LECTURE 2

DISORDERS OF WHITE BLOOD CELLS

OUTLINE:

I. NON – MALIGNANT disorders of white blood cells
   A. Disorders due to a abnormal number of white blood cells
      1. Leucocytosis (↑ number)
      2. Leucopenia (↓ number)
   B. Disorders due to abnormal function of white blood cells

II. MALIGNANT disorders of white blood cells
   A. Introduction and general etiopathogenesis
   B. WHO classification of white cells neoplasms
   C. Leukemias
   D. Lymphomas
   E. Plasma cell neoplasms

I. NON – MALIGNANT DISORDERS OF WHITE BLOOD CELLS

A. Disorders due to abnormal number of white blood cells:

1. Leucocytosis
   = increase of the number of white cells > 10,000/mm³
   Etiology:
       a) Neutrophilia (granulocytosis) – due to:
          - Physiological conditions:
              o Pregnancy (during the 3rd trimester)
              o Increased release of catecholamines (in stress, exercise, after surgery)
          - Pathological conditions:
              o Acute bacterial infections
              o Acute inflammation: acute myocardial infarction, extended burns, active collagen diseases (due to increased production and release from the bone marrow)
              o chronic bacterial infections
                ▪ mech.: growth factors-dependent bone marrow hyperplasia of myeloid line (cytokines such as IL-1, TNF-alpha stimulate the production & release of granulocytes)
              o myeloproliferative disorders – e.g., chronic myeloid leukemia (CML), polycythemia vera
                ▪ mech.: growth factors-independent bone marrow hyperplasia of myeloid line
b) **Lymphocytosis** – due to:
- acute viral infections – mononucleosis, hepatitis, pertussis
- chronic bacterial infections – tuberculosis, syphilis
- malignant disorders – chronic lymphoid leukemia (CLL), lymphomas
- endocrine disorders – thyrotoxicosis, adrenal gland failure

c) **Eosinophilia** – due to:
- Parasitic infestations – trichinosis, giardiasis, echinococcosis
- Allergic disorders: rhinitis, asthma, hives, drug reactions (main cause in hospitalized patients!)
- Hypereosinophilic syndromes (Loeffler syndrome)
- Collagen diseases
- Malignant disorders – chronic myeloid leukemia (CML), metastases or tumor necrosis

d) **Basophilia** – due to:
- chronic inflammation – ulcerative colitis, rheumatoid arthritis
- malignant disorders – CML, polycythemia vera, myelofibrosis
- allergic disorders (type I hypersensitivity reaction: asthma, hives, anaphylactic shock)

e) **Monocytosis** – due to:
- viral infections (mononucleosis)
- bacterial infections – subacute bacterial endocarditis, brucellosis, tuberculosis
- chronic granulomatous infections – sarcoidosis, regional enteritis
- malignant disorders – acute monocytic and acute myelomonocytic leukemias, CML, neoplasms of the breast, ovaries, kidneys, or digestive cancers.

**Infectious mononucleosis**

- **Definition:** benign lymphoproliferative disorder caused by Epstein-Barr (E-B) virus, with oral transmission ("kissing disease") that is particularly common in teenagers and young adults. Typical features of infectious mononucleosis include fever, malaise, pharyngitis, adenopathy, splenomegaly, and hepatomegaly.

- **Pathogenesis:**
  1. E-B viruses invade the lymphoid oropharyngeal tissue with:
     - **Lymphotropism for B lymphocytes:**
       - Destruction of B lymphocytes → with release of virions & further spread of the infection of other B lymphocytes
       - Differentiation of B lymphocytes in plasma cells able to secrete:
         - *Heterophile antibodies (Ab)* - used for diagnosis *(Paul – Bunnel reaction)*
         - *Ab anti-VCA (Viral Capsid Ag)* - IgG and Ig M type
         - *Ab anti-EBNA (EBNA – Ebstein-Barr Nuclear Antigen)* - Ig G type
     - **Activation and proliferation of cytotoxic and natural killer (NK) lymphocytes in lymphoid tissues and spleen** with:
       - The appearance of *atypical lymphocytes* (reactive lymphocytes, Downey cells) in the peripheral blood - > 10% of total lymphocytes has diagnostic value
       - Lymphadenopathy and splenomegaly.
  2. E-B viruses survive in some oropharyngeal B lymphocytes → **latent infection** which favors:
    - relapses (in immunocompromised patients)
    - dissemination of the infection (by asymptomatic patients)
    - the occurrence of malignant disorders: B-cells lymphomas and Burkitt lymphoma (endemic in African children).
2. **Leucopenia**
   = decrease of the number of white cells < 4,000/mm$^3$

**Etiology:**

a) **Neutropenia** (<1,500/mm$^3$) is **always** due to pathological conditions. The most severe is condition is **agranulocytosis** (< 500/mm$^3$) with high mortality rate due to severe bacterial and fungal infections.

**Pathogenesis:** A reduction in circulating neutrophils occurs via 2 major mechanisms:

1. **Decreased bone marrow production** – due to:
   - **stem cell suppression** due to: i) **acquired conditions** (aplastic anemia, tumors, granulomatous disorders), radiotherapy, toxic exposure (in this case, neutropenia will be associated with anemia and thrombocytopenia), and ii) **hereditary conditions** (cyclic neutropenia, congenital neutropenia)
   - **granulocyte precursor suppression secondary to drug toxicity is the main cause for agranulocytosis** and can be:
     - **dose-dependent** (e.g., during chemotherapy with alkylating agents or antimetabolites that may trigger bone marrow suppression – in this case, neutropenia occurs together with anemia and thrombocytopenia);
     - **dose-independent**, when an **idiosyncratic reaction** to chloramphenicol, chlorpromazine or phenylbutazone may develop.
   - **ineffective hematopoiesis** (e.g. in megaloblastic anemias, in which large, dysfunctional precursors are destroyed in the bone marrow)

2. **Increased peripheral destruction** – due to:
   - **antibody production in autoimmune diseases** (systemic lupus erythematosus)
   - **disorders associated with splenomegaly** (sarcoidosis, Felty syndrome characterised by triad: **rheumatoid arthritis + splenomegaly + neutropenia**)
   - **increased use** in severe bacterial (sepsis, typhoid fever, severe tuberculosis) and fungal infections.

b) **Lymphopenia**
   = decreased lymphocyte count <1,000/mm$^3$
   - **acute** – acute myocardial infarction, sepsis, pneumonia, stress
   - **chronic** – due to:
     - **decreased production**
       - B cell/T cell/combined deficiencies
       - post-radiation therapy, chemotherapy, cortisone therapy
       - malignant disorders – bone marrow aplasia, lymphomas
     - **increased destruction**
       - AIDS, autoimmune disease (systemic lupus erythematosus)

B. Disorders due to **abnormal function** of white blood cells:

I. Disorders of chemotaxis

a) **Primary defects**
   - **Chediak-Higashi syndrome** is caused by a decreased chemotactic response, with fusion of the cytoplasmic granulations and altered intracellular digestion
   - **“lazy leucocytes” syndrome** is due to defective contractile proteins, that would normally ensure the movement of neutrophils
b) Secondary defects
   - dysfunctional complement due to decreased complement fractions
   - chemotaxis suppression

Etiology:
   - Hodgkin’s disease
   - liver cirrosis
   - uremia

II. Disorders of phagocytosis
   a) Primary defects
      - chronic granulomatosis, due to an alteration in oxygen dependent phagocytosis, accompanied by an NADH-oxydase and peroxidase deficit, that leads to a decreased production of hydrogen peroxide (antibacterial effect)
      - Chediak-Higashi syndrome – incapacity to form phagozomes
   b) Secondary defects
      - low serum levels of IgG and fraction C3
      - dysfunctional lysosomal enzymes

II. MALIGNANT DISORDERS OF WHITE BLOOD CELLS

A. Introduction and general etiopathogenesis

Hematological malignancies have been classically divided by whether the malignancy is mainly located in the blood (leukemia) or in the lymph nodes (lymphomas). LEUKEMIAS are primary neoplasms of the bone marrow (BM) with characteristic spilling over of the malignant cells into peripheral circulation. LYMPHOMAS are primary neoplasms of lymph nodes/lymphoid tissues that usually do not involve peripheral spilling over of malignant cells.

General etiopathogenesis of leukemias and lymphomas is common:

1. Genetic damage to the cells
   - Cancer promoting genes (oncogenes) may become activated if a chromosome breaks or a translocation occurs ⇒
     • acceleration of the proliferation and increased turnover of the malignant cells (e.g.: mutation of tyrosin-kinases)
     • suppression in the cellular differentiation process, especially when it comes to acute forms of disease (e.g.: abnormal proteins or oncoproteins)
   - Increased incidence of leukemia in the identical twins
   - Risk of developing AML is increased threefold in first-degree relatives of patients with AML

2. Viruses
   There are 3 viruses with tropism for lymphocytes which lead to lymphoid neoplasms:
   - Human Immunodeficiency Virus (HIV) ⇒ B cell lymphoma
   - Human T-cell Lymphotrophic Virus (HTLV-1), a retrovirus similar to HIV ⇒ T cell leukemia/lymphoma
   - Epstein Barr Virus, a virus similar to herpes virus is the causative agent of infectious mononucleosis ⇒ Hodgkin lymphoma, Burkitt lymphoma, and a type of ALL

3. Radiation exposure
   - Nuclear explosion (Japanese survivors of the atomic bomb)
   - Medical exposure:
     ✓ Radiologists exposed to high levels of X-rays prior to the adoption of modern radiation safety practices
4. Cytotoxic drugs/chemicals
   - Chemotherapeutic drugs administrated for solid tumors (alkylating agents, anthracyclines) may induce myeloid leukemia usually after 3-5 years post treatment
   - Chronic exposure to organic solvents, such as benzene or toluene, herbicides, pesticides
   - Heavy smoking (cigarette smoke contains benzene) – it has been shown that acute myeloid leukemia is twice as frequent in smokers

5. Other diseases/pathological states
   - High incidence of leukemias occurs in:
     ✓ Genetic diseases:
       ▪ Down syndrome (10- to 18-fold increase in the risk of AML) and Patau syndrome
       ▪ Fanconi anemia, Bloom syndrome
     ✓ Immune deficiency states (AIDS) or immune system activation (gluten intolerance, infection with Helicobacter pylori) are associated with an increase in hematological malignancies

B. The novel classification of white blood cells malignancies

WHO (World Health Organization) Classification is based on cell type of the neoplasm:

A. Neoplasms involving the myeloid lineage
   I. Acute Myeloid Leukemias (AML)
   II. Myelodysplastic Syndromes
   III. Myeloproliferative Diseases

B. Neoplasms involving the lymphoid lineage:
   1. Precursor B-cell neoplasms (neoplasms of immature B cells)
   2. Peripheral B-cell neoplasms (neoplasms of mature B cells)
   3. Precursor T-cell neoplasms (neoplasms of immature T cells)
   4. Peripheral T-cell and NK-cell neoplasms (neoplasms of mature T cells and NK cells)
   5. Hodgkin lymphoma (neoplasms of Reed-Sternberg cells and variants)

C. LEUKEMIAS

- Definition: malignant disorders resulting from an abnormal myeloid proliferation in the bone marrow, with invasion of the blood stream.
- Classification:
  I. According to the evolution:
     1. Acute leukemias, in which accumulation of immature cells (blasts) suppresses normal hematopoiesis.
     2. Chronic leukemias, characterized by an abnormal proliferation of the mature cells having better prognosis and longer evolution.
  II. According to the abnormal cell type:
     1. Myeloid leukemias (acute, chronic)
     2. Lymphoid (lymphocytic) leukemias (acute, chronic)

The major differences between acute and chronic leukemia are presented in Table 1.

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<tr>
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<th>Acute leukemia</th>
<th>Chronic leukemia</th>
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<tr>
<td></td>
<td>High proportion of immature cells in bone marrow and peripheral circulation</td>
<td>High proportion of mature cells in bone marrow and peripheral circulation</td>
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<tr>
<td>Abrupt onset</td>
<td>Insidious onset</td>
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<td>Marked signs and complications</td>
<td>Mild signs</td>
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<tr>
<td>Bad prognosis</td>
<td>Better prognosis</td>
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Consequences:

1. **Deficiency in blood cell number and function**
   a) **Infections**
      - Most common cause of death
      - Due to severe infections (neutropenia, impairment of phagocytic function and lymphocyte function)
   b) **Hemorrhage**
      - Due to thrombocytopenia or liver disease
   c) **Anemia**
      - Due to replacement of RBC precursors by cancer cells
      - Normochromic-normocytic
      - Severity of anemia reflects severity of disease

2. **Invasion of vital organs** → enlargement of spleen, liver, brain, lung, and lymph nodes

**ACUTE LEUKEMIAS**

- **Definition:** acute leukemias develop as a consequence of an abnormal proliferation of blasts (immature cells) that leads to hematopoietic suppression.

- **Classification:**
  1. **Acute myeloid leukemia (AML)** is a malignant transformation of a myeloid precursor with proliferation of immature “myeloid” cells:
     - 80% of adult leukaemia
     - Rare in childhood (20%)
     - Develops in some patients after chemotherapy & radiotherapy
     - Poor prognosis: 50% of children and 30% of adults achieve long-term survival
     - **FAB (French American British) classification** of AML is based on the degree of differentiation or maturation of the neoplastic cells:
        - M0: Acute Myeloblastic Leukemia with minimal differentiation
        - M1: Acute Myeloblastic Leukemia with no maturation
        - M2: Acute Myeloblastic Leukemia with maturation: Auer rods and primary granules visible
        - M3: Acute Promyelocytic Leukemia
        - M4: Acute Myelomonocytic Leukemia
        - M5: Acute Monocytic Leukemia
        - M6: Erythroleukemia
        - M7: Acute Megakaryocytic Leukemia
  2. **Acute lymphoid leukemia (ALL)** is a malignant disorder of the lymphoid precursor with:
     - B-lineage involvement (80% of cases)
     - T-lineage involvement (20% of cases)
     - ALL represents the most common malignant disease in children - 85% of childhood leukaemia:
        - Peak incidence: 3-7 years
        - Incidence decreases with age, and a secondary rise occurs in middle age.
     - The FAB Classification of ALL recognizes three subtypes:
        - **L1** = small monotonous blasts, B lymphocytes are present (the most frequent form in children: 70% of childhood ALL, good prognosis)
        - **L2** = large varied blasts, T lymphocytes (the most frequent form in adults: 70% of adult ALL, poor prognosis)
• **L3** = blasts with cytoplasmatic vacuoles, B lymphocytes (rare) – is the leukemic form of **Burkitt lymphoma**

! *Obs: Burkitt lymphoma:*
  - is a highly aggressive lymphoma of the lymphoid stem cells either in bone marrow or lymph nodes often presenting at extranodal sites
  - **Features:**
    ✓ confined to children in some parts of Africa
    ✓ high association with Epstein-Barr infection
    ✓ rapidly progressive but responsive to therapy

- **Prognosis:** highly curable in pediatric population, less so in adults. The 5-year survival rate is 85% in children and 30-50% in adults.

- **Clinical manifestations - TYPICAL ASSOCIATION: anemia + hemorrhages + infections:**
  - **Anemia** is due to:
    o erythropoiesis suppression
    o ineffective erythropoiesis and
    o increased spleen destruction (hypersplenism)
  - **Hemorrhages** are the result of:
    o **thrombocytopenia** (caused by medullar infiltration with decreased megakaryocytic production or increased destruction in the spleen) and
    o **disseminated intravascular coagulation** (more frequently in promyelocytic leukemia)
  - **Recurrent infections and fever** are the result of:
    o lowered antibacterial function of the leukocytes and
    o release of endogenous pyrogens
  - **Other clinical manifestations** include:
    - *loss of appetite, weight loss, weakness*
    - **neurological disorders** due to malignant infiltration and cerebral hemorrhages
    - **lymphadenopathy, hepatosplenomegaly, skin invasion, uric nephropathy**
    - **leukostasis** – leukemic blasts tend to invade the blood stream, reaching high levels (over 100,000/mm³), which can lead to blast emboli, that block the small vessels in the lung and brain ⇒ progressive dyspnea, confusion or coma (vital risk and firm indication for performing plasmapheresis)

### CHRONIC LEUKEMIAS

- **Definition:** chronic leukemias develop as a consequence of increased proliferation and accumulation of differentiated, mature leukemia cells.

- **Classification:**
  1. Chronic myeloid leukemia (CML)
  2. Chronic lymphoid leukemia (CLL)

1. **Chronic myeloid leukemia (CML)**
   - abnormal proliferation that affects adults between 30 – 40 years old
   - over 90% of the patients have a chromosomal abnormality called the **Philadelphia chromosome** that is present in the **pluripotent stem cell** and may impair the development of all types of precursors (granulocytic, erythroid and megakaryocytic).
   - Philadelphia chromosome contains an **abnormal oncogene**, as result of a fusion process that takes place between the **BCR gene** (Breakpoint Cluster Region) located on the 22nd chromosome and the **ABL gene** (Abelson Kinase fusion gene = proto-oncogene
and cellular correspondent to the virus that cause leukemia in mice) located on the 9th chromosome; the BCR-ABL fusion gene is responsible for the synthesis of a *constitutional active tyrosine–kinase* that further orchestrates the malignant transformation (and is the major target of tyrosine-kinase inhibitors, the main drugs currently used in CML treatment).

- CML is part of a group of malignancies, called *myeloproliferative disorders*, together with *polycytemia vera, essential thrombocytopenia and idiopathic myelofibrosis*.

**Clinical phases:**

1. **Chronic phase (myeloproliferative phase)** – lasts 2 to 5 five years and is characterized by:
   - *in the bone marrow:*
     * increased myeloid proliferation
     * low level of myeloblasts (under 10% of the total medullar cellularity)
   - *in the peripheral blood:*
     * leukocytosis > 15,000/mm³, myelocytes and metamyelocytes are present (normally these intermediate elements are present only in the bone marrow)
     * anemia, leading to progressive fatigue, effort-induced dyspnea
     * thrombocytopenia or thrombocytosis (in the beginning of the disease)
   - *biochemical abnormalities:*
     * decreased or absent alkaline phosphatase
     * increased B12 vitamin and transcobalamine serum levels

2. **Accelerated phase** – lasts 6 to 18 months and is characterized by:
   - *overproduction and increased destruction of myeloid cells*, leading to:
     * fever, bone pain, weight loss, infections
     * symptomatic splenomegaly
     * invasion of peripheral organs: intestines, kidneys, lungs
     * hyperuricemia, with an increased risk of gout and kidney stones
   - *progressive leukocytosis*, *basophilia*
   - *progressive anemia and thrombocytopenia* due to medullar myeloid invasion

3. **Acute terminal phase (blast crisis)** – lasts approximately 3 months, and is characterized by:
   - marked aggravation of the chronic leukemia
   - *blast transformation* with impaired differentiation of the leukemia cells
   - *in the bone marrow:*
     * over 50% myeloblasts and promyelocytes
   - *in the peripheral blood:*
     * severe leukocytosis > 100,000/mm³, together with a high number of blast cells
   - *chromosomal abnormalities* (other than the Philadelphia chromosome, such as aneuploidy)
   - acute myeloid leukemia complications
   - *highly resistant to therapy*, usually fatal

**2. Chronic lymphoid leukemia (CLL)**

- it usually affects adults between 50 – 70 years old
- blast lymphoid cells levels are less 5% from the total medullar cellularity (maturation occurs)
- leukocyte levels in the blood stream vary between 10,000 – 150,000/mm³
- B cell lymphocytes are proliferating in more 90% of the cases together with a high risk for developing immune hemolytic anemias
- Clinical manifestations:
  - lymph node/spleen invasion leads to peripheral lymphadenopathy and splenomegaly
  - skin lesions
  - progressive invasion of the bone marrow causes anemia, granulocytopenia (risks of infections), thrombocytopenia (risk of bleeding disorders)

D. LYMPHOMAS

- Definition: malignant disorders caused by abnormal proliferation of the cells that reside in the lymphoid tissues. Morphologically, these neoplasms are solid tumors of the lymphoid tissue that do not lead to peripheral blood invasion. However, if such an invasion were to take place, it will be described as a leukemia phase of the lymphoma.

- Classification:
  - Hodgkin’s lymphoma
  - Non – Hodgkin’s lymphoma

Both diseases invade the lymphoid tissues, but the difference between them resides in the biological, clinical and prognosis features.

1. HODGKIN’S LYMPHOMA

- Definition: neoplastic disorder with development of specific infiltrate containing pathologic REED-STERNBERG CELLS (malignant, star-like cells, with multiple nuclei and prominent nucleoli)
- Onset: isolated non-painful asymptomatic adenopathy, located either in the lateral part of the neck, or in the mediastinum (detected through routine radiological investigations) → the adenopathy extends progressively to contiguous groups (via lymphatic pathways)
- Clinical features: general symptoms such as: fever, night sweats, weight loss (due to cytokine release by the malignant cells)
- Pathogenesis:
  ✓ Activation of a transcription factor NF-kB → mutations of B Lf. with proliferation and their transformation in Sternberg-Reed cells
  ✓ Sternberg-Reed cells secrete cytokines, growing factors, and chemokines:
    - Depression of the immune cellular response
    - Activation of the humoral response
    - Inflammatory infiltrate with MO/macrophages & EO
    - Fibroblasts proliferation
- Curability: > 75% of the cases with favorable prognosis

2. NON – HODGKIN’S LYMPHOMA

- Onset: slow, usually with multiple adenopathies, located either in the neck, axillary, inguinal, mesenteric regions, or it locates itself outside the lymphoid tissue, in the nose, pharynx, gastrointestinal tract, testes → high risk for medullar metastases and extralymphatic invasion
- Histologically: NO REED – STERNBERG CELLS can be found
- Clinical features: usually, it is not accompanied by general symptoms
- Pathogenesis: chromosomal abnormalities (translocations, mutations) are responsible for:
  ✓ preventing the apoptotic process
✓ Alteration of mechanisms of DNA repairing at the level of B Lymphocytes
  – Curability: only 25% of the cases, with a poor prognosis

E. PLASMA CELL NEOPLASMS

- **Definition:** malignant disorders, caused by an abnormal proliferation of a clone of plasma cells (plasmocytes).

- **Etiology:**
  - genetic predisposition
  - exposure to chemical substances, such as solvents or pesticides, or radiation
  - bacteria, virus

- **Classification:**
  1. Multiple myeloma
  2. Waldenstrom’s macroglobulinema (lymphoplasmacytic lymphoma)

MULTIPLE MYELOMA

- **Pathogenesis:**
  - the malignant plasma cell clone releases in the blood stream and urine abnormal monoclonal proteins that contain either the entire immunoglobulin molecule (IgG most frequently, IgA occasionally, IgE/M rarely), or just immunoglobulin fragments, usually light chains, types κ and λ. These light chains, are filtered in the kidneys and eliminated through urine.
  - the abnormal proteins can be detected through protein electrophoresis as M proteins, located in the γ – globulin region

- **Clinical manifestations:**
  - invasion and destruction of the bones (plasma cells are over 30% of the total medullar cellularity) → multiple osteolysis regions with bone resorption → bone pain and pathological bone fractures. Bone resorption takes place because of an overexpression of Receptor Activator for Nuclear Factor κ B Ligand (RANKL) that increases osteoclastic activity → hypercalcemia with secondary renal failure.
  - protein M production is responsible for the paraproteinemia in the blood stream (> 3 g/dL) and the Bence – Jones paraproteinuria.

- **Complications:**
  - anemia (normochromic normocytic) and thrombocytopenia, due to plasma cell proliferation within the BM → impairment of blood cells precursors proliferation and maturation
  - defective humoral immune response, due to leukopenia and hipogammaglobulinemia ⇒ recurrent infections (main cause of death)
  - renal failure (second cause of death).