Something different-differential blood count-cytological way

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Given the known changes in cytodiagnostics and the fact that the number of cervical samples will be reduced in the future, but also for the future of cytotechnology as a whole, it is necessary to keep cytotechnologists in cytology laboratories by giving them „new” – „old” jobs.
Differential blood smear

- Despite automation and new technologies, peripheral blood smear still remains finding of great importance in establishing an accurate diagnosis.
- Opposite results obtained by automatic counter - Cytomorphological analysis of blood cells includes morphology and representation (percentage) of each type of leukocytes, erythrocytes and thrombocytes. It helps detect and classify immature or abnormal leukocytes or any other abnormalities that are present, giving us very accurate and useful information to diagnose the specific cause of the disease as well as in monitoring the success of treatment of hematological patients.
• Smears are prepared from capillary blood from the fingertip by cytotechnologists, which can provide high-quality slides for microscopy.

• It is extremely important to make technically correct smear which should be neither dense nor overstretched and cover two-thirds of the base slide length because it significantly affects the morphology and the accuracy of the findings.

• After preparation we mark the smears with pencil on the frosted end, air dry and stain by May-Grünwald-Giemsa staining method.
ISSA computer program for creating a differential blood count

Keyboard is adapted in a way that a certain number on the keyboard corresponds to a certain type of leukocyte. In this way we record / differentiate data about cells that we see in the slide.
Hematopoietic organs

Primary lymphoid organs

- Bone marrow
- Thymus

Secondary lymphoid organs

- Lymph nodes
- Spleen, tonsils
Hematopoiesis

Erythrocytopoiesis

Leucocytopoiesis

Granulocytopoiesis

Agranulocytopoiesis
Lympho/imuno poiesis

Thrombocytopoiesis
• Normal result in adults:
  segmented neutrophils 44 – 72 %,
  non-segmented neutrophils 0– 2 %,
  lymphocytes 20-46 %,
  monocytes 2 – 12 %,
  eosnophils 0 – 7 %,
  basophils 0 -1 %

• Reduced or increased number of a certain type of leukocytes may be a relative or absolute.
Erythropoiesis

pluripotent stem cell

multipotent stem cell

targeted stem cells to erythrocytopoiesis

proerythroblast

basophilic erythroblast

polychromatophilic erythroblast

orthochromatophilic ebl.

reticulocytes

erythrocytes
Disorders of erythrocytes

Variations in color
- hypochromia
- hyperchromia
- polychromasia
- anisochromasia
- anisocytosis
- microcytosis
- macrocytosis
- spherocytosis
- ovalocytosis - elliptocytosis
- leptocytosis
- acanthocytosis
- megalocytosis
- poikilocytosis
- drepanocytosis
variations in color

**Normochromic erythrocyte**

- normal color with a normal concentration of hemoglobin
Hypochromia

- paler colored erythrocyte
- erythrocyte with larger central enlightenment
- erythrocyte in the form of ring – anulocytes
- reduced amount of hemoglobin
- disturbed synthesis of heme and iron metabolism
- intensity of hypochromia we express by graduation from 1 to 3 cross. Where a one cross signifies light, two crosses high and three crosses strong hypochromia.
Hyperchromia

- highly colored red cells, without central enlightenment

- disorder of the central depressions, and not because of an increase in the average concentrations of hemoglobin

- present in megaloblastic anemia
**Polychromasia**

- different coloration of erythrocytes
- effective erythropoiesis
- regeneration
- successful therapy Fe, B12 or folic acid
- hemolysis
- graduation from 1 to 3 crosses
Reticulocytosis

- „young" erythrocytes
- supravital staining with Brilliant cresyl - reticulin mesh (remains of endoplasmic reticulum)
- effective erythropoiesis
- regeneration
- a successfully therapy Fe, B12 or folic acid
- hemolysis
Size Variations

Anisocytosis

- microcytosis
- macrocytosis
- graduation from 1 to 3
Shape variations

Spherocytosis

• small red cells that contain the maximum amount of hemoglobin

• disruption in the structure of erythrocyte membrane

• reduced osmotic fragility

• shortened life expectancy

• hemolytic anemia

• hereditary spherocytosis
Elliptocytosis

- erythrocytes with elliptical appearance
- membrane disorder
- shortened life
- reduced osmotic fragility
- hereditary elliptocytosis
- some cases of myelofibrosis
Leptocytosis – Target cells

- erythrocytes in the form of targets
- hemoglobinopathies
- β thalassemia
- liver diseases
Acanthocytosis

- membrane shoots-star-appearance
- β lipoproteinaemia
- neuromuscular diseases
- azotemia
- gastric cancer
- artifact?
**Stomatocyte**

- shape of the mouth
- membrane disorder
- hereditary stomatocytosis (intracellular reduction in the value of K (potassium) and increased Na (sodium))
- alcoholism
Schistocyte

- fragments of red blood cells, triangular
- congenital hemolytic anemia
- megaloblastic anemia
- microangiopathy
- heart defects
- burns
- carcinoma
- myelofibrosis
Sickle cell

- instead disc shaped, sickle cells are curved and more stiff

- Sickle cell anaemia

- belongs to a group of hemolytic anemia

- an abnormality in the oxygen-carrying protein haemoglobin

- Sickle-cell disease is inherited in the autosomal recessive pattern
Tear drop - dacryocytes

- erythrocytes in the form of tears
- myelofibrosis, anaemia, splenomegaly
**Rouleaux formation**

- erythrocytes in a row such as coins
- increased sedimentation
- dysproteinemia-myeloma, macroglobulinemia
Inclusions
Howell Jolly bodies

• remains of the nucleus
• round dark purple dots
• posthemorrhagic anemia
• post-splenectomy
• also seen in: decreased splenic function, severe hemolytic anemia, megaloblastic anemia, hereditary spherocytosis, myelodysplastic syndrome (MDS), in premature infants
Cabot’s ring

- erythrocytes with the remains of nuclear membranes in the form of a ring or eights
- severe anemia (megaloblastic)
- the impact of chemotherapy
Nuclear dust in erythrocytes

- gently visible reddish-blue granules
- nuclear residue, smaller than Howell-Jolly bodies
- reflect heavy anemias
Basophilic stippling

- basophil granulation in the cytoplasm
- dots are the visualization of ribosomes
- lead poisoning or other heavy metals, MDS, sideroblastic anemia etc.
Pappenheimer bodies-

siderotic granules

• dense, blue-purple granules

• abnormal granules of iron

• in sideroblastic anemia, hemolytic
  anemia, sickle cell disease, post
  splenectomy

• they are seen by May-Grünewald
  Giemsa and Prussian blue staining
  methods
Plasmodium malariae

- is a parasitic protozoa that causes malaria
- inclusion in erythrocytes depend on the type and stage of the malaria parasite
Iron deficiency anemia:
- microcythosis
- hypochromia
- anulocytes

Megaloblastic anaemia: macro-megalocytes, large metamyelocytes and large non-segmented granulocytes, pancytopenia, hypersegmented granulocytes - segment index greater than 3.5

Myelodysplastic syndromes: neutropenia, anemia and thrombocytopenia, macro and megalocytes, hypogranular myelocytes, pseudo Pelger anomaly
targeted stem cells to granulopoiesis

- myeloblast
- promyelocyte
- myelocyte
- metamyelocyte
- band neutrophil
- segmented neutrophil
- eosinophil
- basophil
Granulocytes morphology disorders

- Pelger–Huët anomaly
- Pseudo Pelger-Huët anomaly
- Hypersegmented neutrophil
- Alder Anomaly (Alder-Reilly Anomaly)
- Chédiak–Higashi anomaly
- May–Hegglin anomaly
- Döhle bodies
- Toxic granules
- Neutrophil’s drumstick
- Auer rods
- “Left shift”

Nuclei changes

Cytoplasm changes of granulocytes
Pelger–Huët anomaly

• neutrophils contain a single, round nucleus with clumped chromatin, little or no nuclear segmentation (eosinophils and basophils also show rounded nuclear lobes with dense chromatin)

• report of shift to the left and large number of immature neutrophils can lead to incorrect diagnosis of sepsis

• benign, genetic disorder with no reflection on the function of granulocytes
• autosomal dominant inheritance pattern as a result of mutations in the lamin B receptor gene
• heterozygotes are clinically normal, although their neutrophils may be mistaken for immature cells (up to two nuclei), homozygotes tend to have neutrophils with rounded (rod) nuclei (>90%) that do have some functional problems
Pseudo Pelger-Huët anomaly

- neutrophils with bilobed nuclei
- absence of these findings in other family members, a low percentage of affected cells suggest acquired anomaly
- infections
- often appear after chemotherapy
- myelodysplasia
- multiple myeloma
- metastatic process in the bone marrow
- acute leukemia with acquired multilineage dysplasia
Hypersegmented neutrophils

• at megaloblastic and iron deficiency anemia (including folic acid deficiency and pernicious anemia), during chemotherapy or long-term chronic infections

• acquired
  - megaloblastic anemia

• heritable

• homozygotes > 80% granulocytes

• heterozygotes > 69%
Alder Anomaly (Alder-Reilly Anomaly)

- purple to black granules in all types of mature white blood cells, sometimes found in immature forms

- rare autosomal recessive disorder

- mucopolysaccharidosis

- osteoclasts, the cells of the liver and spleen

- mesothelial cells
Chédiak–Higashi anomaly

• formation of large granules as a result of lack of lysosomes

• accumulation of lysosomal acid phosphatase which does not react with bacteria

• neutropenia

• autosomal recessive disorder resulting from mutations in one of the lysosomal trafficking regulator protein, which leads to decrease phagocytosis
May–Hegglin anomaly

• hereditary disorder with presence of bluish, rod-shaped inclusions in the cytoplasm of:
  - myelocytes, metamyelocytes, neutrophils, eosinophils, basophils and monocytes

• macro platelets

• variable thrombocytopenia
Döhle bodies

• light blue-gray, basophilic oval inclusions in the cytoplasm of neutrophils

• the rest of immature cytoplasmic RNA

• severe infections and toxic state

• may follow malignancies, burns, aplastic anemia and cytostatic treatment
Toxic granules

- primary azurophil granules persist through most mature form
- neutrophil maturation disorder
- severe bacterial infections and intoxications
Neutrophil’s drumstick

• drumstick consists of a small nuclear mass attached to the body of the nucleus by a thin tread

• neutrophils in women contain drumstick which is an expression of an X-chromosome
Auer rods

- a thin stick in the cytoplasm of blast, clumps of azurophilic granular material

- disorder of development of azurophil granules

- can be seen in the myeloblasts and promyelocytes in the acute myeloid leukemia
Left shift and leukemoid reaction

• increased number of non-segmented granulocytes in the peripheral blood

• sometimes metamyelocytes and myelocytes

• a reflection of accelerated granulopoiesis

• regeneration

• infection
Leukoerythroblastic picture – Myelophthisic type of peripheral blood smear

- the presence of hematopoietic development forms that are not found in the peripheral blood smear under normal conditions:
- development forms of the granulopoiesis
- erythroblasts
- megakaryocytes
- occurs due to the presence of foreign cells in the bone marrow:
- metastatic processes
- infiltration of lymphoma cells
- infiltration of plasma cells with plasmacytoma
- development of leukemia
Leukemoid reaction versus chronic myeloid leukemia

**Leukemoid reaction**
- left shift, usually to the myelocytes stage
- toxic granules and Döhle bodies
- increased alkaline phosphatase value - APLc
- multiplied granulopoiesis in the bone marrow
- morphologically normal erythro- and thrombocytopoiesis
- no splenomegaly
- no chromosomal abnormalities

**Chronic myeloid leukemia**
- all developmental forms of granulocyte order mbl -seg neut.
- basophilia
- eosinophilia
- reduced alkaline phosphatase value in granulocytes - APLc
- hyperplasia granulo- and thrombopoiesis in the BM (suppressed erythropoiesis)
- mononuclear forms of megakaryocytes in the BM
- mild splenomegaly
- t9; 22 (Philadelphia chromosome)
Development forms of the monocytes

- Pluripotent stem cell
- Multipotent stem cell
- Targeted stem cells to monocytopoiesis
- Monoblast
- Promonocyte
- Monocyte
- Macrophage

Bone marrow

Peripheral blood

Tissue
Monocytosis

- regeneration
- chronic diseases
- CMML (MDS / MPN)
- acute myelomonocytic leukemia and monocytic leukemia
T cells are produced in the bone marrow, pass through the thymus, mature forms of T cells enter the peripheral blood. T cells - involved in cell-mediated immunity.

B cells are responsible for humoral immunity (relating to antibodies).
Lymphocytosis

- relative normal number of leukocytes and domination of lymphocytes

- granulocytopenia

- absolute virosis infectious mononucleosis infectious lymphocytosis

- chronic and sub-chronic inflammation

- lymphoproliferative diseases
  - CLL
  - ALL
Pancytopenia

- anemia
- leukopenia
- thrombocytopenia
### Pancytopenia Clinical Implications

<table>
<thead>
<tr>
<th>Pancytopenia</th>
<th>Smear Type</th>
<th>Bone Marrow</th>
<th>Spleen</th>
<th>Possible Diagnosis</th>
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<td>+</td>
<td>Myelophthisic type</td>
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<td>-</td>
<td>Metastatic process</td>
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</tbody>
</table>
Thrombocytopoiesis

- Pluripotent stem cell
- Multipotent stem cell
- Targeted stem cells to thrombocytopoiesis
- Megakaryoblast
- Promegakaryocyte
- Megakaryocyte
- Thrombocytes

BM → PB
Thrombocyte disorders

quantitative changes
- thrombocytosis
- thrombocytopenia

qualitative changes
- anisothrombocytosis
- aggregates of platelets
- megakaryocyte nucleus
- megathrombocytes
Quantitative changes

Thrombocytosis

• condition with wicket or permanent increase in the number of platelets

• posthemorrhagic

• after splenectomy

• chronic myeloproliferative diseases
  ET
  PRV
  CML
Thrombocytopenia

- the situation with a reduced number of platelets
- megakaryocytic and nonmegakaryocytic
- myelodysplastic syndrome
- damage of the bone marrow caused by physical, chemical or infectious agents
- hypersplenism
- immunothrombocytopenia
Anisothrombocytosis

- presence of different sized platelets
- posthemorrhagic regeneration
- various hematological diseases
Aggregates of platelets

- the presence of platelet clumps due to artifacts
- increased aggregation of platelets in the circulating blood
- "false thrombocytopenia"
- EDTA anticoagulant reaction
- coldness
Megakaryocyte nuclei

- MPN - myelofibrosis
Macro thrombocytes

- May-Hegglin anomaly
- MDS
- MPN
• With proper training and education in the field of non-gynecological cytotechnology and with its unique competence in morphology and laboratory technologies, Cytotechnologists are the most competent choice for making findings of peripheral blood smear.

• Findings made and analyzed by educated personnel – cytotechnologists and proper interpretation, will certainly contribute to accuracy of diagnosis.
Especially big thanks to prof. Ika Kardum Skelin on support and help!
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