UNIT 2

INVESTIGATION OF WHITE CELL DISORDERS

LEARNING OBJECTIVES

At the end of this chapter, students are expected to:
1. Recognize the alterations of leukocyte count.
2. Ask for the usual investigations needed to diagnose leukemias.
3. Discuss the differential diagnosis of acute and chronic leukemias.
4. Ask for the usual investigations in lymphomas (including lymphopasmacytic lymphomas)

Alterations of leukocyte count and function occur in a wide variety of hematological, infectious, inflammatory, metabolic and neoplastic diseases. Because leukocytes are affected by so many diseases, the routine laboratory evaluation of many patients begins with determination of the white cell or leukocyte count and the examination of a stained blood smear. Alterations of leukocytes can be divided in 2 major groups: non-malignant and malignant disorders. To the former group belong abnormalities of either leukocyte count (most frequently encountered in pathology) or function (rare diseases of abnormal phagocytosis). The latter group comprises leukemias and lymphomas.

The automated white cell count is provided as part of the full blood count (FBC). The red cells in the analyzed sample are lysed before counting the white cells. The differential white count breaks down the white cell count to identify the level of each of the five peripheral white cell lines: neutrophils, lymphocytes, monocytes, eosinophils and basophils. This is automated and uses stains, cell size and light scatter to differentiate between the different cell types.

The five types of circulating white cells can also be identified by their morphology on blood smears: neutrophil granulocytes (polymorphonuclear leukocytes), eosinophil granulocytes, basophil granulocytes, lymphocytes and monocytes. The peripheral blood smear if the patient’s indices are abnormal or if we suspect an underlying blood disorder. Normal values for blood leukocytes are presented in Table 1.

<table>
<thead>
<tr>
<th>Table 1. Normal values for blood leukocytes</th>
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<tbody>
<tr>
<td>WBCs count</td>
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<tr>
<td>Neutrophils (N)</td>
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<tr>
<td>Non-segmented (NN)</td>
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<td>Segmented (SN)</td>
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<td>Eosinophils (EO)</td>
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<td>Basophils (BA)</td>
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<td>Lymphocytes (LY)</td>
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<td>Monocytes (MO)</td>
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I. NON MALIGNANT WHITE CELL DISORDERS

1. ALTERATIONS OF LEUKOCYTE COUNT

Increased number of circulating leukocytes >10,000/mm³ is referred as leucytosis, while a number of leucocytes < than 4,000/mm³ is defined as leucopenia.

1.1. Neutrophils

1.1.1 Neutrophilia is defined as an absolute neutrophil count greater than 10,000 cells/mm³.

Causes of raised circulating neutrophils are:
- Physiological: exercise, stress, epinephrine
- Infections: chiefly bacterial (also fungal and parasitic)
- Inflammations: burns, necrosis in myocardial infarction, collagen vascular disease, hypersensitivity states
- Myeloproliferative diseases: myelocytic leukemia, polycythemia vera
- Metabolic disorders: acute renal failure, ketoacidosis, acute poisoning
Other: leukemoid reaction, acute hemorrhage or hemolysis, corticosteroid therapy.

Observation:
Most acute bacterial infections are associated with neutrophilia. This neutrophilia:
- initially occurs because of accelerated release of cells from the bone marrow reserves and is often accompanied by an increase in the number of non-segmented (young) neutrophils in the blood, (i.e. "shift to the left")
- with prolonged inflammation from any cause neutrophil production is stimulated and the bone marrow shows granulocytic hyperplasia.

1.1.2 Leukemoid reaction
This term describes persisting high neutrophilia with counts greater than 30,000 to 50,000/mm$^3$ (this degree of leukocytosis usually suggests leukemia).
It is a reversible reactive leukocytosis (a defense reaction) due to:
- severe bacterial infections: tuberculosis, pneumonias, meningitis
- organ necrosis (liver, colon)
- side effect of drugs (growth factors, corticosteroids in high doses)
The raised count is predominantly due to an increase in mature neutrophils (neutrophilia) with only a minor increase of nonsegmented neutrophils and metamyelocytes (early forms) in the peripheral blood
Other characteristics of the leukemoid reaction:
- elevation of leukocyte alkaline phosphatase
- normal or slight increase in serum level of vitamin B12
- lack of Philadelphia chromosome (a small chromosome 22)
These criteria are used to differentiate leukemoid reactions from chronic myeloid leukemia where:
- there is a malignant neoplastic proliferation of hematopoietic cells in the bone marrow
- there is a markedly decreased neutrophil alkaline phosphatase level
- the serum level of vitamin B12 is highly increased
- Philadelphia chromosome is present in over 90% of cases.

1.1.3 Neutropenia and agranulocytosis
Neutropenia is defined as a circulatory neutrophil count below 2,000/mm$^3$. A virtual absence of neutrophils is called agranulocytosis.
Causes of neutropenia are:
- Racial: neutropenia is common in black races, with no apparent disease
- Viral infections: infectious mononucleosis, infectious hepatitis, measles, influenza, HIV (inhibits progenitor cells)
- Severe bacterial infections: typhoid fever, brucellosis, tuberculosis, overwhelming sepsis
- Nutritional deficiencies: vitamin B12 and folic acid, especially in alcoholics
- Hematologic diseases: leukemia, lymphoma, aplastic anemia
- Diseases associated with splenomegaly: Felty syndrome (rheumatoid arthritis complicated by splenomegaly and profound neutropenia), sarcoidosis, Gaucher’s disease.
- Drugs:
  - alkylating agents: chlorambucil, cyclophosphamide, busulfan
  - antimetabolites: methotrexate, 6-mercaptopurine
  - anti-inflammatory: phenylbutazone
  - phenothiazine: chlorpromazine
  - anticonvulsants: phenytoin, carbamazepine
Patients are very liable to infections and occasionally septicemia complicates the condition.

1.2 Eosinophils

1.2.1 Eosinophilia is said to occur when the number of eosinophils is more than 500/mm$^3$ in the peripheral blood.
Causes of eosinophilia are as follows:
- Parasitic infections: infections with helminths, trichinosis, echinococcosis, schistosomiasis
- Allergic diseases: allergic rhinitis, asthma, eczema, angioedema, serum sickness, allergic vasculitis, as well as drug allergy
- Collagen vascular diseases: rheumatoid arthritis, dermatomyositis, periarteritis nodosa
- Malignant disorders: Hodgkin’s disease, chronic myeloid leukemia, melanoma
1.3 Basophils
These are the least common blood leukocytes; usually none are seen in the routine examination of a blood smear.

1.3.1 Basophilia
These cells are thought to be involved in:
- Certain acute hypersensitivity reactions: life threatening urticaria, asthma and anaphylactic shock
- Chronic myeloid leukemia, myelofibrosis and polycythemia vera (basophilia helps to distinguish these diseases from leukemoid reactions).

1.4 Monocytes
Blood monocytes are phagocytic cells with bactericidal capacities and they are precursors of tissue macrophages; they spend only a few hours in blood, but can continue to proliferate in the tissues for many years. Monocytes have a critical role in processing of antigen, essential for both cellular and humoral immunity.

1.4.1 Monocytosis
Causes for increased circulating number of monocytes are:
- Certain infections: tuberculosis, subacute bacterial endocarditis, infectious mononucleosis, brucellosis, malaria
- Granulomatous diseases: sarcoidosis, regional enteritis
- Malignancies: leukemias, lymphomas, myeloproliferative syndromes, neoplasms of breast, kidney, ovary.

1.4.2 Monocytopenia
Causes for (acute) reduction in blood monocytes are:
- Stress and following corticosteroid administration
- Aplastic anemia, acute leukemia
- The direct effect of myelotoxic and immunosuppressive drugs.

1.5 Lymphocytes
Circulating lymphocytes are small cells with a dark-staining central nucleus. There are two main types: the thymus-dependent or T lymphocytes which are concerned with cellular immunity and the bursa dependent or B lymphocytes which are concerned with humoral immunity.

1.5.1 Lymphocytosis
An increase in the absolute lymphocyte count occurs in:
- Certain infections: infectious mononucleosis, infectious hepatitis, pertussis, brucellosis, tuberculosis, syphilis
- Thyrotoxicosis
- Adrenal insufficiency
- Chronic lymphocytic leukemia

1.5.2 Lymphocytopenia
Acute lymphocytopenia occurs with acute stressful illness: myocardial infarction, severe pneumonia and sepsis.
Chronic lymphocytopenia is due to: lymphomas (especially Hodgkin’s disease), aplastic anemia, lupus erythematosus, primary immune deficiencies (of either B or T lymphocytes or both) or acquired immunodeficiency syndromes (particularly AIDS with a typically decrease of the T helper - CD4+ lymphocytes).

II. MALIGNANT WHITE CELL DISORDERS

1. Leukemias

1.1 Definition: malignant proliferations of hematopoietic cells in the bone marrow.

1.2 Classification:
A. According to the onset and clinical course:
   1. Acute leukemia
   2. Chronic leukemia
B. According to the cell type:
   1. Lymphocytic leukemia
   2. Granulocytic (myeloid) leukemia
   3. Other cell types (rare)
C. According to the cell blood picture:
   1. Leukemic: white count elevated, blood leukocytes >20,000/mm³; abnormal cells present
   2. Subleukemic: white count elevated, blood leukocytes between 7,000 - 20,000/mm³; abnormal cells present
   3. Aleukemic: total white count normal; no abnormal cells detectable in peripheral blood.
1.3. Acute leukemias

Clinical characteristics:
- Sudden onset; rapid progression; bad prognosis
- Increased proliferation of primitive precursors (blast cells) while maturation and differentiation are blocked
- Rapidly developing anemia
- A decrease in mature functioning granulocytes with relapsing infections, fever and mucous membrane ulcerations
- Thrombocytopenia with purpura.

Diagnosis:
- The total white count is increased
- Abnormal primitive (blast) cells are present in the peripheral blood in most cases (exceptions: aleukemic leukemia)
- The diagnosis is confirmed by examination of the bone marrow, which shows:
  - extreme hypercellularity
  - infiltration with primitive blasts.

Types:
1.3.1 Acute lymphocytic leukemia (ALL)
- Acute leukemia characterized by lymphoblast proliferation in bone marrow and lymphoid tissue
- Lymph node enlargement common
- ALL occurs in young children
- Classification FAB (French-American-British) based on morphologic features includes 3 types of ALL:
  a) L1: this is the usual type of childhood leukemia: the lymphocytes do not mark either as T or B cells (hence null cells). This type has the best prognosis.
  b) L2: this is less common in children; it is the usual type in adults. The cells frequently mark as T cells and have convoluted nuclei. This type is frequently associated with mediastinal mass and has a bad prognosis.
  c) L3: Burkitt type; cells frequently mark as B cells; bad prognosis.

1.3.2 Acute granulocytic leukemia (AGL)
- Acute leukemia characterized by myeloblast proliferation
- AGL occurs in any age, but especially in young adults
- Classification FAB based on morphologic and cytochemical features comprises 8 types:
  a) M0: myeloblasts with minimal differentiation
  b) M1: myeloblasts without maturation
  c) M2: myeloblasts with some maturation into more differentiated granulocytic cells
  d) M3: promyelocytic leukemia
  e) M4: myelomonocytic leukemia
  f) M5: monocytic leukemia
  g) M6: erythroleukemia
  h) M7: megakaryocytic leukemia
- Particular features:
  - Auer rods are characteristic rod-shaped purple-staining cytoplasmic inclusions in myeloblasts and promyelocytes (M0 to M4)
  - Promyelocytic leukemia (M3): Granules in promyelocytes have coagulant material that causes disseminated intravascular coagulation (DIC)
  - Erythroleukemia (M6) is characterized by the proliferation of erythroid as well as myeloid precursors.

1.4 Chronic leukemias

1.4.1 Chronic lymphocytic leukemia (CLL)
- CLL is a disease of the elderly (over 60 years)
- Insidious onset and slow progression
- Lymph node, spleen, and liver enlargement are common.
- The peripheral blood shows increase in the absolute lymphocyte count, often exceeding 100,000/mm³.
- The lymphocytes are well differentiated, resembling "mature" B lymphocytes (95% of cases) or T lymphocytes (5% of cases)
- Anemia and thrombocytopenia due to bone marrow infiltration are later features
- The bone marrow is infiltrated by small lymphocytes
- Diffuse leukemic infiltration is common in other organs (skin, liver, heart, kidneys, etc)
- Average survival is 5 years after diagnosis.
1.4.2 Chronic granulocytic (myeloid) leukemia (CGL, CML)

- CGL is a disease of the adults (30-50 years)
- Insidious onset, with sweating, fever, weight loss (as the result of high metabolic rate) and the effects of splenic enlargement (gastrointestinal pain or discomfort)
- Characteristic biphasic evolution with:
  - The chronic proliferative phase shows:
    - The white cell count is greater than 50,000/mm$^3$ (until 500,000/mm$^3$)
    - Peripheral blood shows marked increase in granulocytes (all types), especially neutrophils, but intermediary forms such as myelocytes and metamyelocytes are also common. Primitive myeloblasts account for less than 5% of cells in the periphery
    - The platelet count is elevated initially, but later falls

CGL is differentiated from other causes of neutrophilia by:
- markedly decreased leukocyte alkaline phosphatase level
- increased serum levels of vitamin B12 and B12 binding proteins (transcobalamins)
- Philadelphia chromosome (a small 22 chromosome due to translocation of part of 22 to 9) is present in over 90% of cases
- Bone marrow shows marked hypercellularity with increased numbers of granulocytes and megakaryocytes (myeloproliferative disease)
- The spleen is massively enlarged. (spleen infarction may occur due to vascular occlusion by aggregates of granulocytes).

- The accelerated phase:
  - Progressive leucocytosis (± basophilia)
  - Progressive anemia and thrombocytopenia (due to bone marrow infiltration with granulocytes)
  - 15% - 30% blasts
  - Hyperuricemia with risk of gout and nephrolithiasis

- The blast crisis:
  - occurs after a median time of about 3-4 years of onset
  - is characterized by an increased number of blast cells in the bone marrow and peripheral blood over 50% of the cells (acute blasts crisis).
  - the median survival after blasts crisis is 2 months.
  - can be predicted by the appearance of new karyotype abnormalities.

1.5 Myeloproliferative disorders

This is a group of diseases of unknown origin in which there is proliferation erythroid, granulocytic, and megakaryocytic cell lines in the bone marrow.
Peripheral blood shows an increase in all cell lines. Usually, one cell line dominates:
- Chronic granulocytic leukemia (granulocytes)
- Polycythemia vera (erythrocytes)
- Idiopathic thrombocytopenia (platelets)
In evolution progressive fibrosis of the bone marrow (myelofibrosis) occurs.

2. Lymphomas

2.1 Definition: solid tumours of lymph nodes and most frequently, unlike leukemia, lymphocytes are not found in circulation. However, a number of lymphomas may present in a leukemic phase.

2.2 Classification

Traditionally, lymphoma is classified in:
1. Hodgkin`s lymphoma
2. Non-Hodgkin`s lymphoma

Current classification:
1. Neoplasms with immature B-cells
2. Neoplasms with mature B-cells
3. Neoplasms with immature T-cells
4. Neoplasms with mature T cells and natural killer cells (NK)
5. Hodgkin`s lymphoma

2.3 Diagnostic procedures

2.3.1 Blood Tests
- Blood tests help rule out infection and other diseases.
- Such tests include those blood counts and blood chemistries for kidney and liver
function, uric acid, calcium, and phosphate levels.

- The erythrocyte sedimentation rate (ESR) is sometimes elevated in Hodgkin's disease (although it is not specific for this condition).
- In a patient already diagnosed with lymphoma, blood tests that measure the enzyme LDH (lactate dehydrogenase) are important in determining the prognosis; elevated levels indicate bulkier tumors.

2.3.2 Biopsy
A biopsy of the suspicious lymph node is the most definitive way to diagnose lymphomas and can be used to tell the difference between non-Hodgkin's versus Hodgkin's disease.

Procedure: during a biopsy, the physician usually removes the node and checks the surrounding areas. The tissue in the node is then examined under a microscope for signs of infection and abnormalities indicating cancer or other conditions. The presence of Reed-Sternberg cells, a multinucleated, abnormal lymphocyte with a clear halo around the nucleoli is thought to be specific to Hodgkin's lymphoma.

2.3.3 Imaging Techniques
- **Chest X-Ray.** A chest X-ray allows a view of the lymph nodes in the chest and neck area. It is particularly useful in detecting Hodgkin's disease and is a useful step for detection of enlarged lymph nodes.
- **Computer Tomography.** Computed tomography (CT) scans are more accurate than X-rays and can detect abnormalities in the chest and neck area, as well as revealing the extent of the cancer and whether it has spread outside the nodes.
- **Other Advanced Imaging Techniques.** A number of advanced imaging techniques, including gallium scintigraphy and positron emission tomography (PET) are proving to be very helpful. Magnetic resonance imaging (MRI) may be used to detect the spread of the disease to the brain, spine, chest, pelvic, and abdomen.
- **Lymphangiography.** Lymphangiography is an X-ray of the lymph glands and vessels after injection of a dye. It provides additional information on lower parts of the body and is a good complement to CT scans if the latter does not reveal abnormal lymph nodes but they are still suspected, but it is generally not performed routinely.

2.3.4 Bone Marrow Aspiration and Biopsy
- Bone marrow aspirate and biopsy are routinely performed to determine whether the disease has spread.
- With bone marrow aspirate, bone marrow cells are sucked out through a special needle; during a biopsy, a special needle removes a core of the marrow that is structurally intact.

2.3.5 DNA Tests
- Tests of lymphoma's DNA are in use or are being developed to detect particular genetic abnormalities that help determine outlook and may eventually lead to new treatments.
- Examples of such abnormal genetic arrangements are those that affect normal cell death, resist chemotherapy, or trigger aggressive cancer growth.

3. Multiple myeloma (MM)

3.1. Definition: proliferation of a clone of malignant plasma cells and a subsequent overabundance of monoclonal paraproteins (monoclonal gammapathy, paraproteinemia). The malignant plasma cells produce only one type of intact immunoglobulin (Ig) in large amounts - usually IgG or IgA or an excess of the light chains of Ig. These identical immunoglobulins or light chains are also known as monoclonal proteins or M proteins and are responsible for the abnormal protein level.

3.2 Diagnostic procedures
Tests used as a follow-up to abnormal protein level include one or more of the following:

3.2.1. Protein level and Immunofixation Electrophoresis
- large amounts of an abnormal immunoglobulin protein (M-protein) will show up as a large peak on the electrophoresis graph; amount of normal immunoglobulins may decreased
- a blood and a urine sample will be tested during the diagnosis of multiple myeloma because some proteins, such as the Bence Jones proteins (free light chains), may not show up in significant quantities in blood samples,
while those with only intact immunoglobulins may not have the abnormal protein in urine.
- Immunofixation electrophoresis is done to identify the specific protein produced by the malignant plasma cells.

3.2.2 Bence-Jones proteinuria (free light chains)
- Use of a 24-hour urine sample because the total amount of Bence Jones protein in 24 hours is related to the amount of tumor that is present.
- Measurement of kappa or lambda light chains (not both in the same patient).

3.2.3 Bone marrow aspiration and biopsy
- Evaluates the amount of malignant plasma cells present in the bone marrow, and to what degree they have affected the production of normal WBCs, RBCs, and platelets.

3.2.4 Other tests and procedures that may be done as part of an initial diagnostic workup, to monitor the progress of the disease, and to help detect and address complications include:

- Comprehensive Metabolic Panel (CMP) a group of tests used to evaluate kidney (urea, creatinine) and other organ function, electrolyte status, and to determine calcium and total protein levels.
- Complete Blood Count (CBC) in order to assess the severity of anemia
- Uric acid levels: hyperuricemia
- Calcium levels: hypercalcemia
- ESR and CRP: increased
- Imaging techniques:
  - X-ray: detects cavities in bones, extent of bone damage, and the number and size of tumors in the bones.
  - Magnetic Resonance Imaging (MRI): more sensitive than X-ray for evaluating bone destruction.
  - Computed tomography (CT): evaluation of bone tumors.
CHECKPOINT

1. Philadelphia chromosome is specific for which type of leukemia?
   A. CML
   B. CLL
   C. AML
   D. ALL
   E. Hodgkin lymphoma

2. Which of the following features are characteristic for multiple myeloma:
   A. Hyperuricemia
   B. Increased B2 and transcobalamins
   C. Bence-Jones proteinuria
   D. Elevated ESR
   E. Hypocalcemia

3. The following are true about CLL:
   A. Auer rods are characteristic for the final diagnosis
   B. The bone marrow is infiltrated by small lymphocytes
   C. Peripheral blood smear and bone marrow aspirate examination show an increased number of lymphoblasts
   D. Peripheral blood shows marked increase in granulocytes (all types), especially neutrophils
   E. The peripheral blood shows well differentiated, resembling "mature" B lymphocytes or T lymphocytes

4. A 28-year-old patient complains about weight loss, moderate fever and general malaise. His laboratory tests were:
   - Hb = 7,5 g/dl, Hct = 25%, RBC count = 2,5 millions/mm³,
   - Reticulocyte count = 0,1%,
   - Platelet count = 180,000/mm³,
   - WBC count = 32,000/mm³ (90% lymphoblasts)
   - ESR = 60 mm

   What is the most likely diagnosis?
   A. Acute granulocytic leukemia M₃
   B. Acute lymphocytic leukemia
   C. Leukemoid reaction
   D. Chronic lymphocytic leukemia
   E. Polycythemia vera.

5. Laboratory investigations of a patient showed:
   - Hb = 10 g/dl, Hct = 30%, RBC count = 3,1 millions/mm³,
   - WBC count = 15,000/mm³ (70% small lymphocytes),
   - Platelet count = 300,000/mm³.

   The diagnosis is:
   A. Acute lymphocytic leukemia
   B. Acute granulocytic leukemia
   C. Chronic myeloid leukemia
   D. Chronic lymphocytic leukemia
   E. Leukemoid reaction.

6. A patient with:
   - RBC count = 1,8 millions/mm³,
   - Hct = 18 %,
   - Reticulocyte count = 0%,
   - WBC count = 60,000/mm³ (N = 2%, S =8%, lymphocytes = 10%, myeloid blasts = 80%),
   - Platelet count = 30,000/mm³.

   has the followings:
   A. Anemia
   B. Thrombocytopenia
   C. Acute granulocytic leukemia
   D. Acute lymphocytic leukemia
   E. Chronic myeloid leukemia.