Diagnostic methods in hematology I – Complete blood count

Doc. MUDr. Jan Živný, Ph.D.

jzivny@LF1.cuni.cz

22496-5865

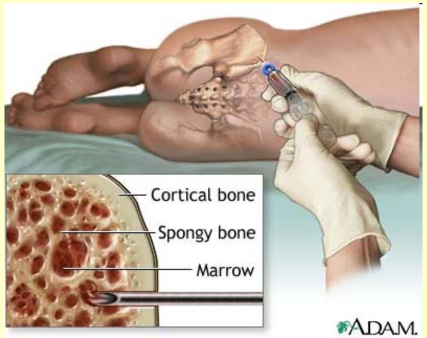
Institute of Pathophysiology 1.LF UK

Outline

- Diagnostic methods in hematology
  - General manifestation of hematologic diseases
  - Complete blood count
    - Basic evaluation of anemia
    - White blood cell count

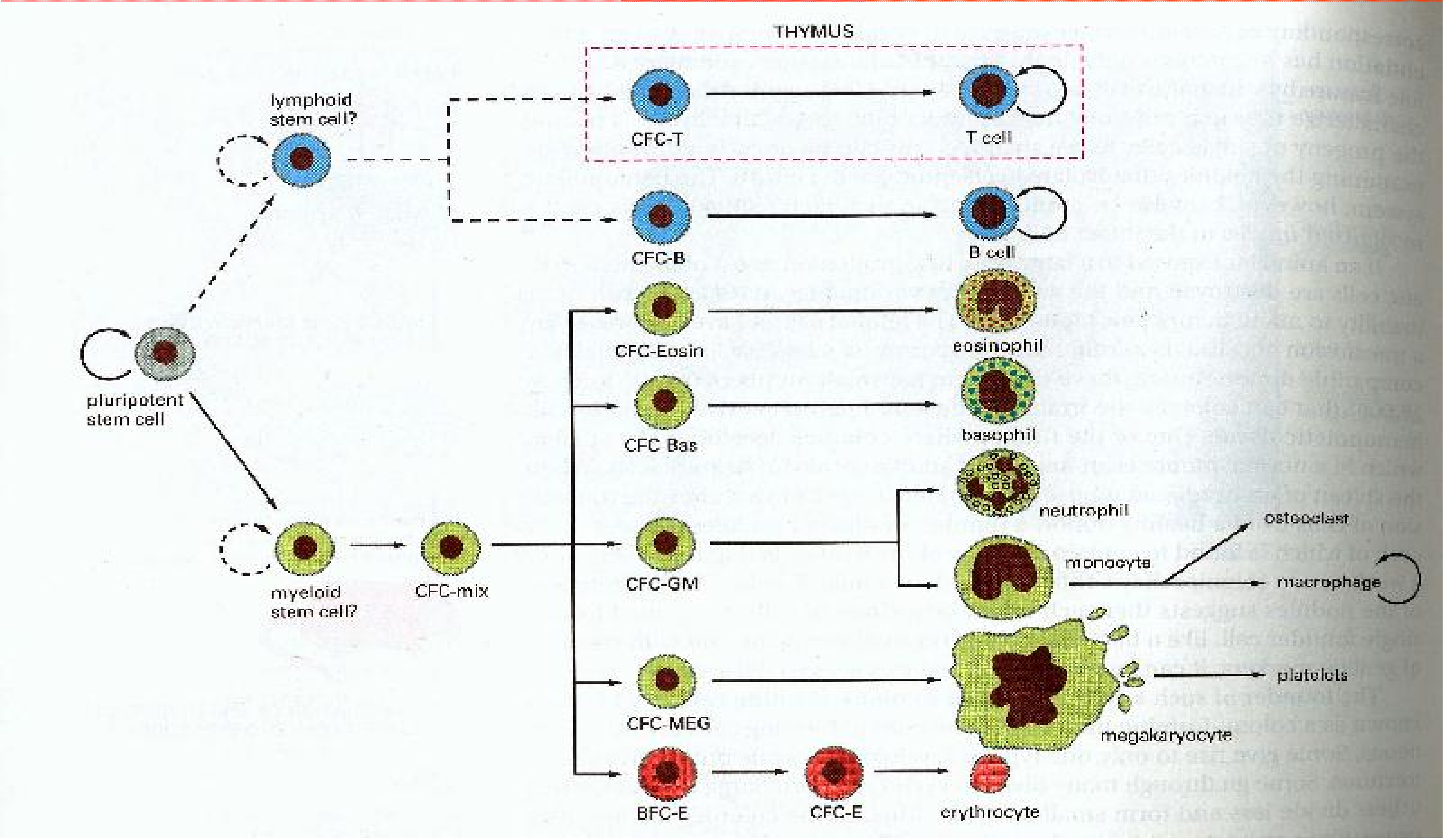
What we analyze?

1. Blood
2. Bone marrow
   - bone marrow aspiration
   - trepanobiopsy
3. Lymph nodes – extirpation



Hematopoietic Cells

Bone marrow | Blood / Lymph nodes



Manifestation of hematologic diseases

Manifestation of hematologic diseases

- **Hypoxia**

  local

  – tissue ischemia – tissue damage – Inflammation - necrosis

  systemic

  – cardiovascular signs, dyspnea, acidosis

- **Chronic or Frequent infections**
- **Bleeding / Thrombosis**
- **Inflammation (autoimmunity)**

Basic mechanisms of hematologic disease manifestation

- **Polycytemia**

  → hyperviscose blood → thrombosis → embolia → hypoxia

- **Cytopenia**
  - **Leukopenia** → frequent infections
  - **Anemia** → hypoxia
  - **Thrombocytopenia** → bleeding → hypovolemia → shock → tissue hypoxia

- **Pathologic leukocytosis (blasts)**
  - →obstruction of lung capillaries → dyspnea → pulmonary hypertension → hypoxia

Laboratory Tests

Basic:

- Complete blood count

Specialized:

- Tests for iron metabolism
- Measurement of soluble factors (e.g. cytokines, antibodies, complement subunits.....)
- Cytogenetic and genetic analysis (mutations)
- Immunophenotyping of BM or PB cells
- Detection of antibodies to self antigens (e.g. RBC)
- Histochemical analysis of cell enzymatic activity
- Functional tests (Clonogenic assay)

Complete Blood Count (CBC)

Complete Blood Count (CBC)

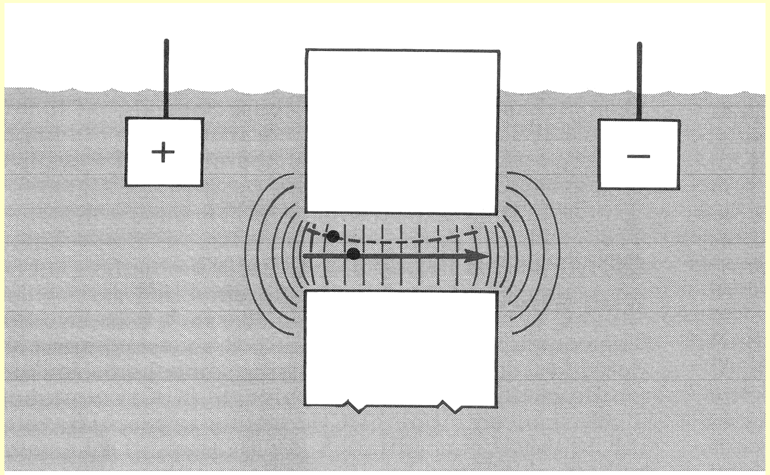
- **Hemoglobin concentration (Hb)**
- **Hematocrit (Hct)**
- RBC count
- **RBC parameters**
- **WBC count**
- **WBC differential count**
- **Platelet count**
- Platelet parameters
- **Description of blood smear**

- Hematology Analyzers

First automated cell counters came out in the 1950s

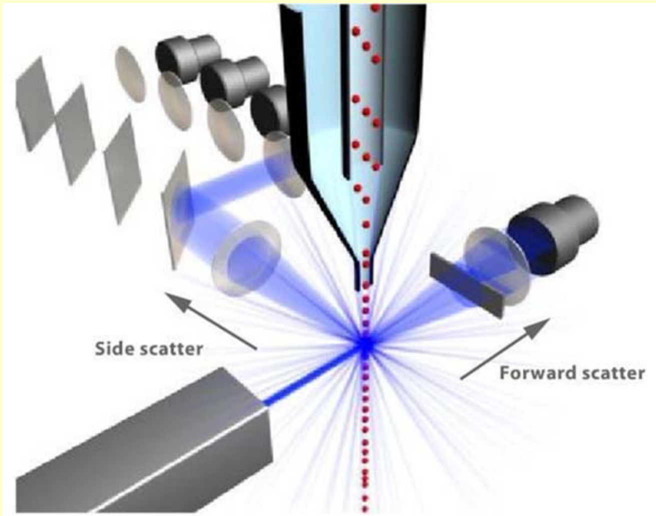
How the analyzers work ?

- Electrical impedance principle (Coulter)
  - cells break an electric circuit as they pass though the aperture between electrodes
  - indicate the presence of a cell (number) and the size of a cell



How the analyzers work ?

- Optical principle
  - cells break the laser beam the number of events (cell number) and light scatter property of the cells are recorded
    - size of the cell (forward scatter)
    - granularity of the cell (side scatter)



When to do CBC?

- **Suspected disease**
  - hematologic, inflamatory, neoplastic, or infection
- **Screening**
  - infants (<1yr.), pregnant women, elderly patients, and patients with nutritional abnormalities
- Controversial values during routine patient evaluation

Hemoglobin concentration (Hb) and Hematocrit (Hct)

- Depends on age and sex of the patient
- Depends on hydratation of the patient (e.g. pregnancy)
- F: Hb 121-151 g/L Hct 36-44%
- M: Hb 138-170 g/L Hct 41-50%
- Less then 70 g/L usually symptomatic tissue hypoxia

ANEMIA

- **Anemia is clinical sign!! not a disease**
  - is considered to be present if the Hb (or Hct) is below the lower limit of 2 standard deviations (-2SD) or the 95% confidence interval for the normal population
- **Statistics:** 2.5% of normal individuals are classified as anemic.

- WHO criteria: Hb < 125 g/L in adults
- US criteria:
  - M: Hb < 135 g/L
  - F: Hb < 125 g/L
- 1.LF criteria
  - M Hb < 135 g/L
  - F Hb < 116 g/L

Anemia

- Absolute if the RBC mass is decreased

- Relative if associated with an increased plasma volume
  - e.g.
    - overhydration (volume overload)
    - pregnancy
    - macroglobulinemia
    - postflight astronauts ☺

General consequences of anemia

hemoglobin loss

↓

impaired delivery of oxygen to the tissues

↓

tissue hypoxia

↓

Symptoms: fatigue, dyspnea, paleness...
Signs: tachycardia

Causes of anemia

- **Insufficient RBC production:** deficient erythropoiesis
- **Excessive RBC loss**

Insufficient RBC production

- Nutritional deficiencies (iron, folate, vitamin B12, vitamin B6)
- Anemia of chronic disease
- Renal, liver, or endocrine disease
- Bone marrow failure (aplastic anemia, pure red cell aplasia, sideroblastic anemia)
- Bone marrow infiltration (myelophthisic anemia)

- **Complete loss of erythropoiesis results in Hb decline of** ????????

Insufficient RBC production

- **Complete loss of erythropoiesis** results in Hb decline of about 10% / wk

Wk	1	2	3	4
M [g/L]	160	144	129	116
F [g/L]	140	126	113	102

  – WHY??

Insufficient RBC production

- Complete loss of erythropoiesis results in Hb decline of about 10% / wk
  - WHY??
    - Physiologically ~ 1 – 2 % of RBC are reticulocytes
    - Reticulocytes mature in 1 day to erythrocytes

Excessive RBC loss

- Bleeding
  - **Acute:** shortly after massive blood loss Hb normal due to vasoconstriction (normochromic - normocytic)
  - **Chronic** – leads to depletion of iron which results in **insufficient RBC production**
- **Hemolysis**
  - intrinsic
  - extrinsic
- **Hemoglobin disorders** (hemoglobinopaties, thalasemia)

Red blood cell (RBC) count

- **F: 3.9 – 5.0 x 10^{12} erythrocytes / L**
- **M: 4.5 –5.7 x 10^{12} erythrocytes / L**

RBC parameters (indices) - 1

Differential diagnosis of anemia (morphologic criteria)

MEAN CORPUSCULAR VOLUME = MCV

- MCV (fL) = Hct / RBC count
- Histological classification of anemias
  - microcytic anemia (< 80 fL)
  - normocytic anemia (80 – 95 fL)
  - macrocitic anemia (> 95 fL)

- Not useful to detect anisocytosis = variation in cell size
- Reticulocytosis may increase MCV

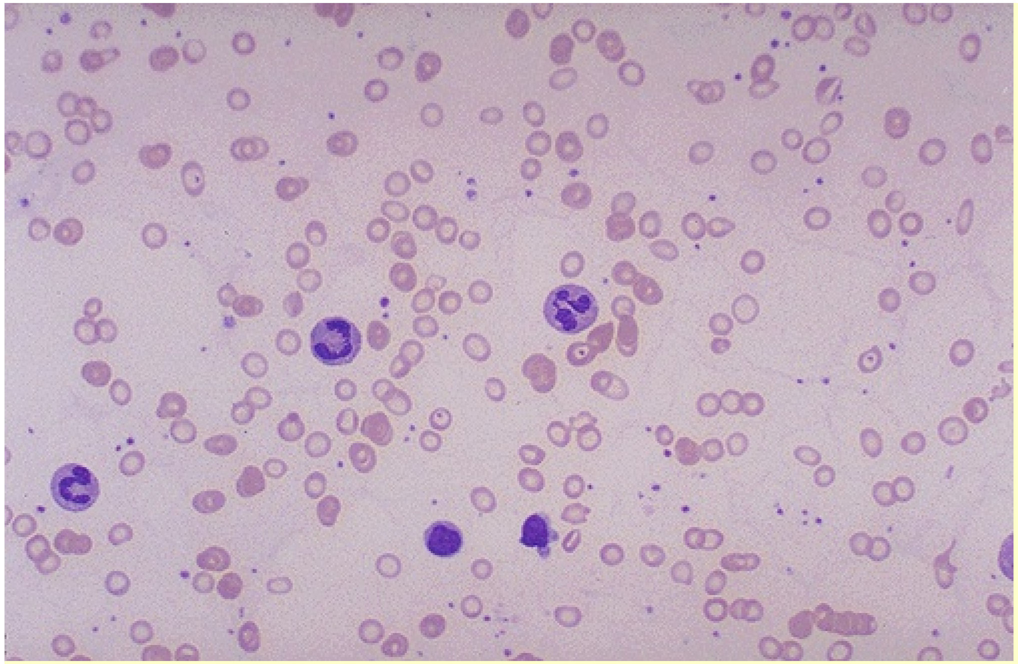
RBC parameters (indices) - 2

MEAN CORPUSCULAR HEMOGLOBIN = MCH

- MCH (pg/cell) = Hb / RBC count
- MCH 32.7 – 33.7 pg / cell
- Hypochromia MCH < 27 pg / cell

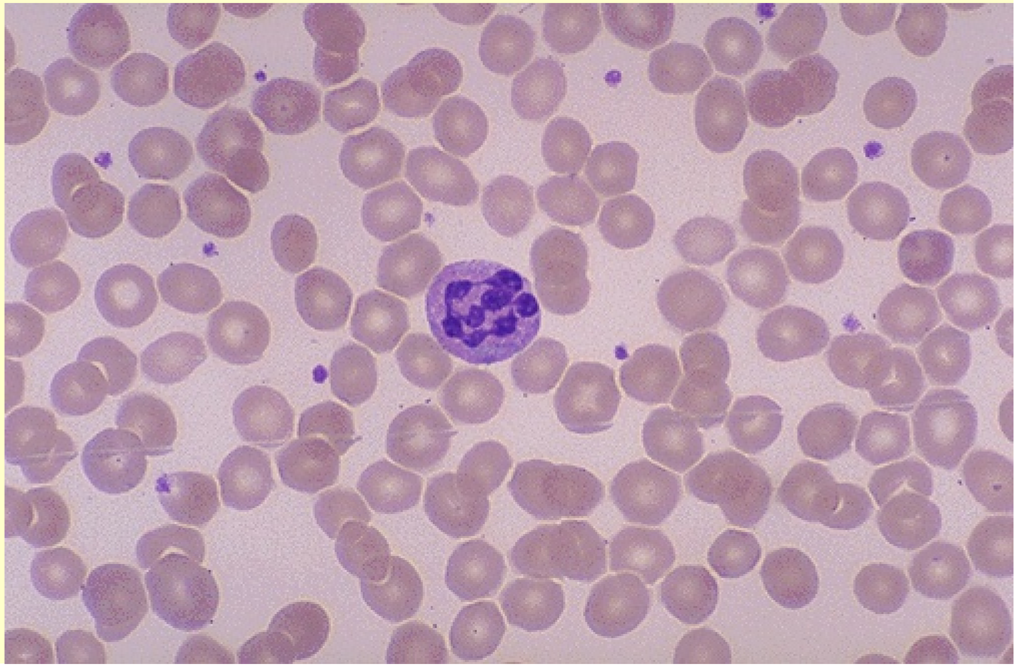
MEAN CORPUSCULAR HEMOGLOBIN CONCENTRATION = MCHC

- MCHC (g/L of RBC)= Hb / Hct
- MCHC: 267 – 355 g / L



hypochromic microcytic anemia

macrocytic anemia
hypersegmented neutrophil



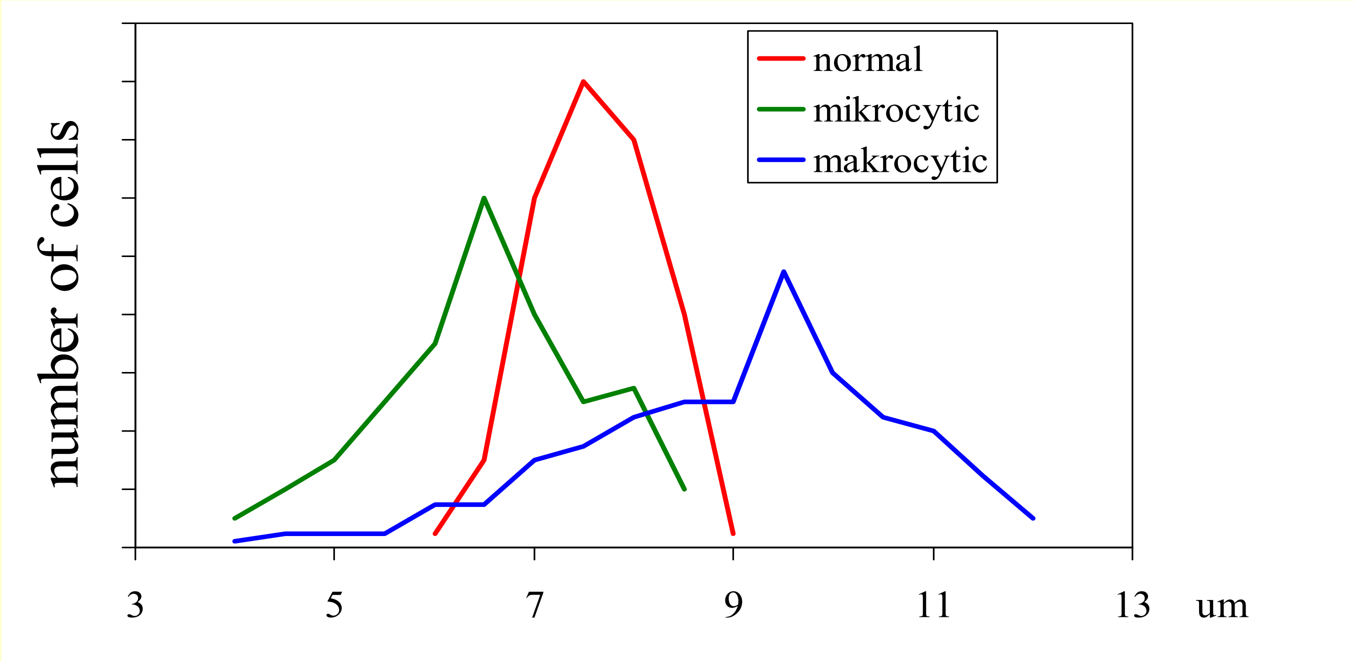
RDW – red cell distribution width

- Measure the variation of red blood cell (RBC) size (volume)

- RDW can be reported statistically as
  - coefficient of variation (RDW-CV) in %
    - = 1 standard deviation of RBC volume / MCV x 100%
    - affected by the average RBC size
  - standard deviation (RDW-SD) in fL
    - is measured by calculating the width (in fL) at the 20% height level of the RBC size distribution histogram

RDW – red cell distribution width

- Normal RDW
  - RDW-CV 11.6 – 14.6 %
  - RDW-SD 39-46 fL
- **Higher RDW** values indicate greater variation in RBC size = **anisocytosis**



- **WHY TO CALCULATE RDW??**

WHY TO CALCULATE RDW?

- More sensitive for diagnosis of early nutritional deficiency (iron, folate, or vitamin B12)
- Along with MCV narrowing the cause of anemia
- Flagging samples that may need manual peripheral blood smear examination (red cell fragmentation, agglutination, or dimorphic red blood cells)

Narrowing the cause of anemia using RDW and MCV

EXAMPLES

- **Elevated RDW and normal MCV:**
  - Early iron, vitamin B12, or folate deficiency
  - Dimorphic anemia (for example, iron and folate deficiency)
  - Sickle cell disease
  - Chronic liver disease
  - Myelodysplastic syndrome

Narrowing the cause of anemia using RDW and MCV

EXAMPLES

- **Elevated RDW and low MCV:**
  - Iron deficiency
  - Sickle cell-β-thalassemia

- **Normal RDW and low MCV:**
  - Anemia of chronic disease
  - Heterozygous thalassemia
  - Hemoglobin E trait

Narrowing the cause of anemia using RDW and MCV

EXAMPLES

- **Elevated RDW and high MCV:**
  - Folate or vitamin B12 deficiency
  - Immune hemolytic anemia
  - Cytoxic chemotherapy
  - Chronic liver disease
  - Myelodysplastic syndrome
- **Normal RDW and high MCV:**
  - Aplastic anemia
  - Chronic liver disease
  - Chemotherapy/antivirals/alcohol

Reticulocyte count

- Daily RBC replacement 40,000 – 50,000 /μL
  - 0.5 – 1.5% of RBC count
  - Maturate within 1 day in peripheral blood
- **Criteria of marrow activity**
  - Reticulocytosis
    - response to blood loss (hemolytic anemias, severe bleeding)
    - response to therapy of anemia (e.g. B12 or Fe def.)
  - Reticulocytopenia
    - deficient erythropoiesis (nutrient , hormonal, etc.)

Reticulocyte count

- **Reticulocyte index** = RI corrects the reticulocyte count for the severity of anemia

RI < 2% indicates hypoproliferative component of anemia

RI = Reticulocyte Count x (HCT / normal HCT)

Flow cytometry
Reticulocyte count

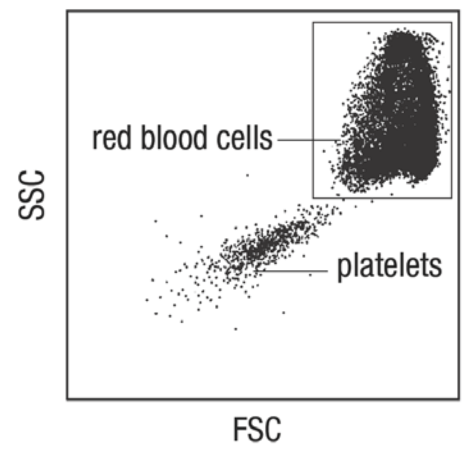
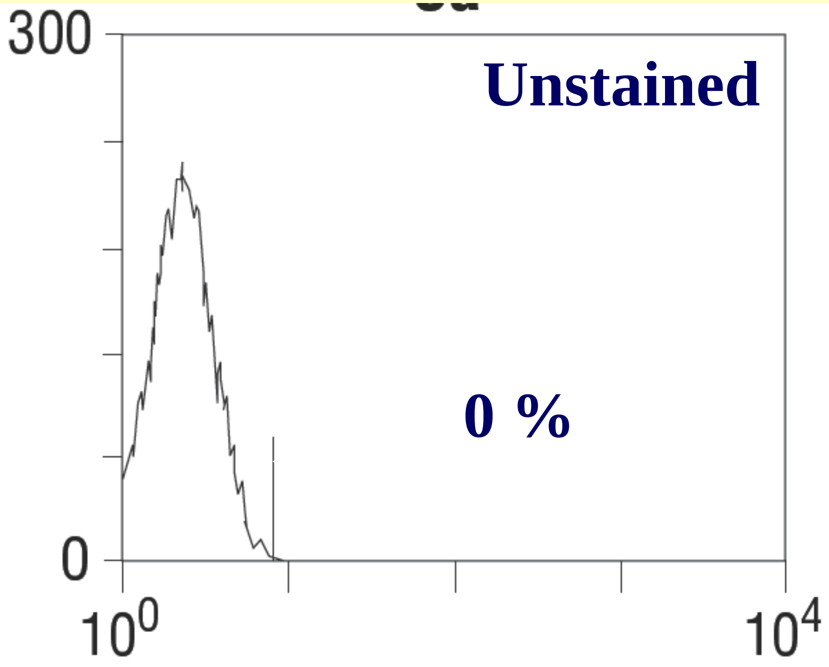
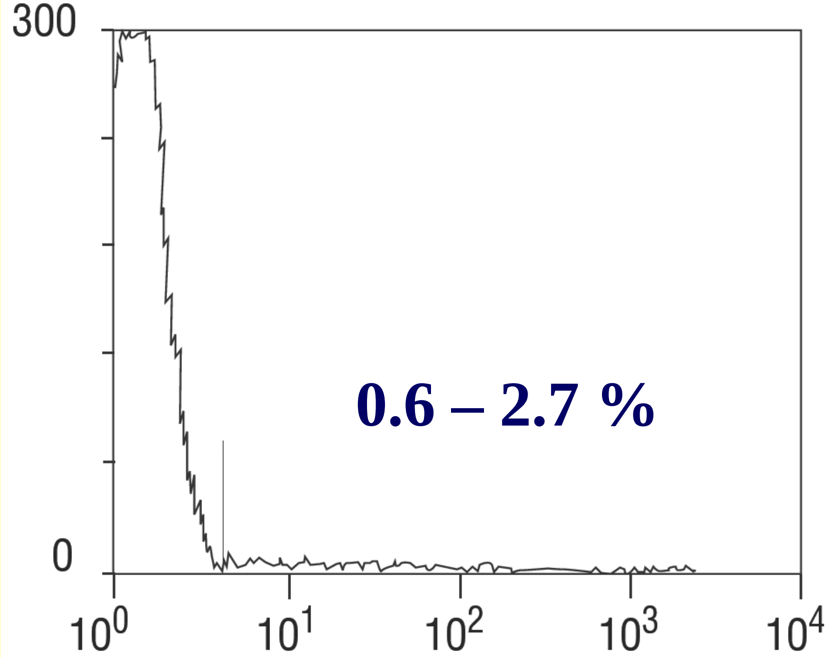


Figure 2 FSC vs SSC dot plot showing gate drawn around red blood cell population.





Platelet count and indices

- **Highly dependent on blood collection method**
- **Platelet count**
  - **140 – 440 K /μL**
- **Platelet indices**
  - **MPV**
- **Platelet function**

Will be discussed in week 3

WBC count

- **Total leukocytes: 4 – 11 x 10^9 / L**
  - **> 11 x 10^9 / L: Leukecytosis**
  - **< 4 x 10^9 / L: Leukepenia**
  - In normal pregnancy
    - total leukocytes <14.5 x 109/L (increase of neutrophils)
  - Neutrophils: 2.5–7.5 x 10^9 / L
  - Lymphocytes: 1.5–3.5 x 10^9 / L
  - Monocytes: 0.2–0.8 x 10^9 / L
  - Eosinophils: 0.04-0.4 x 10^9 / L
  - Basophils: 0.01-0.1 x 10^9 / L

Leukopenia

- Supply of leukocytes is depleted
  - e.g. infection or treatment (chemotherapy or radiation therapy)
- Hematopoietic stem cell abnormality
  - growth/maturation is affected
    - myelodysplastic syndrome
    - leukemia
- Most often due to a lower number of neutrophils, i.e. neutropenia (neutrophil count $< 1.5 \times 10^9$ /L)

Leukocytosis

- Reactive leukocytosis
  - response to infection, stress, inflammatory disorders
- Abnormal production
  - leukemia
- Individual cell component or a combination, depending on the cause

Clinical manifestation of leukopenia and leukocytosis

- Malaise
- Chills
- Fever (related to infection)

- Extreme leukocytosis
  - capillary obstruction (leukemia blasts)

Reactive leukocytosis

- can be classified on the basis of the white blood cell type affected.

Neutrophilic leukocytosis (>7.5 x 109/L)

- Acute bacterial infections
- Sterile inflammation/tissue necroses
  - myocardial infarction
  - burns
  - crush injuries

Eosinophilic leukocytosis (> 0.4 x 109/L)

- Allergic disorders
  - asthma
  - hay fever
- Parasitic infections
- Drug reactions

Basophilic leukocytosis (> 0.1 x 109/L)

- Allergic reactions (IgE mediated)
- Blast crisis of AML

Monocytosis (> 0.8 x 109/L)

- Chronic infections
  - tuberculosis
  - Bacterial endocarditis
  - Rickettsiosis
  - Malaria
- Collagen vascular disease
- Inflammatory bowel disease

Lymphocytosis (> 3.5 x 109/L)

- Accompanies monocytosis (chronic infections)
- Viral infections
  - e.g. hepatitis A, cytomegalovirus (CMV), Epstein-Barr virus (EBV)
- Bordetella pertussis

Abnormal clones of white blood cells

- Lymphoid and myeloid neoplasms depending on the type of white cell proliferation
- Characterized by
  - maturity and differentiation of the individual cell types
  - genetic abnormalities

- Divided into
  - acute leukemias
    - acute myeloid leukemia
    - acute lymphoblastic leukemia
  - chronic leukemias
    - chronic myeloid leukemia
    - chronic lymphocytic leukemia

WBC differential count

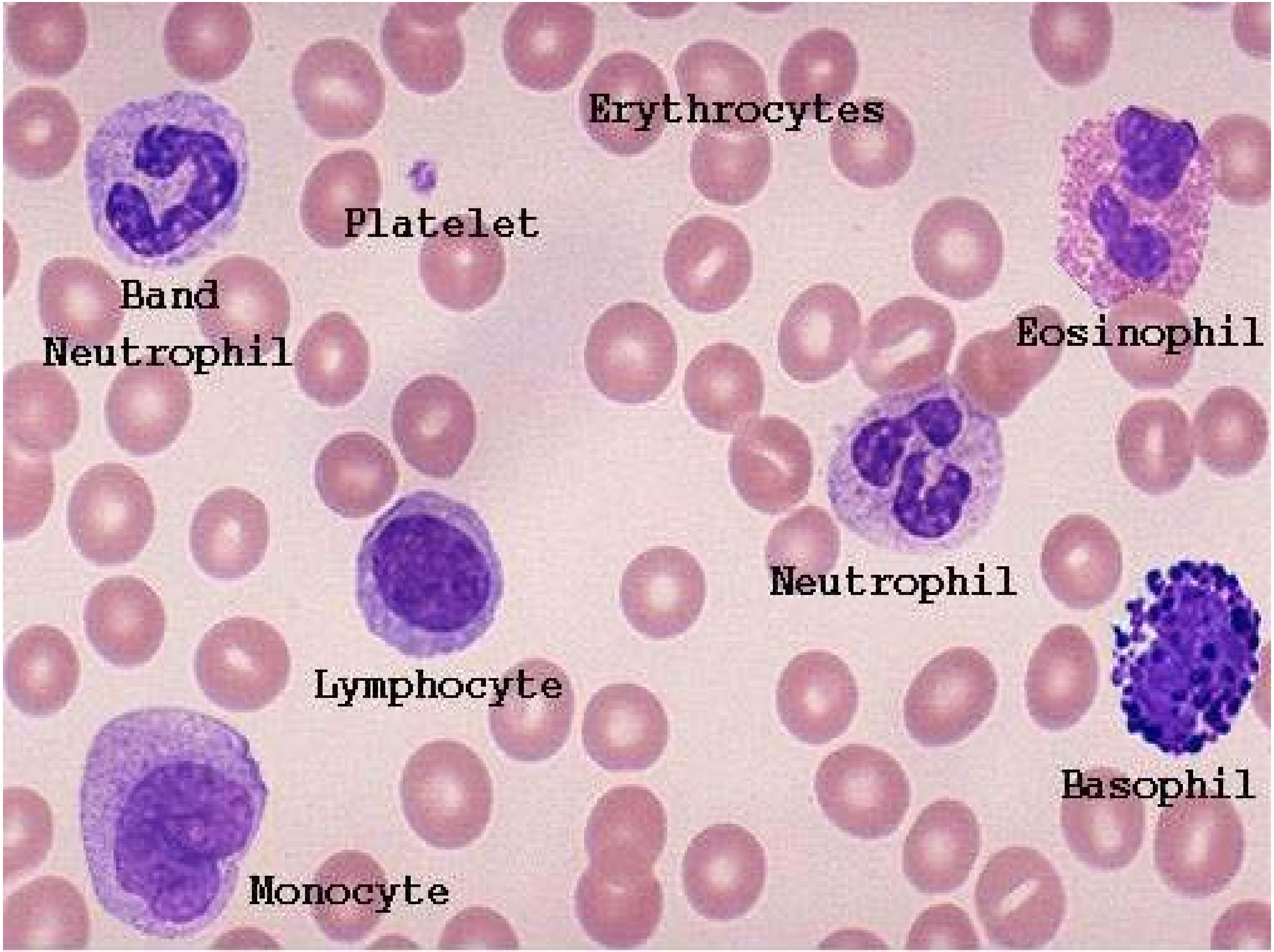
– Segmented neutrophils: 34-75%;
– Band neutrophils ≤ 8%;
– Lymphocytes: 12 – 50%;
– Monocytes: 3-15%;
– Eosinophils ≤ 5%;
– Basophils ≤ 3%.

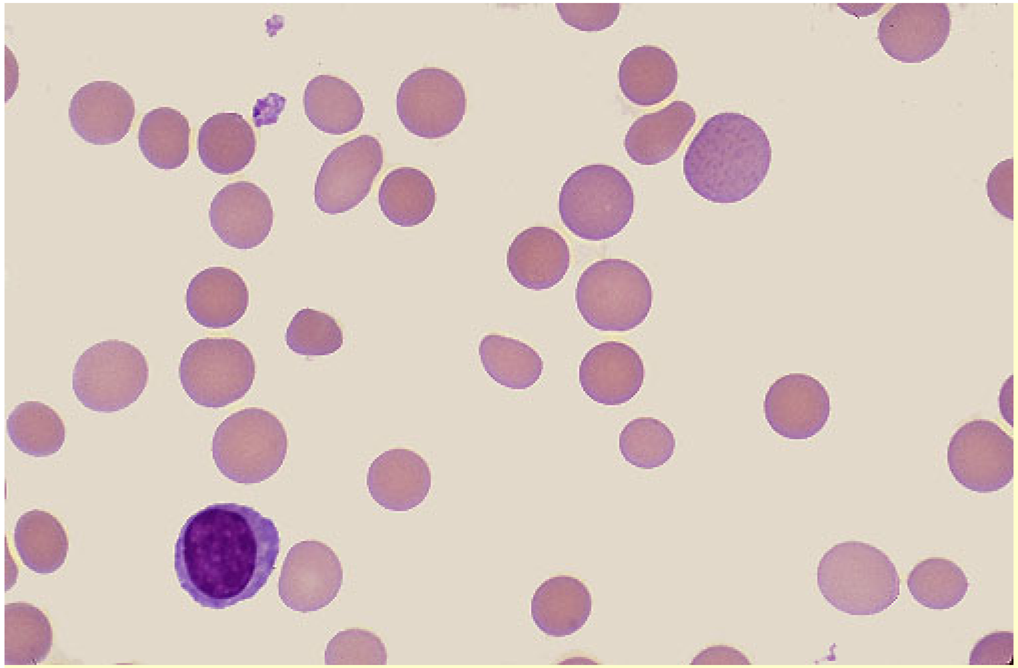
Immature granulocytes "left shift" of differential count

- Include metamyelocytes, myelocytes, promyelocytes, and/or blasts
  - Infections
  - growth factor therapy
  - chronic leukemia
  - acute leukemia

Blood smear

- In case of pathologic values in automated analysis of blood count
- Morphology of blood elements
  - Anisocytosis = variation in size
  - Poikilocytosis = variation in shape (schistocytes = RBC fragments; ovalocytes; spherocytes)
  - Atypical leucocytes (e.g. blasts)

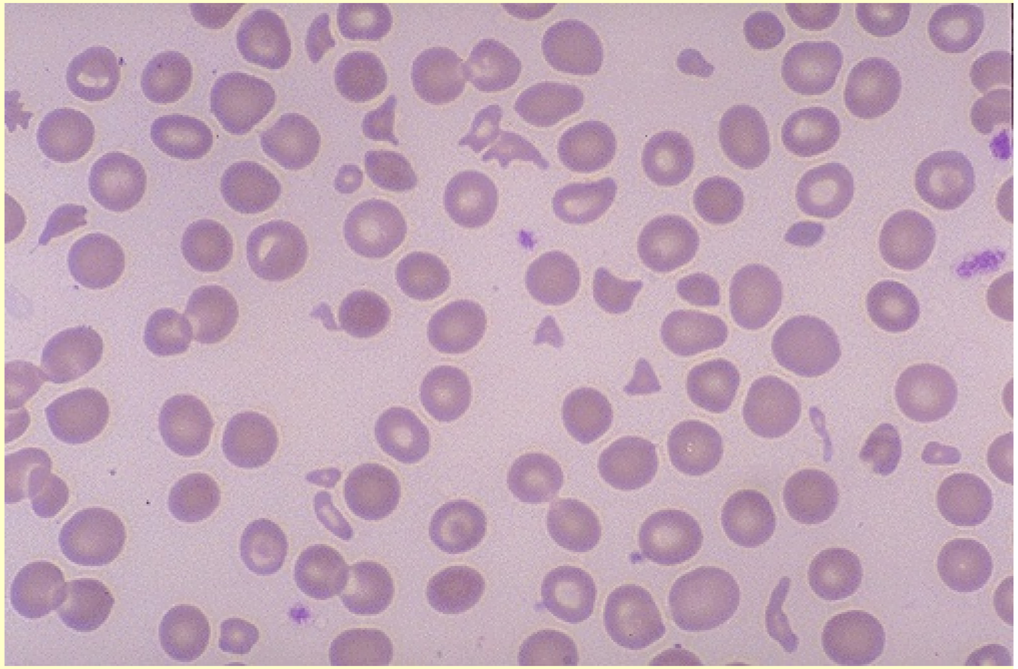

Erythrocytes
Platelet
Band
Neutrophil
Eosinophil
Neutrophil
Lymphocyte
Basophil
Monocyte



← **spherocytes**
(RBC spherical in shape
w/o area of central pallor)

schistocytes →

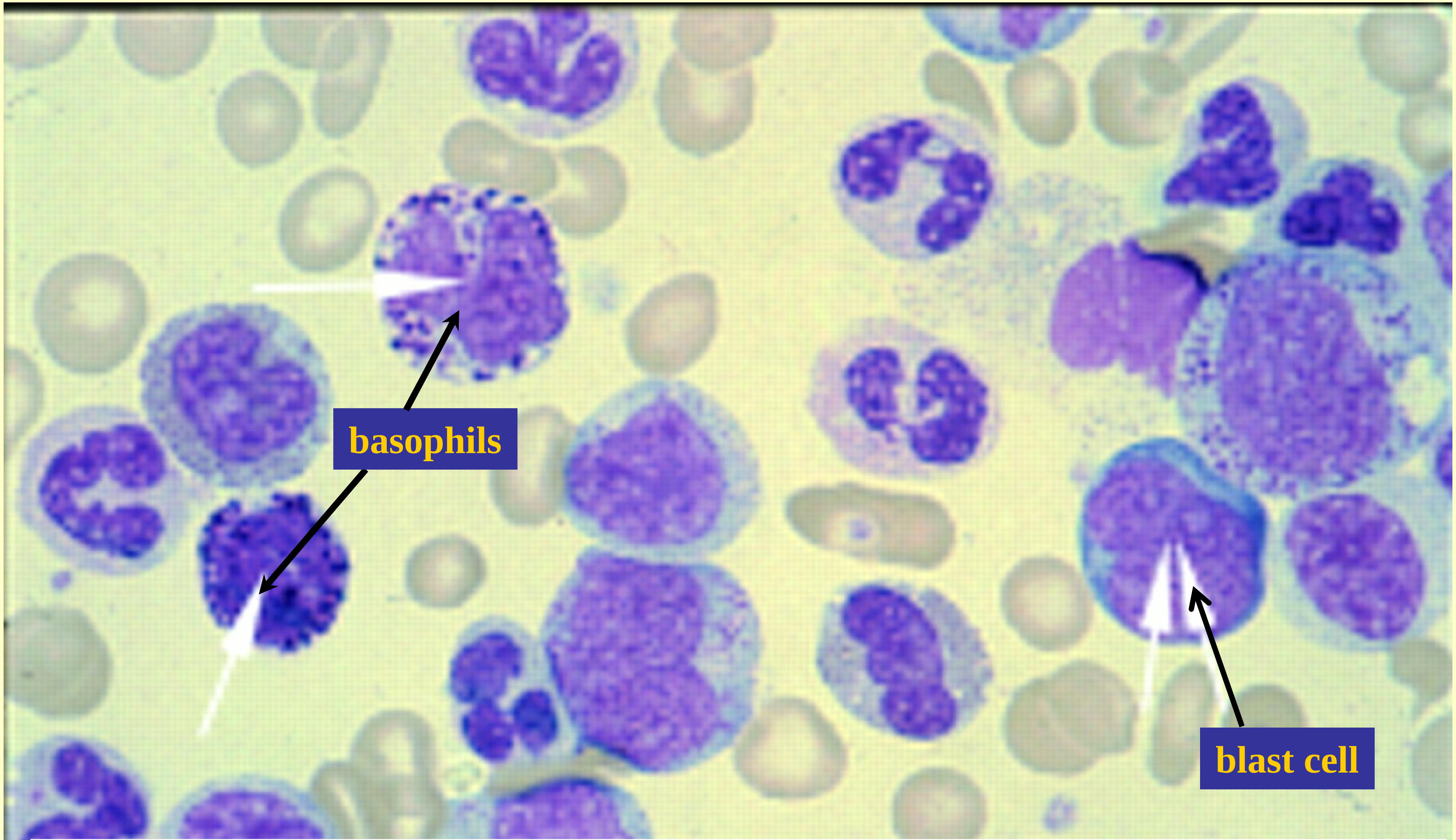
(RBC fragments)



Sickle Cell Disease

Hemoglobin (Hb) S: záměna valinu za glutamin v pozici 6 Hb beta řetězce

Peripheral blood film chronic myeloid leukemia



many mature granulocytes (arrow) and occasional blast cell (double arrow)

END