

**MINISTRY OF HEALTHCARE OF UKRAINE
HSEEU "Ukrainian Medical Stomatological Academy"**

"Approved"

at the meeting of internal
medicine №1 department

Head of Department

Prof. Skrypnyk I.M.

Protocol № 4 from 13.10.2016

**GUIDELINES
FOR STUDENTS
INDEPENDENT WORK
IN THE PRACTICAL CLASSES PREPARING**

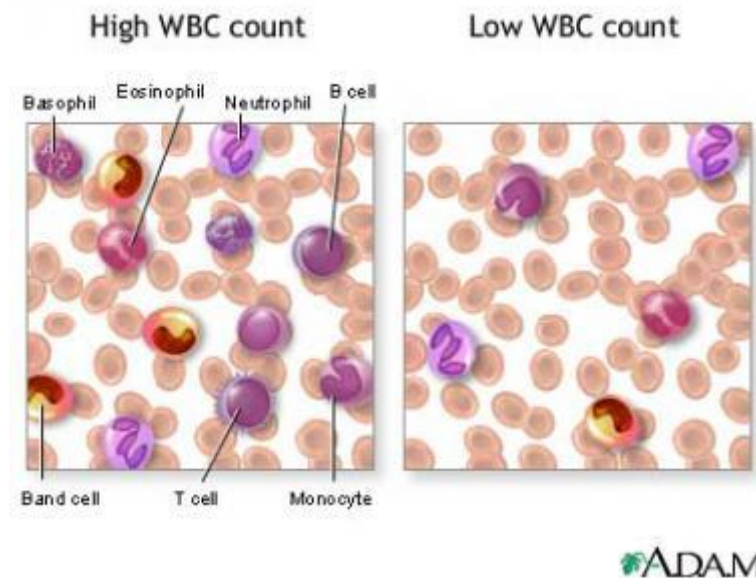
<i>Academic discipline</i>	Internal medicine
<i>Module</i>	Current practice of internal medicine
<i>Content module</i>	Management of the patients with main symptoms and syndromes in hematology clinic
<i>Study subject</i>	Management of the patients with leukocytosis that leukopenia
<i>Course</i>	VI
<i>Faculty</i>	of foreign students training

Poltava 2016.

The subject of the lesson: Leukocytosis and leukopenia

Leukocytosis refers to an increase in the total number of WBCs due to any cause. From a practical standpoint, leukocytosis is traditionally classified according to the component of white cells that contribute to an increase in the total number of WBCs. Therefore, leukocytosis may be caused by an increase in (1) neutrophil count (ie, neutrophilia), (2) lymphocyte count (ie, lymphocytosis), (3) monocyte count (ie, monocytosis), (4) eosinophilic granulocyte count (ie, eosinophilia), (5) basophilic granulocyte count (ie, basophilia), or (6) immature cells (eg, blasts). A combination of any of the above may be involved.

The image below is an illustration of high and low WBC counts.



Neutrophilia also is divided into 4 categories based on the mechanism of neutrophilia: (1) increased production, (2) decreased egress from vascular space (demargination), (3) increased mobilization from the marrow storage pool, and (4) reduced margination into the tissue.

Clinically, dividing leukocytosis on the basis of its causes is more convenient. By dividing it according to causes, leukocytosis can be immediately applied for diagnostic purposes. Leukocytosis can be caused by infection, inflammation, allergic reaction, malignancy, hereditary disorders, or other miscellaneous causes

Educational goal:

The student must know:

1. Aetiology and pathogenesis of leukocytosis and leukopenia.
2. Clinical symptoms of leukocytosis and leukopenia.
3. Modern classification of leukocytosis and leukopenia.
4. Methods of diagnostics of leukocytosis and leukopenia.
5. Methods of treatment of leukocytosis and leukopenia.

The student must be able:

1. To choose the symptoms of leukocytosis and leukopenia from the history data.
2. In examination of the patient to choose the symptoms of leukocytosis and leukopenia.
3. To make the scheme of investigation for the determination leukocytosis and leukopenia.
4. To define the cause and the severity of leukocytosis and leukopenia.
5. To assess the haemologic study results.

The main problems of the lesson:

1. Pathogenesis of leukocytosis and leukopenia.

2. Clinical symptoms of leukocytosis and leukopenia.
3. Modern classification of leukocytosis and leukopenia.
4. Methods of diagnostics of leukocytosis and leukopenia.
5. Differential diagnosis of leukocytosis and leukopenia.

The aim: The students must be able to diagnose leukocytosis and leukopenia, determine the types, severity, and prescribe the proper treatment.

Topicality: The incidence of leukaemia of all types in the population is approximately 10/100 000 per annum, of which just under half are acute leukaemia. Males are affected more frequently than females, the ratio being about 3:2 in acute leukaemia, 2:1 in chronic lymphocytic leukaemia and 1.3:1 in chronic myeloid leukaemia.

CONTENTS OF THE TRAINING MATERIALS

Leukocytosis can be a reaction to various infectious, inflammatory, and, in certain instances, physiologic processes (eg, stress, exercise). This reaction is mediated by several molecules, which are released or upregulated in response to stimulatory events that include growth or survival factors (eg, granulocyte colony-stimulating factor, granulocyte-macrophage colony-stimulating factor, c-kit ligand), adhesion molecules (eg, CD11b/CD18), and various cytokines (eg, interleukin-1, interleukin-3, interleukin-6, interleukin-8, tumor necrosis factor).

The peripheral leukocyte count is determined by several mechanisms, including (1) the size of precursor and storage pool of myeloid and lymphoid cells, (2) the rate of release of the cells from the storage pool in the bone marrow, (3) the rate of marginating cells out of blood vessels into the tissues, and (4) the rate of consumption of the cells in the tissues (ie, cell loss). The growth factors, adhesion molecules, and cytokines control all 4 mechanisms listed above. For a detailed discussion, see *Robbins Pathologic Basis of Disease*.

Hyperleukocytosis (WBC count $>100 \times 10^9/L$, or $>100 \times 10^3/\mu L$) occurs in leukemia and myeloproliferative disorders. This is certainly due to its inherent autonomous growth potential of malignant cells. Hyperleukocytosis often causes vascular occlusion, resulting in ischemia, hemorrhage, and edema of the involved organs. The problem is most commonly observed in acute myelogenous leukemia with high WBC counts. Individuals often clinically present with mental status changes, stroke, and renal or pulmonary insufficiency. If the neutrophil count exceeds $30,000/\mu L$ as a reaction to extrinsic factors, such as infection, it is sometimes called a leukemoid reaction.

In a person with sickle cell disease, the baseline WBC count is elevated with a mean of $12-15 \times 10^9/L$ ($12-15 \times 10^3/\mu L$). This change mainly is due to a shift of granulocytes from the marginated pool to the circulating compartment. The segmented neutrophil count increases in both vaso-occlusive crisis and in bacterial infection in patients with sickle cell disease.

Neutrophilia

Neutrophilia (ie, neutrophil count that exceeds the reference range for age; see the Absolute Neutrophil Count calculator) may be due to the following conditions:

- Infection (most common cause)
 - Most bacterial infections cause neutrophilia with bandemia (number of bands exceeds the reference range). Some bacterial infections do not cause neutrophilia. For example, typhoid fever causes leukopenia, neutropenia, or both. Other bacterial infections that are known to cause neutropenia include *Staphylococcus aureus*, brucellosis, tularemia, rickettsia, *Mycobacterium tuberculosis*, ehrlichiosis, and leishmaniasis. Infants, preterm infants in particular, have small storage pools of neutrophils in the bone marrow. Therefore, neutropenia develops in severe or chronic infections because the neutrophilic demand is greater than the supply.

- Neutrophilia alone or with an increased band count had variable sensitivity and specificity in numerous studies as a possible predictor of bacteremia in young children with fever. A study by Lee and Harper was unique in that they selected infants and toddlers aged 3-36 months with fever ($\geq 39^{\circ}\text{C}$) who appeared well and who were sent home from the emergency department. They excluded patients who were admitted, transferred, or died to select a population who potentially had truly occult bacteremia. The study showed a significantly positive correlation between the frequency of blood cultures positive for *Streptococcus pneumoniae* and the WBC and absolute neutrophil counts.
- In another study, Brown et al focused on febrile neonates (aged ≤ 28 d) who visited the emergency department. They calculated the sensitivity and specificity of various WBCs for the detection of bacterial infection. They found modest discriminatory power of the WBC count; the area under the receiver operator characteristic [ROC] curve was 0.7231.
- Immunization practice with heptavalent pneumococcal conjugate vaccination (now 13-valent) seems to have reduced incidence of bacteremia with this organism in infants aged 2-6 months. Accordingly, extreme leukocytosis, which is a common characteristic of pneumococcal bacteremia, has decreased in frequency.
- Urinary tract infection and pneumonia due to other organisms are more prevalent in infants with fever and typically cause less leukocytosis than an infection with *S pneumoniae*. Therefore, the algorithm that uses the total white cell count to gauge bacteremia risk in infants may not apply to the new generation of children with fever.
- In general, the WBC and neutrophil counts alone are not sensitive or specific enough to accurately predict bacterial infection. Although viral infections generally do not cause neutrophilia, it can occur during the early phases of infection (see below under "lymphocytosis").
- Inflammation: This includes inflammatory bowel disease, rheumatoid arthritis, and vasculitis (eg, Kawasaki syndrome).
- Extremely low birth weight: A higher frequency of leukemoid reaction (neutrophils $>30,000/\mu\text{L}$) was reported in extremely low birth weight (≤ 1000 g) infants without obvious causes of leukocytosis and in association with longer ventilatory support and a higher frequency of bronchopulmonary dysplasia (BPD). A prospective study of preterm infants showed a significant correlation between the infant's leukemoid reaction (neutrophil count $>40,000/\mu\text{L}$) and histological evidence of chorioamnionitis. In this study, the incidence of BPD was significantly higher in infants who had leukemoid reaction compared with those without leukemoid reaction.
- Prostaglandin (PGE_1): In neonates with ductus-dependent congenital heart disease, administration of PGE_1 caused reversible elevation in neutrophil count by an average of $6000/\mu\text{L}$. This was later confirmed in a retrospective study with more than 2 weeks of infusion of PGE_1 .
- Lithium: Lithium carbonate, commonly used for depression and bipolar disorder, is known to cause modest leukocytosis and neutrophilia (up to twice as many as the baseline count). The increase is due to increased production of neutrophils.
- Heparin: Heparin induces leukocytosis, mainly lymphocytosis, but in some cases, neutrophilia as well. One in every 230 patients treated with heparin had leukocytosis.^[10]
- Other: Medications that are known to cause leukocytosis and leukemoid reaction along with eosinophilia are antiepileptic drugs, including carbamazepine, phenobarbital, and phenytoin. Minocycline, which is commonly used for the treatment of acne, have been reported to cause a severe hypersensitivity reaction called drug rash with eosinophilia and systemic symptoms (DRESS) syndrome. The neutrophilia and leukocytosis are secondary to hypersensitivity reaction to the drug. Some patients developed lymphocytosis or leukopenia instead of neutrophilia and leukocytosis with antiepileptic drugs. The antipsychotic drug clozapine has been known to cause agranulocytosis, but it also causes dose-related elevation in leukocytes and neutrophil counts.

- Familial cold autoinflammatory syndrome (familial cold urticaria) is characterized by development of multiple purpuric raised erythema a few hours after exposure to cold, fever, chills, arthralgia, and consistent elevation of neutrophil and WBC counts. It is transmitted in autosomal dominant fashion.
- Malignancy and myeloproliferative disorders
 - These are rare causes of neutrophilia in children.
 - Hodgkin lymphoma typically causes mild-to-moderate neutrophilia.
 - Patients with chronic phase of adult-type chronic myelocytic leukemia and a positive Philadelphia chromosome present with neutrophilia with immature forms, eosinophilia, basophilia, and thrombocytosis.
 - Juvenile myelomonocytic leukemia causes leukocytosis and monocytosis with bizarre-shaped monocytes rather than neutrophilia alone.
 - Infants with Down syndrome frequently have leukocytosis, neutrophilia, differential shift to the left, and immature forms (blasts) in the blood (myeloproliferative disorder) during the postnatal period. In most cases, this change is transient (referred to as transient myeloproliferative disorder); however, some develop acute leukemia.
 - Some solid tumors (most commonly described in carcinoma of the lung and in undifferentiated carcinoma) cause neutrophilia by the tumor cells called paraneoplastic leukemoid reaction. This is rare in children, but has been well described in adult patients. The presumed mechanism is production of cytokines, such as granulocyte colony-stimulating factor (G-CSF) and granulocyte macrophage colony-stimulating factor (GM-CSF), by tumor cells or metastatic cells. However, in some patients, cytokines measured were not elevated.
- Decreased egress from circulation
 - The neutrophil count is a balance between its production and release into blood circulation and its destruction and departure from circulation into tissue. Anything that affects any component of this balance affects the neutrophil count.
 - Decreased egress from circulation may occur with the administration of corticosteroids, splenectomy, or congenital leukocyte adhesion molecule deficiency. Persistent leukocytosis and thrombocytosis are commonly seen in patients postsplenectomy. Leukocyte adhesion molecule deficiency (LAD) has 3 subtypes (LAD1, LAD2, LAD3), characterized by delayed separation of umbilical cord and neutrophilia with an increased susceptibility to infection. LAD 1 is caused by a mutation of *ITGB2* gene coding for the $\beta 2$ (CD18) subunit responsible for membrane expression of the leukocyte integrins. Flow cytometric demonstration of the absence of CD11b/CD18 on the patient's leukocytes is diagnostic. Patients with LAD3 have abnormal bleeding in addition to increased risk of infections.
- Decreased neutrophil margination, including steroid administration, exercise, epinephrine administration, and other stressful situations (eg, trauma, severe pain)
 - Neutrophilia due to these causes is generally short lived (ie, minutes to hours, not days). Transient but significant elevation in white cell numbers and neutrophil counts have been described after a brief period of exercise, afebrile seizure including status epilepticus, and mild head trauma with Glasgow Coma Scale of 15. See the Glasgow Coma Scale calculator.
 - A significant elevation in the leukocyte count (and lymphopenia) during the first week after isolated spinal cord injury was observed in patients with neurological impairment compared with controls who had isolated spinal cord injury without neurological impairment. This elevation was not due to steroid administration. Authors speculated that alpha adrenergic stimuli, endogenous corticosteroid increase, or both may be the cause. Contrary to the simultaneous lymphopenia in this study, lymphocytosis was observed after a brief exercise. Neutrophilia and leukocytosis were also observed during abdominal attack in patients with hereditary angioneurotic edema. Attacks of other organs were not associated with leukocytosis.

- Increased release of neutrophils from marrow: This occurs in infection, stress, and hypoxia; it also occurs due to endotoxin stimulation and steroid administration.
- A mutation in the *CSF3R* gene: A familial neutrophilia (neutrophil count $\leq 22,900/\mu\text{L}$) has been described due to a mutation in the transmembrane domain of G-CSF receptor (T617N).
- Therapeutic repetitive injections of pegylated G-CSF or G-CSF–caused hyperleukocytosis
- Lymphocytosis conventionally refers to a lymphocyte count greater than $4 \times 10^9/\text{L}$ ($4000/\mu\text{L}$); however, a lymphocyte count that exceeds this is physiologically present in infants and young children. The upper normal limit of lymphocyte count in this age group has not been well defined in a healthy population.
- Marked lymphocytosis is observed in individuals infected with pertussis (total leukocyte count of $40\text{--}50 \times 10^9/\text{L}$, or $40\text{--}50 \times 10^3/\mu\text{L}$). An exceedingly high lymphocyte count such as $100 \times 10^9/\text{L}$ indicates poor prognosis.
- Viral infection generally causes lymphocytosis (relative or absolute) with or without neutropenia. Typical examples include infectious mononucleosis or cytomegalovirus infection, respiratory syncytial virus infections, and infectious hepatitis. On the other hand, some viral infection results in remarkable leukemoid reaction with a shift to left. An example is the Hantavirus pulmonary syndrome.^[25] The highest WBC count during the 1993 outbreak was reported to be $65,000/\mu\text{L}$ with shift to left. The author has seen neutrophilia and leukocytosis in the early phase of Epstein-Barr virus infection in children.
- Chronic lymphocytic leukemia that is routinely characterized by mature lymphocytosis is extremely rare in children and is usually not considered in the differential diagnosis of lymphocytosis.

Eosinophilia

An increase in absolute eosinophil count greater than $0.5 \times 10^9/\text{L}$ ($500/\mu\text{L}$) is generally considered eosinophilia. The following are common causes of eosinophilia.

- Allergy and drug hypersensitivity: This includes asthma, hay fever, angioneurotic edema, urticaria, atopic dermatitis and eczema, anticonvulsant hypersensitivity reaction, allergy to drugs, eosinophilic esophagitis and enteritis, and other allergic conditions (see above under the heading of neutrophilia for familial cold autoinflammatory syndrome).
- Parasitic infections: The most commonly observed parasitic infection causing marked eosinophilia in the United States is caused by visceral larva migrans due to *Toxocara canis*. *Toxocara cati* also causes visceral larva migrans, but this is rare. Other parasitic infections that cause tissue invasion also cause marked eosinophilia.
- Other infections: Scarlet fever (recovery phase), viral infections (recovery phase), and chlamydial infection cause an absolute increase in eosinophils but generally do not cause leukocytosis.
- Dermatologic disorders: Dermatitis herpetiformis, pemphigus, and erythema multiforme cause eosinophilia.
- Hypereosinophilic syndrome
- Other conditions: Most other conditions that cause eosinophilia rarely lead to leukocytosis and, therefore, are not listed. However, other rare disorders that should be considered include eosinophilia associated with malignant disease. Pulmonary infiltration with eosinophilia (PIE) and a combination of eosinophilia, leukocytosis, and hepatosplenomegaly may be noteworthy. PIE is characterized by bilateral pulmonary infiltrates and eosinophilia. The symptoms are similar to those of chronic pneumonia. The etiologies are multiple and include various infections (bacterial, viral, fungal, and parasitic) and neoplastic conditions (eg, Hodgkin lymphoma). The combination of leukocytosis, eosinophilia, and hepatosplenomegaly could be true eosinophilic leukemia (with blasts observed in the peripheral blood) or marked eosinophilia with a chronic indolent course.
- Hyperleukocytosis: This disorder refers to a WBC count $100 \times 10^9/\text{L}$ ($100 \times 10^3/\mu\text{L}$). It is observed almost exclusively in leukemia and myeloproliferative disorders. Hyperleukocytosis

may cause life-threatening complications (eg, cerebral infarct, cerebral hemorrhage, pulmonary insufficiency). The frequency of complications is higher in acute myelocytic leukemia than in acute lymphoblastic leukemia because myeloblasts are larger and more adhesive than lymphoblasts.

A study by Drago et al indicated that the presence of peripheral eosinophilia may be associated with greater severity of adverse cutaneous drug reactions (ACDRs). The report included 63 ACDR patients, including 11 with peripheral eosinophilia, with ACDRs in the latter marked by longer recovery times and diffuse severe cutaneous reactions. In addition, all 11 patients with peripheral eosinophilia required systemic therapy, while just 41% of the other patients did.^[26]

Monocytosis

Monocytosis is defined as a monocyte count that exceeds the upper limit of the reference range of $0.95 \times 10^9/L$ ($950/\mu L$). Monocytosis is commonly caused by the following conditions:

- Bacterial infections: These include tuberculosis, subacute bacterial endocarditis, and brucellosis.
- Other infections: Syphilis, viral infections (eg, infectious mononucleosis), and many protozoal and rickettsial infections (eg, kala azar, malaria, Rocky Mountain spotted fever).
- Malignancies: Malignancies include chronic myelomonocytic leukemia, monocytic leukemia, Hodgkin disease, and myeloproliferative disorders; in adults, they include metastatic carcinoma, lung cancer, and other malignant neoplasms (paraneoplastic leukemoid reaction).
- Recovery phase of neutropenia or an acute infection.
- Autoimmune disease and vasculitis: These include systemic lupus erythematosus, rheumatoid arthritis, ulcerative colitis, and inflammatory bowel disease.
- Miscellaneous causes: Sarcoidosis and lipid storage disease are included.

A study by Cherfane et al indicated that a finding of monocytosis, along with a low lymphocyte/monocyte ratio, can identify the presence of active ulcerative colitis, as opposed to ulcerative colitis in remission. According to the investigators, a monocyte count of 483 and a lymphocyte/monocyte ratio of 3.1 had a sensitivity of 60% for active ulcerative colitis, along with a specificity of 61% and 53%, respectively. It was also found that a monocyte count of greater than 860 and a lymphocyte/monocyte ratio of less than 1.6 had a positive predictive value of 75% for active ulcerative colitis.^[27]

Basophilia

A basophil count that exceeds $0.10\text{--}0.15 \times 10^9/L$ ($100\text{--}150/\mu L$) that leads to leukocytosis is rare. Chronic myelogenous leukemia (adult type) typically exhibits basophilia and leukocytosis as described above (see Malignancy and myeloproliferative disorder).

Leukaemias are malignant disorders of the haematopoietic stem cell compartment, characteristically associated with increased numbers of white cells in the bone marrow and/or peripheral blood.

The cause of the leukaemia is unknown in the majority of patients. Factors, which are associated with the development of leukaemia: Ionising radiation, Cytotoxic drugs, Exposure to benzene in industry, Genetic, Immunological.

Leukemias were originally termed acute or chronic based on life expectancy but now are classified according to cellular maturity.

Leukaemias are traditionally classified into four main groups:

- acute lymphoblastic leukaemia (ALL)
- acute myeloid leukaemia (AML)
- chronic lymphocytic leukaemia (CLL)
- chronic myeloid leukaemia (CML).

In acute leukaemia there is proliferation of primitive stem cells leading to an accumulation of blasts, predominantly in the bone marrow, which causes bone marrow failure. In chronic leukaemia the malignant clone is able to differentiate, resulting in an accumulation of more mature cells.

Acute leukemias are malignant disorders of the haematopoietic stem cell with increase of proliferation and accumulation of predominantly immature, poorly differentiated cells (usually blast forms).

Acute leukemia occurs when a hematopoietic stem cell undergoes malignant transformation into a primitive, undifferentiated cell with abnormal longevity.

Symptoms have usually been present for only days to weeks before diagnosis. The most common:

Anemia – pallor, fatigue, tachycardia, chest pain

Infection as a result of granulocytopenia - fever, malaise, weight loss

Bleeding: petechiae, easy bruising, epistaxis, bleeding gums, or menstrual irregularity. Hematuria and GI bleeding are uncommon.

Bone marrow and periosteal infiltration may cause bone and joint pain, especially in children with ALL.

Initial CNS involvement or leukemic meningitis (manifesting as headaches, vomiting, irritability, cranial nerve palsies, seizures, and papilledema) is uncommon.

Extramedullary infiltration by leukemic cells may cause lymphadenopathy, splenomegaly, hepatomegaly, and leukemia cutis (a raised, nonpruritic rash).

Diagnosis - CBC and peripheral smear - pancytopenia and peripheral blasts, Blast cells in the peripheral smear may approach 90%, unless the WBC count is markedly decreased; Blast cells in the bone marrow are 20 - 95%.

Histochemical studies, cytogenetics, immunophenotyping, and molecular biology studies - Specific B-cell, T-cell, and myeloid-antigen monoclonal antibodies, together with flow cytometry, are very helpful in classifying ALL vs AML, which is critical for treatment.

Management of Leukaemias - The aim of treatment of Leukaemias is to destroy the leukaemic clone of cells without destroying the residual normal stem cell compartment from which repopulation of the haematopoietic tissues will occur.

There are three phases: Remission induction; Remission consolidation; Remission maintenance.

Although basic principles in treating ALL and AML are similar, the drug regimens differ. The complex nature of patients' clinical situations and the available treatment protocols necessitate an experienced team. Whenever possible, patients should be treated at specialized medical centers, particularly during critical phases (remission induction).

Specific therapy (Chemotherapy) - is generally aggressive, has a number of side effects, and may not be appropriate for the very elderly or patients with other serious disorders.

DRUGS COMMONLY USED IN THE TREATMENT OF ACUTE LEUKAEMIA

Phase	ALL	AML
Induction	Vincristine (i.v.)	Daunorubicin (i.v.)
	Prednisolone (oral)	Cytarabine (i.v.)
	L-asparaginase (i.m.)	Etoposide (i.v. and oral)
	Daunorubicin (i.v.)	
	Methotrexate (intrathecal)	
Consolidation	Daunorubicin (i.v.)	Cytarabine (i.v.)
	Cytarabine (i.v.)	Amsacrine (i.v.)
	Etoposide (i.v.)	Mitoxantrone (i.v.)
	Methotrexate (i.v.)	
Maintenance	Prednisolone (oral)	
	Vincristine (i.v.)	

	Mercaptopurine (oral)	
	Methotrexate (oral)	

Disease which relapses during treatment or soon after the end of treatment carries a poor prognosis and is difficult to treat. The longer after the end of treatment that relapse occurs, the more likely it is that further treatment will be effective.

Supportive therapy

Anaemia is treated with packed RBC transfusions (red cell concentrate infusions) to maintain Hb above 100 g/l.

Bleeding - Transfusions of platelets, RBCs, and granulocytes are administered as needed in patients with bleeding, anemia, and neutropenia, respectively.

Infection - Fever ($> 38^{\circ}\text{C}$) lasting over 1 hour in a neutropenic patient (absolute neutrophil count $< 1.0 \times 10^9/\text{l}$) indicates possible septicaemia. Granulocyte transfusions may help neutropenic patients with gram-negative or other serious sepsis but have no proven benefit as prophylaxis. Parenteral broad-spectrum antibiotic therapy is essential.

Metabolic problems Continuous monitoring of renal, hepatic and haemostatic function is necessary, together with fluid balance monitoring. Renal toxicity occurs with some antibiotics and antifungal agents. Cellular breakdown during induction therapy increases uric acid production, which may cause renal failure. Allopurinol and intravenous hydration are given to try to prevent this, along with close monitoring of biochemistry.

Chronic myelogenous leukemia (CML; chronic myeloid leukemia) (ICD-10 C92.1) – the hematopoiesis system clonal disorder, which develops from the pluripotent hematopoietic stem cell, is characterized by granulocytic leukocytosis, basophilia, thrombocytosis and splenomegaly. CML specific cytogenetic marker – Philadelphia chromosome (Ph-chromosome), it represents a balanced translocation involving the long arms of chromosomes 9 and 22, $t(9;22)$, produces the BCR-ABL chimeric gene, which encodes a protein p210 with the tyrosine kinase activity.

Etiology. Proven risk factor for CML is ionizing radiation. Excess morbidity occurs within 7-12 years after exposure with no significant differences in age groups. At risk of occurrence affect chemical agents, including professional factors (gasoline), drugs (cytostatics), hereditary tendency to instability of chromosomes or DNA repair system failure (Down, Patau, Klinefelter, Turner, Fanconi syndromes et al.), long-term smoking.

Pathogenesis. CML develops as a result of the Ph-chromosome formation, which is the product of the transfer of the chromosome 22 long arm's greater part on the long arm of chromosome 9 and a short terminal segment of the long arm of chromosome 9 – on the chromosome 22 long arm (reciprocal translocation). As a result, the long arm of chromosome 9 is increased in length and the long arm of chromosome 22 is shortened. This shortened long arm belonging to 22th pair is called Ph-chromosome. The protooncogene ABL resides on the long arm of chromosome 9, and it encodes the protein formation with molecular weight of 145 kDa ($p145^{\text{ABL}}$) – tyrosine protein kinase, which catalyzes the amino acids phosphorylation processes in the cell cycle. At (9, 22) translocation part of the ABL gene is fused with part of the BCR gene ($p160^{\text{BCR}}$) with the chimeric gene BCR-ABL formation on the chromosome 22, which generates a chimeric protein with a molecular mass 210 kDa – $r210^{\text{BCR-ABL}}$, which has much more powerful tyrosine kinase activity than its normal prototype $p145^{\text{ABL}}$. In such a way the cell predecessor proliferation increases, that is independent of growth factors (increased mitotic activity) with the following differentiation infringement, the adhesion of cell predecessor to stroma reduces (increase circulation cells predecessors with the extramedullary lesions formation), the apoptosis inhibition and cell genomic instability development take place.

According to CML pathogenesis the disease occurs in two phases:

- monoclonal (meets the chronic phase in clinic), benign;
- polyclonal (the acceleration phase and blast crisis in clinic), malignant.

Clinic and diagnostics. CML is often divided into three phases based on clinical characteristics: chronic phase, acceleration and blast crisis (terminal).

1. Chronic phase CML is characterized by a gradual increase in leukocytosis with a shift to myelocytes, metamyelocytes, promyelocytes and increase the number of platelets in the peripheral blood. For a long time CML is asymptomatic and can sometimes be found accidentally. During the detailed clinical manifestations the patients complain of general weakness, increased sweating, heaviness and pain in the left upper quadrant, weight loss, arising only after 1-3 years of onset. With the spleen size increasing the dyspeptic symptoms appear: discomfort, postprandial heaviness in the epigastric region of the abdomen. High leukocytosis and thrombocytosis lead to the hyperviscosity syndrome development with brain and vision dysfunction, spleen infarction, veinocclusive liver disease. An objective examination the skin paleness (in the presence of anemia), splenomegaly can be revealed.

Diagnostic criteria for chronic phase CML:

1) in hemogram – leukocytosis (15 to $800 \times 10^9/L$), the granulocytes percentage increase in the leukocyte formula up to 85-95%, possibly to blast cells (unfavorable prognostic sign), basophilic-eosinophilic association (basophils $<20\%$ and eosinophils $>5-8\%$); 30% of cases – mild normocytic normochromal anemia, 30% – thrombocytosis $400-800 \times 10^9/L$ or more, rarely – thrombocytopenia, which is caused by the treatment;

2) myelogram – hypercellular bone marrow with an increased number of young granulocytes (percentage of myeloblasts $<15\%$), the percentage of myeloblasts + myelocytes $<30\%$);

3) trepanobiopsy (microscopic examination of the bone marrow) – hypercellular bone marrow with myeloid hyperplasia, leuco-erythroblast ratio is more than 10:1, in 40-50% megakaryocytosis is detected;

4) cytogenetic and molecular genetic study – available Ph-chromosome $t(9, 22)(q34; q11)$ in 95-100% of metaphases and gene BCR-ABL;

5) absence of myeloid lesions in the other organs and tissues except the spleen and liver.

The chronic phase CML treatment is effective under conditions of adequate pharmacological therapy. The clinical and hematologic manifestations of the disease may be restrained for a long time.

2. The acceleration phase develops when the monoclonal stage transits into polyclonal, it characterized by decreased sensitivity to the previous specific therapy, even to full resistance. Diagnosed acceleration phase CML provided that one or more of the following symptoms, according to the ESMO recommendations (2008):

- Increasing the number of leukocytes, myelocytes, metamyelocytes, promyelocytes;
- 10-29% blast cells in the hemogram and / or myelogram;
- Progressive thrombocytosis (resistant to treatment), sometimes up to $1500-2000 \times 10^9/L$ or progressive thrombocytopenia $<100,0 \times 10^9/L$, doesn't caused by treatment;
- The basophils number in peripheral blood $> 20\%$;
- The growth of the tumor clone, according to cytogenetic and molecular genetic study.

Clinically, in the acceleration phase no specific symptoms are observed. The patient's general condition may remain satisfactory. In some cases, patients complain of increasing general weakness, body temperature, the spleen enlargement. In the later stages of acceleration phases there can be pain in bones and joints, increased susceptibility to recurrent infectious processes.

3. The blast crisis phase is the terminal stage of CML.

The blast crisis phase diagnostic is based on the following criteria:

- In hemogram and/or myelogram the blast cells number is above 20% of total nucleated cells number;
- Extramedullary proliferation of blast cells.

The blast crisis phase in peripheral blood is usually manifested by leukocytosis, increased basophils and eosinophils number, normochromal anemia, thrombocytopenia. In cytochemical, morphological, immunological studies of blast cells the blast crisis type is defined: in 50% of patients the myeloid variant is diagnosed, 25% – lymphoblastic, 25% – undifferentiated variant. The bone marrow fibrosis presence is diagnosed in 50% of patients.

Clinically in CML blast crisis phase the *tumor intoxication syndrome* is observed – severe weakness, decrease in working capacity, intermittent fever to $38-39^{\circ}C$, fever, heavy sweats, significant

weight loss; the *tumor proliferation syndrome* – bones and joints pain, heaviness and pain in the epigastric region, the left and right upper quadrant of the abdomen, hepatomegaly (liver extends at 15-20 cm below the costal arch), splenomegaly (spleen much enlarged, firm, sometimes occupies the whole left half of the abdomen), enlarged peripheral and mediastinal lymph nodes; *anemic syndrome* – skin paleness; *hemorrhagic syndrome* – petechiae, bruising, hemorrhage, bleeding.

Therapeutic tactics. According to the contemporary viewpoint, the first-line therapy in newly diagnosed CML is a tyrosine kinase inhibitor of the 1st generation – imatinib (Gleevec) 400 mg daily per os, which is permanently assigned to as long as the patient is sensitive to the drug. Imatinib represents targeted therapy and in 96% of patients with CML achieved a complete hematological response. In the context of insensitivity to imatinib at standard dosage it is necessary to raise the dose up to 600-800 mg per day. If no effect the prescriptions of tyrosine kinase inhibitors of the 2nd generation (dasatinib, nilotinib) can be considered. The hydroxycarbamide (hydroxyurea), anagrelid or interferon assignment as the first-line therapy should be used in elderly patients and patients who have contraindications for the imatinib treatment.

In the blast crisis phase patients taking imatinib should increase its dose up to 600-800 mg per day. With the ability the 2nd generation of tyrosine kinase inhibitors is prescribed or the transplantation is recommended. The treatment of blast crisis is held by PCT assignment as needed depending on the blasts variant (myeloblastic or lymphoblastic).

Prognosis. CML belongs to chronic diseases; in case of the application of modern treatment methods the recovery is possible; in blast crisis phase – unfavorable prognosis.

Tests for the determining of basis knowledge

Tests of the 2 level.

1. The most common symptoms of Acute leukemias are:
 - A. fever, malaise, weight loss
 - B. petechiae, easy bruising, epistaxis,
 - C. pallor, fatigue, tachycardia
 - D. all of above
2. Symptoms of bone marrow failure in patients with Acute leukemias include all, except:
 - A. bone pain
 - B. multiple ecchymoses
 - C. fatigue
 - D. fever
3. Leukostasis is:
 - A. leukemic gap
 - B. palpable lymphadenopathy
 - C. respiratory distress and altered mental status due to markedly elevated WBC counts
 - D. gingivitis due to neutropenia with swollen gums

Real-life situations to be solved:

1) Patient M., 44 years, female, has complaints of general weakness, fatigue, flicker "flies" before her eyes, fever up to 38.4°C, sore throat, presence of ulcers on the mucosa of the mouth, pain in the ribs and sternum. The skin is pale, with the presence of petechiae and bruises. Pulse 107/min. Hepato- and splenomegaly. In the mucosa of the mouth and throat ulcers with necrotic many edges. In a blood test: RBC $-2,3 \times 10^{12}/L$, HGB - 72 g/L, CI - 0.89, WBC - $26,0 \times 10^9/L$, blasts - 66%, segments - 6%, lymphocytes - 24%, monocytes - 4%, PLT $19,0 \times 10^9/L$, ESR - 68 mm/h. What diagnosis do you think is most likely?

2) Woman P., 45 years old, came to the doctor with complaints of general weakness, pain in the throat when swallowing, fever up to 39.2°C. Acutely ill a week ago. Objectively: pale skin color. Single bruises on the thighs. Gingival hyperplasia. Necrotic changes on the tonsils. Sternalhiya. The liver is not enlarged. The spleen is +3 cm from the edge of the costal arch. In the blood: RBC $-2,1 \times 10^{12}/L$,

HGB 76 g/L, platelets $48 \times 10^9/L$, WBC - $0,7 \times 10^9/L$, bands -1%, segments - 38%, lymphocytes -53%, monocytes 8%. ESR 64 mm /h. In myelogram - 25% blast cells. Your diagnosis?

3) Patient K., 45 years, during the year noticed weakness, sweating, heaviness in the upper abdomen. The examination revealed hepato- and splenomegaly. In the CBC: WBC - $51,0 \times 10^9/L$, basophils -2%, eosinophils - 7%, myeloblasts - 3%, promyelocytes - 5%, myelocytes - 9%, metamyelocytes - 4%, bands - 6 %, segments - 49%, lymphocytes - 11%, monocytes - 4%. Cytogenetic test of the bone marrow found Philadelphia chromosome. What is the most likely diagnosis?

Recommended literature for students:

A. Main:

1. Davidson's Principles and practice of medicine (21st revised ed.) / by Colledge N.R., Walker B.R., and Ralston S.H., eds. – Churchill Livingstone, 2010. – 1376 p.
2. Harrison's Principles of Internal Medicine, 19th Edition / by Longo D.L., Kasper D.L., Jameson J.L. et al. (eds.). – McGraw-Hill Professional, 2015. – 4012 p.
3. The Merck Manual of Diagnosis and Therapy (nineteenth Edition)/ Robert Berkow, Andrew J. Fletcher and others. – published by Merck Research Laboratories, 2011.

B. Additional:

1. H.Löffler, J.Rastetter, T.Haferlach. 6th edition. Atlas of clinical hematology. Springer Berlin Heidelberg New York, 2006, - 429 p.
2. Chronic Lymphocytic Leukemia / edited by Susan O'Brien, John G. Gribben. – CRC Press, 2008. – 312 p.
3. Chronic Myeloid Leukemia: New Insights for the Healthcare Professional: 2013 Edition / by Q. Ashton Acton. – ScholarlyEditions, 2013. – 124 p.

Composed by

as. Lymanets T.V.