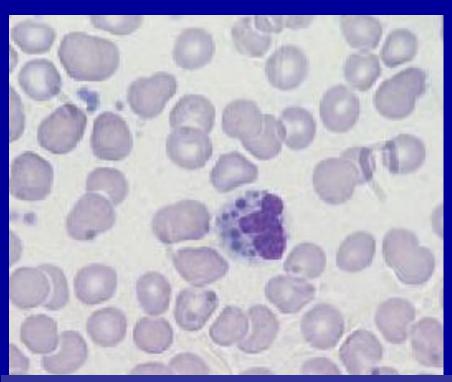
Rarely, bilirubin crystals are seen within neutrophils of infants and children with a markedly elevated plasma bilirubin; they are refractile and faintly yellow; they have been found to form in vitro when EDTA-anticoagulated blood is allowed to stand for at least 30 minutes

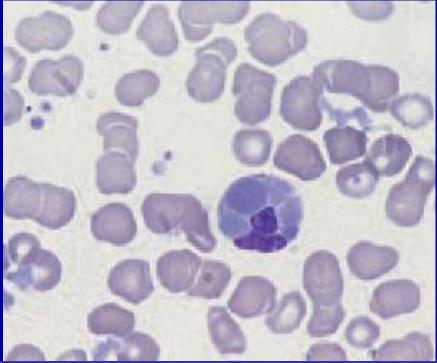


Erythrophagocytosis



Cryoglobulinaemia





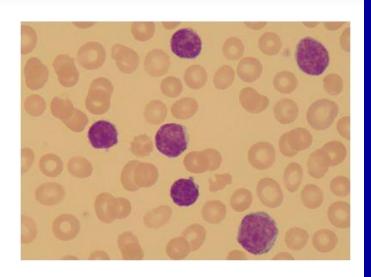
Peripheral blood film of a patient with *cryoglobulinaemia* showing cryoglobulin that has been ingested by neutrophils and appears as: (a) *small round inclusions*; and (b) *large masses* filling the cytoplasm and displacing the nucleus. Some extracellular cryoglobulin is also present.

Lymphocytosis

Non-Malignant causes

Virus infections:

Infectious mononucleosis
Infectious lymphocytosis
Cytomegalovirus infection
Occasionally mumps, varicella,
hepatitis, rubella, influenza



Bacterial Infections:

Pertussis

Occasionally cat-scratch fever, tuberculosis, syphilis, brucellosis

Protozoal infections:

Toxoplasmosis
Occasionally malaria

Other rare causes:

Hyperthyroidism, congenital adrenal hyperplasia

Lymphocytes, Variant Forms

- A normal differential count usually includes *up to* 6% of variant forms.
- Transitional forms between normal and variant lymphocytes are also found.
- In children in apparently good health, more immatureappearing lymphocytes with clear nucleoli are sometimes found.
- The nuclear chromatin may be dense, lumpy, or "blocked" with clearer areas of parachromatin; nucleoli may be visible.

Atypical lymphocytes (LVF)

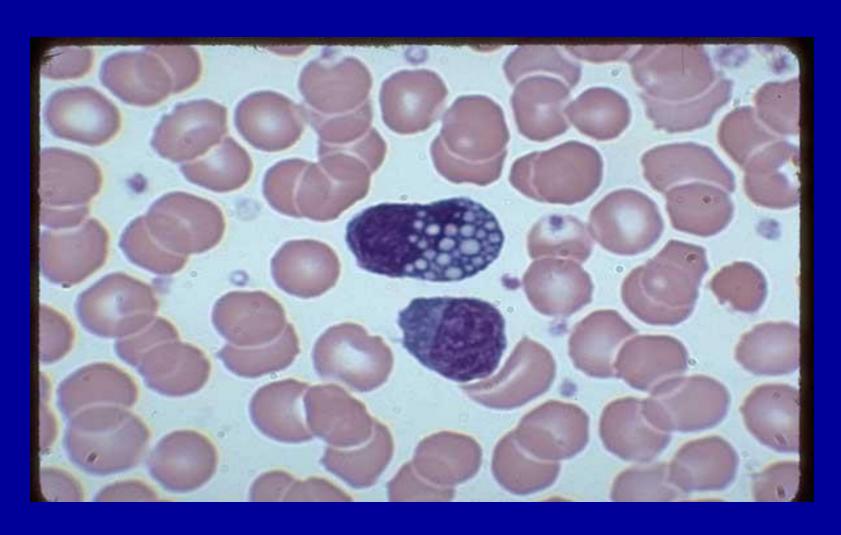
- These cells can be normal physiologic variants or abnormal forms.
- These cells are large and quite variable in appearance.
- The terms "atypical, reactive, Downey cell, virocyte" etc., have been used to identify these cells.
- Because of confusion about the relationship of these cells to either benign or malignant processes, the subcommittee chose the new term-lymphocytes, variant forms.

A normal differential count usually includes up to 6% of variant forms. Transitional forms between normal and variant lymphocytes are also found. In children in apparently good health, more immature-appearing lymphocytes with clear nucleoli are sometimes found.

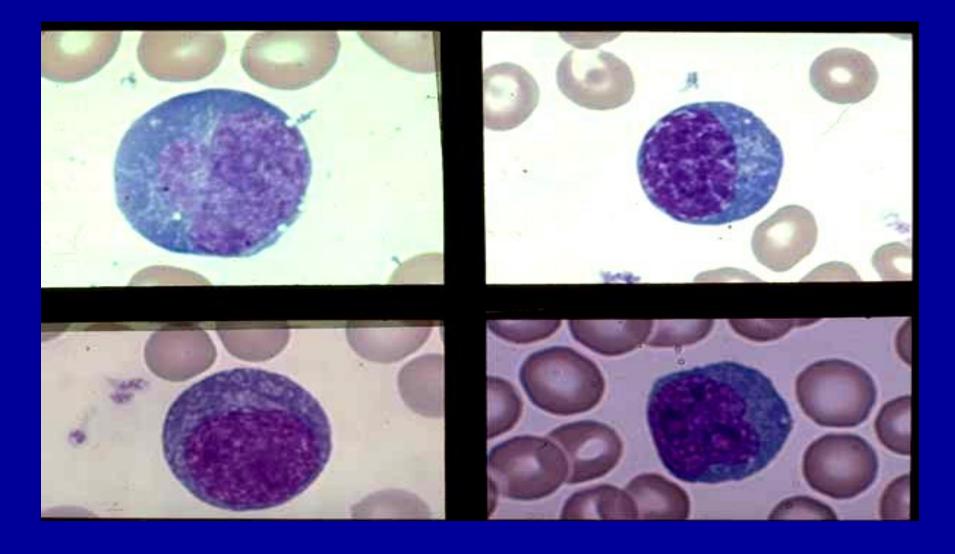
LVF

- LVF (atypical lymph) :Few(<6%)</p>
- **♦ > 20%**
- Infectious Mononucleosis
- Viral Hepatitis
- Cytomegalovirus Infections
- Post Transfusion Syndromes
- Drugs (Phenytoin, PAS)

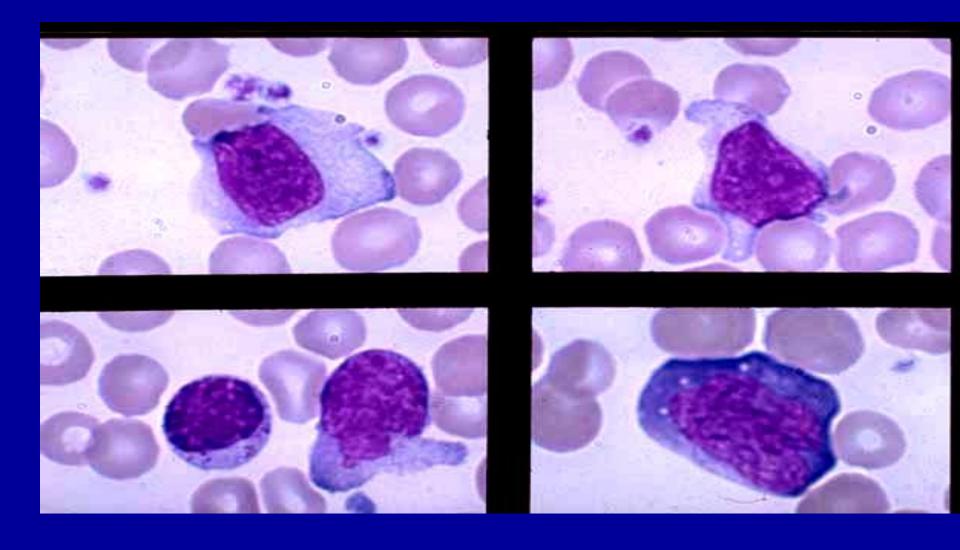
Plasmocytoid Lymphocyte (Mott cell)



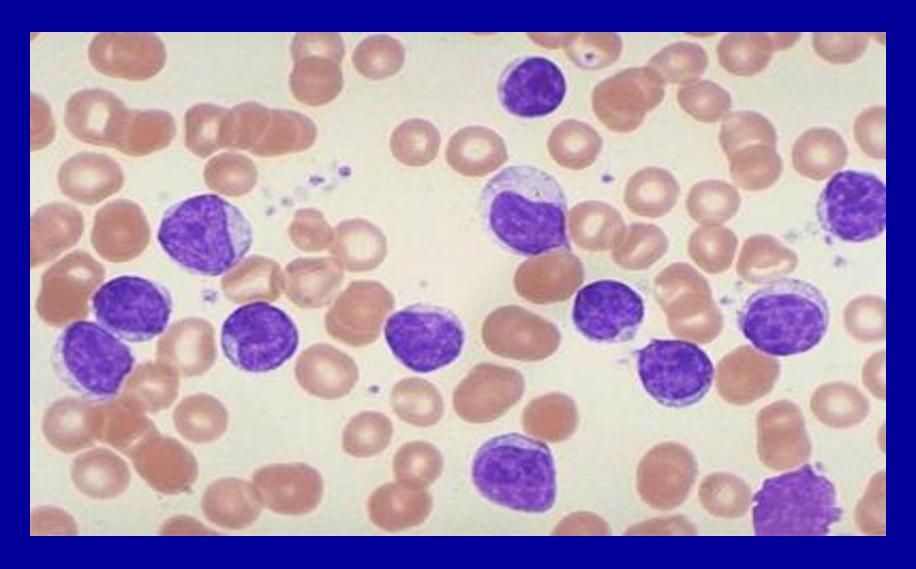
Plasmocytoid lymphocytes



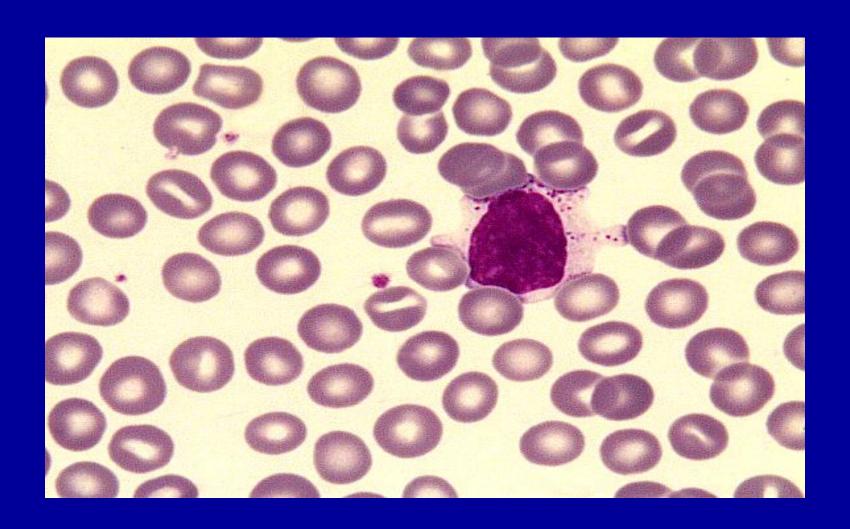
Reactive lymphocytes



CLL



Large Granular lymphocyte



What is the name of this leukocyte?



The hairy lymph. comes from a person suffering from active infection (without any malignant disease). The granules identify it as a T or NK cell

Monocytes qualitative Changes

Increased size

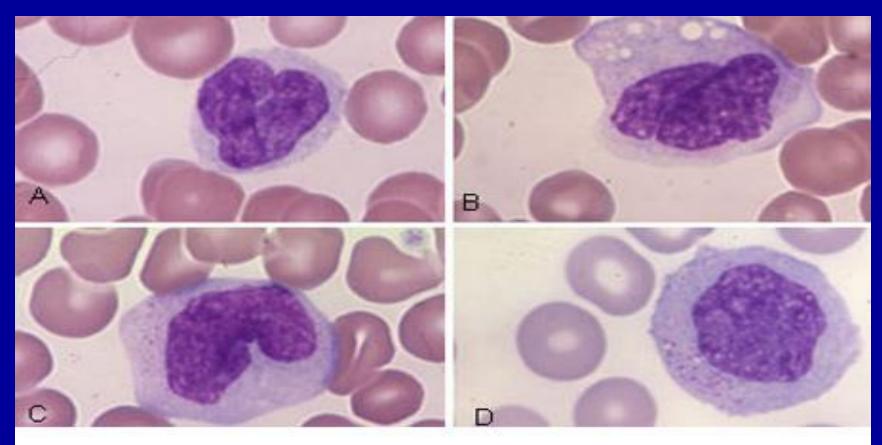
Cytoplasmic vacuolation

Intracellular debris



Irregular cytoplasmic borders

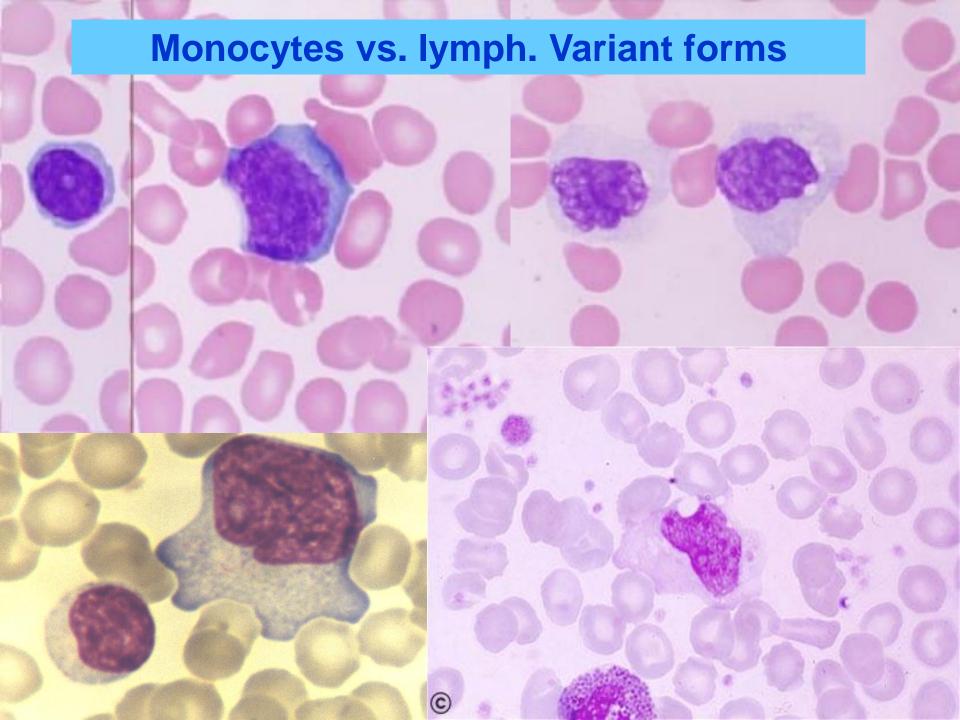
Normal Monocytes

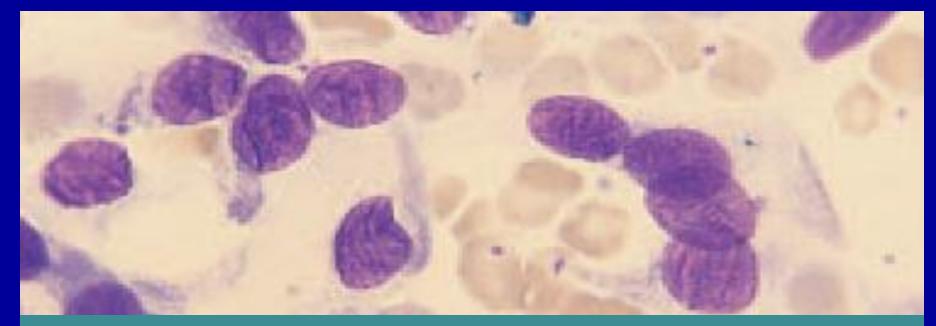


Source: Lichtman MA, Shafer MS, Felgar RE, Wang N:

Lichtman's Atlas of Hematology: http://www.accessmedicine.com

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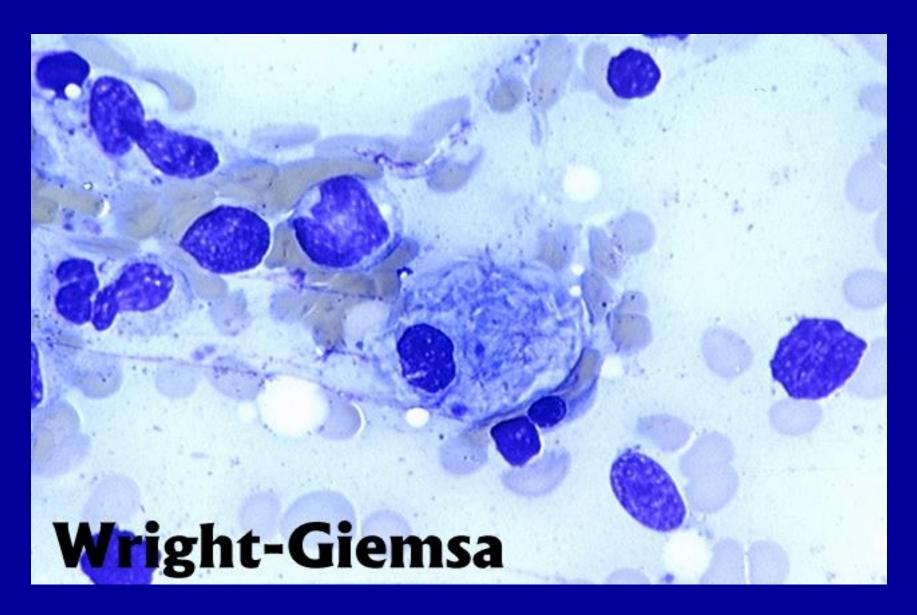
Increased numbers of endothelial cells are present in conditions with *vascular injury* (e.g. rickettsial infection, peripheral vascular disease, CMV infection, thrombotic thrombocytopenic purpura (TTP), sickle cell disease and following coronary angioplasty) but even in such

circumstances they are very infrequent.
Endothelial cells in a peripheral blood film made
from a venous blood sample.

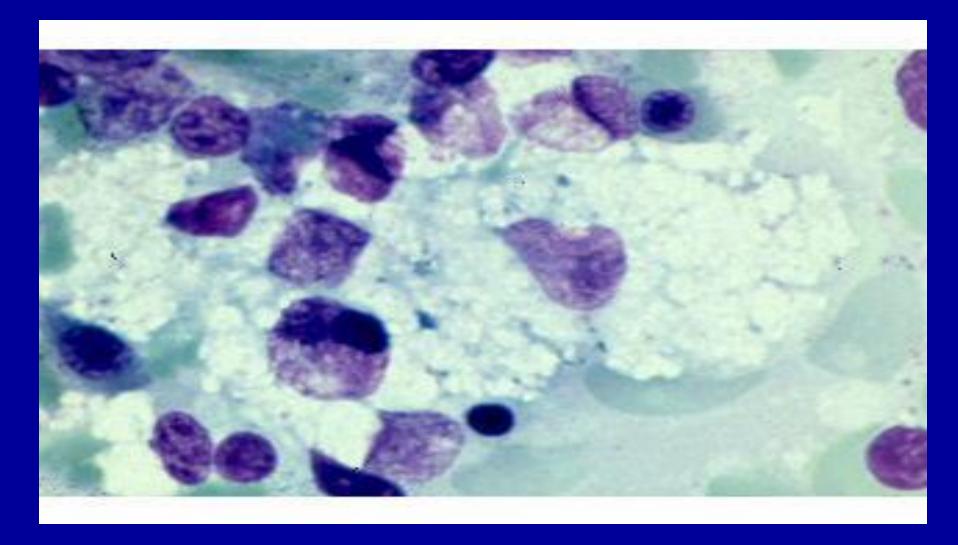
Morphologic alternations of Monocytes/macrophages

- ✓ Also called Lipid Storage Disorders
- Gaucher's Disease: deficiency of ß-glucocerebrosidase
- Niemann-Pick Disease:deficiency of the enzyme sphingomyelinase
 - accumulation of unmetabolized lipid
 - large cells filled with lipid droplets.
 - Tay-Sachs disease:deficiency of the enzyme hexosaminidase A.
- Sea-blue histiocytosis: accumulation of phosphosphingolipids in cytoplasm

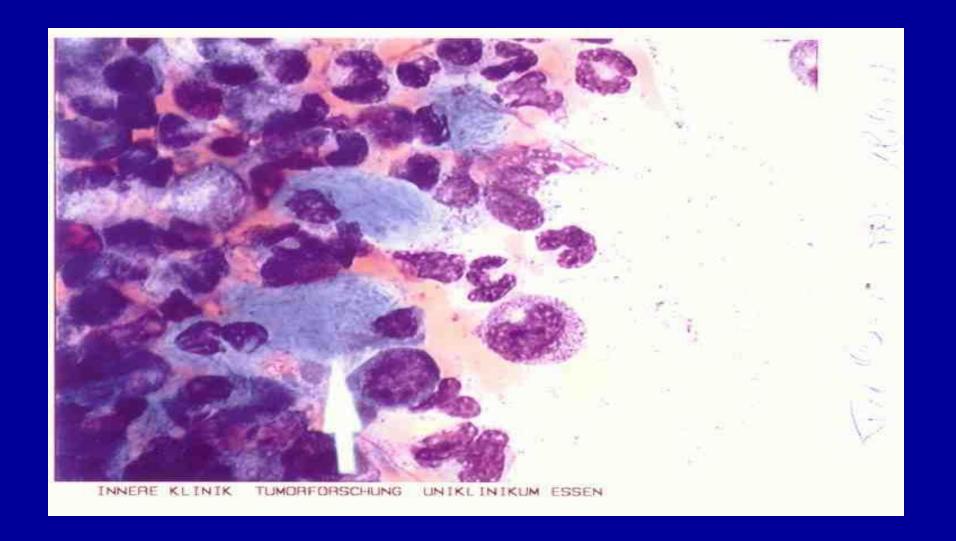
Gaucher Cell

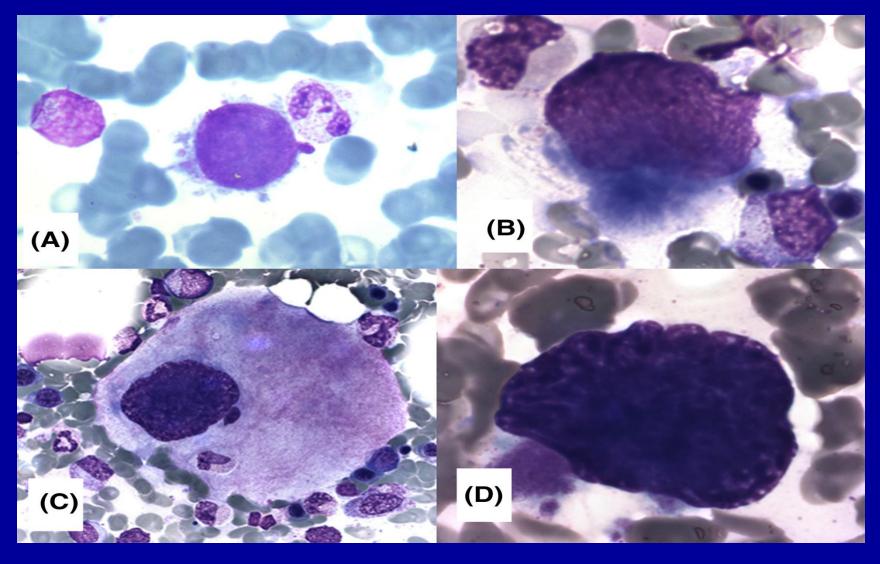


Nimen - pick

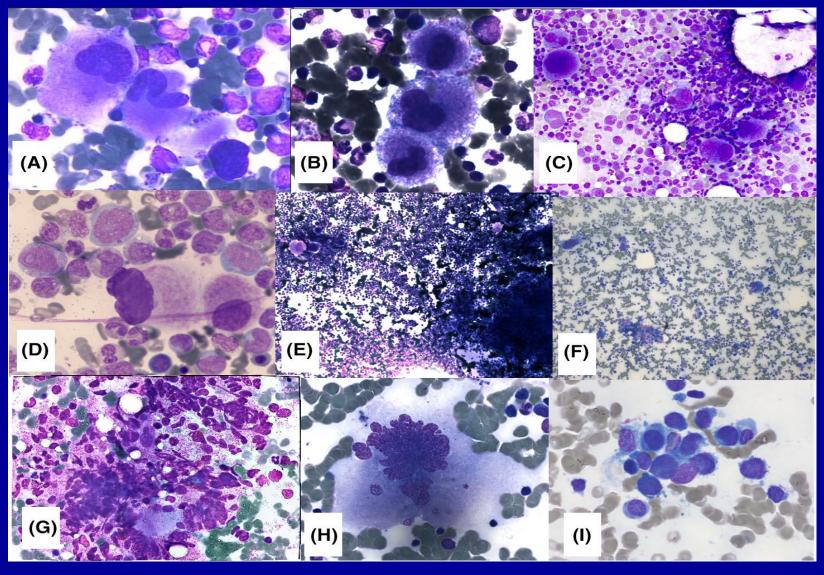


Sea Blue Histocytosis





Int J Lab Hematology, Volume: 43, Issue: S1, Pages: 23-28, First published: 20 July 2021, DOI: (10.1111/ijlh.13536) Morphologic features of normal megakaryocytes (MGK) at different maturation stages. A, Early MGK /megakaryoblast. B, Immature MGK /proMGK. C, Mature/granular MGK with emperipolesis of one neutrophil. D, Late MGK/ bare nucleus

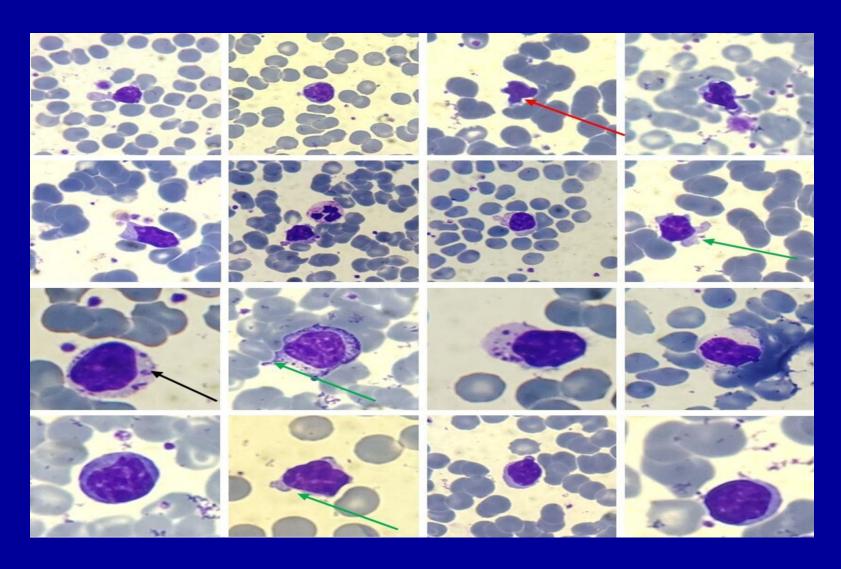


Int J Lab Hematology, Volume: 43, Issue: S1, Pages: 23-28, First published: 20 July 2021, DOI: (10.1111/ijlh.13536)

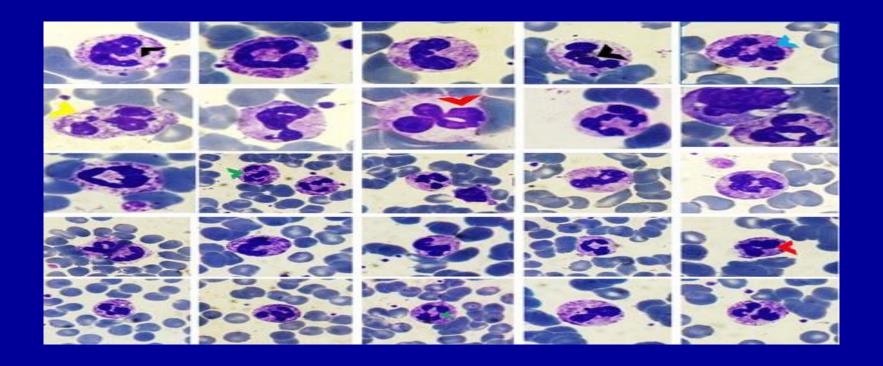
Morphologic features of different megakaryocytes (MGK) with atypical morphology on bone marrow smears. A, Reactively increased MGKs in acquired hemolytic anemia. B, Increased MGKs with a left shift in a patient with immune thrombocytopenia. C, Increased hypolobulated and often clustering MGKs in one patient with immune thrombocytopenia. D, Dwarf MGK in CML ABL-BCL1 positive. E, Increased number of MGKs in PV. F-G, Pleiomorphic MGK in PMF. H, Giant cauliflower MGK in ET. I, Megakaryoblasts in acute megakaryoblastic leukemia

Covid -19 and WBC morphology

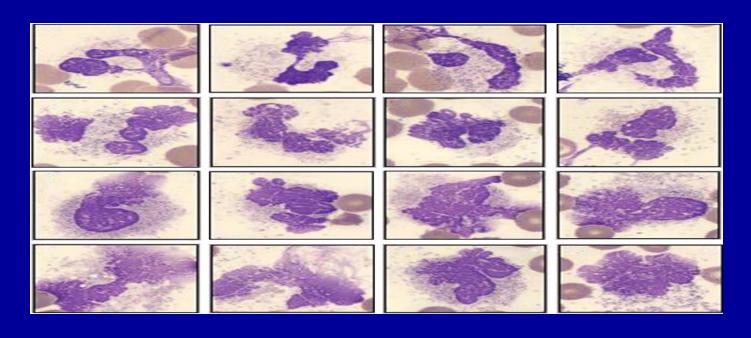
Peripheral blood films showing large granular lymphocytes. Round to indented nuclei, condensed chromatin, prominent nucleoli in a few, along with abundant pale blue cytoplasm with distinct variably sized azurophilic granules are present (long black arrow). Cytoplasmic pod formation (long green arrows) and apoptotic lymphocytes (long red arrow) are highlighted. Giemsa ×200–400.



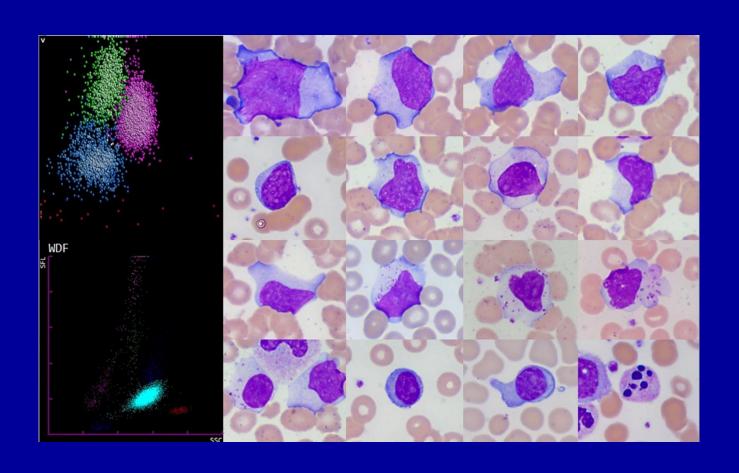
Peripheral blood films showing various neutrophils with C-shaped, fetus-like COVID nuclei (black arrowheads) with aberrant nuclear projections (blue arrowhead). Toxic granulations and vacuolations (yellow arrowhead), ring nuclei (red arrowheads) and elongated nucleoplasm (green arrowheads) are highlighted. Giemsa ×200–400.



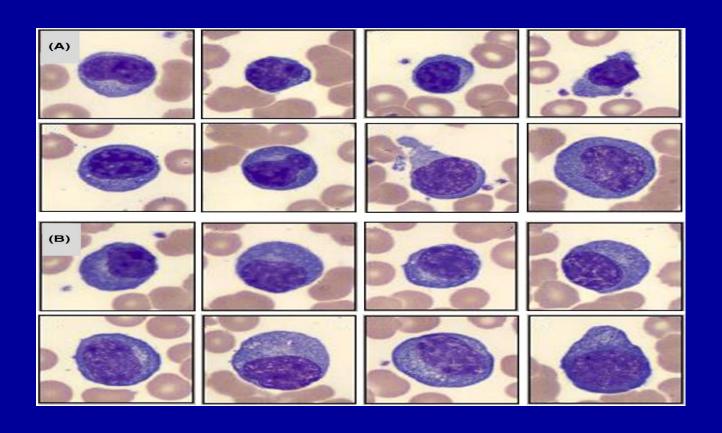
Increase in smudged granulocytes in the peripheral blood of patients with COVID-19



Covid -19 a new ethology for atypical lymphocytes



Spectrum of peripheral blood morphologic findings in patients with COVID-19 including (A) plasmacytoid lymphocytes and (B) plasma cell



Peripheral blood films showing activated monocytes with prominent cytoplasmic vacuolisation and a few granules (small red arrow).

Nuclear blebbing (small green arrow) is also seen. Giemsa ×200–400.

