**Ministry of Healthcare of Ukraine**

Poltava State Medical University

**«Approved»**
at the meeting of the
Chair of Internal medicine №1
Head of the Chair
_____ G.S. Maslova
(signature) (PhD, MD., Assoc.Prof.)

# Methodical instruction for the students self-training during preparing for practical classes (seminars)

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| ***Subject*** | **Internal medicine** |
| ***Module №1*** | **FUNDAMENTALS OF INTERNAL MEDICINE** |
| ***Topic*** | **Modern methods of examination in hematology** |
| ***Course*** | **IV** |
| ***Faculty*** | **Medical** |

***The subject of the lesson*: Modern methods of examination in hematology**

***Educational goal:*** to develop skills and to acquire experience relevant to manage patients with hematological diseases.

**Professional orientation of students:** Abnormalities detected in the blood can be caused ether by primary hemopoietic disorders or by diseases affecting other system of the body.

***The student must know:***

1. Normal complete blood count (CBC), normal hematopoiesis in the bone marrow.
2. The main clinical symptoms and signs of blood cells abnormalities.
3. Standard methods of peripheral blood and bone marrow examination.
4. Modern methods of blood cells (and bone marrow cells) examination: immunophenotyping by flow cytometry, cytogenetic by karyotyping, moleculargenetic tests by FISH or polymerase chain reaction (PCR), cytochemistry.
5. Histological bone marrow (BM) or lymph node examination and immunohisto-chemistry.
6. Instrumental methods of hepatosplenomegaly and lymphadenopathy evaluation.

***The student must be able:***

1. To choose the symptoms of anemic, hemorrhagic, tumor growth, tumor intoxication syndromes from the history data.
2. To assess the complete blood count (CBC) results and the blood smear.
3. To assess the flow cytometry results.
4. To assess the BM aspiration and biopsy results.
5. To assess the genetic tests (FISH, PCR, karyotyping) results.
6. To assess the computer tomography (oncological screening) and MRI results.

***The main questions of the topic:***

1. Blood composition and function.
2. Identification of the main clinical syndromes in hematology.
3. Basic laboratory (general clinical, biochemical, immunoassay, cytological) research methods and clinical hematological syndromes.
4. Indications, contraindications and complications of instrumental (ultrasonographic, radiological) methods of examination in hematology.
5. Structure and function of the bone marrow.
6. Bone smear examination. Myelogram count, bone marrow indexes and their evaluation.

## Contents of the training materials

*Hematopoiesis* is the process by which the formed elements of blood are produced. The process is regulated through a series of steps beginning with the hematopoietic stem cell. Stem cells are capable of producing red cells, all classes of granulocytes, monocytes, platelets, and the cells of the immune system.

## PERIPHERAL BLOOD EXAMINATION

The examination of a peripheral blood smear is one of the most informative exercises a physician can perform. Although advances in automated technology have made the examination of a peripheral blood smear by a physician seem less important, the technology is not a completely satisfactory replacement for a blood smear interpretation by a trained medical professional who also knows the patient's clinical history, family history, social history, and physical findings. It is useful to ask the laboratory to generate a Wright's-stained peripheral blood smear and examine it.

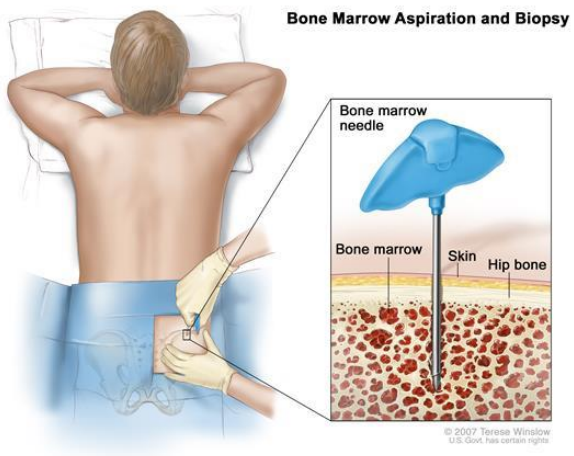
The best place to examine blood cell morphology is the feathered edge of the blood smear where red cells lie in a single layer, side by side, just barely touching one another but not overlapping. The author's approach is to look at the smallest cellular elements, the platelets, first and work his way up in size to red cells and then white cells. Using an oil immersion lens that magnifies the cells 100-fold, one counts the platelets in five to six fields, averages the number per field, and multiplies by 20,000 to get a rough estimate of the platelet count. The platelets are usually 1–2 μm in diameter and have a blue granulated appearance. There is usually 1 platelet for every 20 or so red cells. Of course, the automated counter is much more accurate, but gross disparities between the automated and manual counts should be assessed.

Next one examines the red blood cells. One can gauge their size by comparing the red cell to the nucleus of a small lymphocyte. Both are normally about 8 μm wide. Red cells that are smaller than the small lymphocyte nucleus may be microcytic; those larger than the small lymphocyte nucleus may be macrocytic. Macrocytic cells also tend to be more oval than spherical in shape and are sometimes called macroovalocytes.

Finally, one examines the white blood cells. Three types of granulocytes are usually present: neutrophils, eosinophils, and basophils, in decreasing frequency. Neutrophils are generally the most abundant white cell. They are round, are 10–14 μm wide, and contain a lobulated nucleus with two to five lobes connected by a thin chromatin thread. Bands are immature neutrophils that have not completed nuclear condensation and have a U-shaped nucleus. Bands reflect a left shift in neutrophil maturation in an effort to make more cells more rapidly.

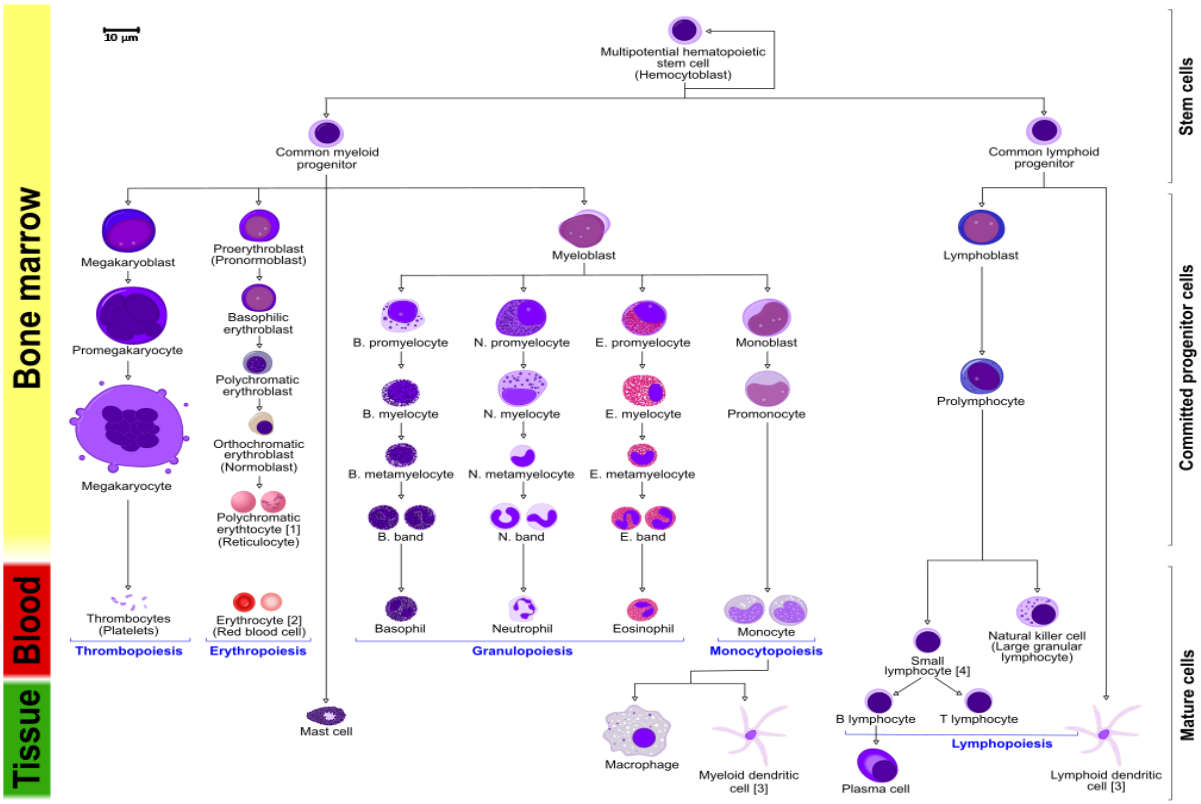
## BONE MARROW EXAMINATION

Bone marrow samples can be obtained by aspiration and trephine biopsy. Sometimes, a bone marrow examination will include both an aspirate and a biopsy. The aspirate yields semi-liquid bone marrow, which can be examined by a pathologist under a light microscope and analyzed by flow cytometry, chromosome analysis, or polymerase chain reaction (PCR). Frequently, a trephine biopsy is also obtained, which yields a narrow, cylindrically shaped solid piece of bone



marrow, 2 mm wide and 2 cm long (80 μL), which is examined microscopically (sometimes with the aid of immunohistochemistry) for cellularity and infiltrative processes. Trephine biopsy (BM biopsy) with immunohistochemistry is mandatory for aplastic anemia, myeloprolipherative Ph-negative diseases diagnostic and BM involvement in lymphoma patients assessment.

**Hematopoiesis**




**FLOW CYTOMETRY**

Flow cytometry provides a well-established method to identify cells in solution and is most commonly used for evaluating peripheral blood, bone marrow, and other body fluids. Flow cytometry studies are used to identify and quantify the cells of the immune system and to characterize hematological malignancies.

Fluorescently-tagged antibodies that target specific antigens on the cell surface can be used to identify and segregate various sub-populations. The basic steps include passing the cells through a narrow channel, such that each cell is illuminated by a laser one at a time. A series of sensors then detect the refracted or emitted light, and this data is integrated and compiled to generate information about the sample.

The B-cell lymphoproliferative disorders often have specific antigen patterns. The use of a wide range of antibodies allows clinicians to make specific diagnoses based on patterns of antigen expression. Not only is the presence or absence of antigens useful in making specific diagnoses, the strength of antigen expression can also aid in diagnosis. One example is the weak expression of CD20 and lymphocytic leukemia. Flow cytometry is particularly good at identifying clonality in B-cell populations. Although T-cell neoplasms may exhibit a predominance of antigens CD4

or CD8, these antigens should not be considered as surrogate markers of clonality. The use of antibodies to the T-cell receptor family may occasionally be helpful in a small percentage of cases; however, many reactive processes can show expansion of particular T-cell receptor clones. Antigen deletions are common in T-cell lymphomas and may suggest neoplasia, but the only way to definitively diagnose T-cell clonality is by molecular methods.

Flow cytometry is not recommended for the diagnosis of Hodgkin lymphoma, chronic myelogenous leukemia, or myelodysplastic syndrome, although disease progression in the latter two conditions can often be monitored.

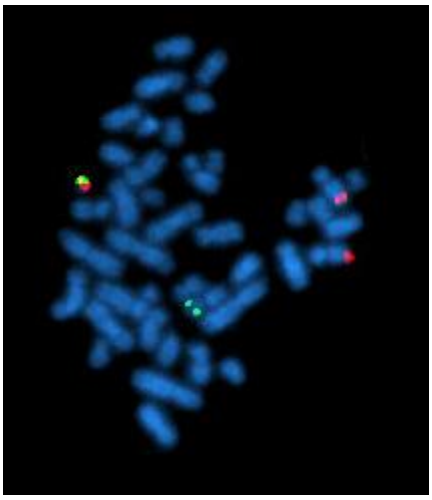
**Common phenotypes of B-cell lymphoproliferative disorders according to flow cytometry**

| Diagnosis | CD5 | CD10 | CD19 | CD20 | CD23 | CD79b | FMC-7 | CD25 | CD11c | CD103 |
|---|---|---|---|---|---|---|---|---|---|---|
| SLL/CLL | + | - | + | + (w) | + | - | - | -/+ | +/- | - |
| Mantle cell lymphoma | + | - | + | + | - | + | + | - | - | - |
| Follicle cell lymphoma | - | + | + | + | -/+ | +/- | +/- | - | - | - |
| Marginal zone lymphoma | - | - | + | + | - | +/- | +/- | -/+ | + | - |
| Hairy cell leukemia | - | - | + | + | - | +/- | +/- | +/- | + | + |

## CYTOGENETIC TESTS

***Karyotyping*** involves analysis of the entire chromosome complement through the microscope. Dividing cells are harvested during metaphase, the time of greatest chromosome condensation, by disruption of the spindle using drugs such as colchicine. Chromosomes are visualized by staining, including the use of special stains to elicit banding patterns.

***Fluorescence in situ hybridization*** (FISH) is widely used for the localization of genes and specific genomic regions on target chromosomes, both in metaphase and interphase cells. The applications of FISH are not limited to gene mapping or the study of genetic rearrangements in human diseases. Indeed, FISH is increasingly used to explore the genome organization in various organisms and extends to the study of animal and plant biology. We have described the principles and basic methodology of FISH to be applied to the study of metaphase and interphase chromosomes. A metaphase cell positive for the bcr/abl rearrangement using FISH to diagnose chronic myeloid leukemia (see picture).



**LYMPHADENOPATHY.** A variety of imaging techniques (computed tomography (CT), magnetic resonance imaging (MRI), ultrasound, color Doppler

ultrasonography) have been used to differentiate benign from malignant lymph nodes. CT and MRI are comparably accurate (65–90%) in the diagnosis of metastases to cervical lymph nodes. Ultrasonography has been used to determine the long axis, short axis, and a ratio of long to short (L/S) axis in cervical nodes. An L/S ratio of <2.0 has a sensitivity and a specificity of 95% for distinguishing benign and malignant nodes. The indications for lymph node biopsy are imprecise, yet it is a valuable diagnostic tool. The decision to biopsy may be made early in a patient's evaluation or delayed for up to 2 weeks. Prompt biopsy should occur if the patient's history and physical findings suggest a malignancy (Hodgkin or non-Hodgkin lymphoma diagnosis – mandatory); examples include a solitary, hard, nontender cervical node in an older patient who is a chronic user of tobacco; supraclavicular adenopathy; and solitary or generalized adenopathy that is firm, movable, and suggestive of lymphoma.

There are different types of biopsy, including:

- an excision biopsy, which removes a whole lymph node (preferable for Hodgkin or non-Hodgkin lymphoma diagnosis)
- an incisional biopsy, which removes part of a lymph node
- a core needle biopsy, which takes a small sample of a lymph node; this type of biopsy is also known as a 'core biopsy' or a 'needle biopsy'
- a laparoscopic (keyhole) biopsy, which removes all or part of a lymph node.

**Test evaluation and situational tasks.**

Choose the correct answer/statement:

1. The most informative examination for aplastic anemia diagnosis:
   A. Complete blood count
   B. Flow cytometry
   C. BM biopsy with immunohistochemistry
   D. BM aspiration
   E. Physical examination
2. Preferable examination for lymphoma diagnostic is:
   A. BM aspiration
   B. Blood smear examination
   C. Excision lymph node biopsy with immunohistochemistry
   D. Physical examination
   E. Core needle lymph node biopsy
3. Most sensitive and specific test for diagnosis of iron deficiency is:
   A. Serum ferritin level
   B. Serum iron level
   C. Serum transferrin receptor population
   D. Transferrin saturation
   E. Hb, Ht
4. Laboratory finding in autoimmune haemolytic anemia is
   A. Positive direct Coombs test
   B. Serum iron decreased

C. Ferritin decreased
D. Hypoalbulinemia
E. Macrocytic anemia

5. Reduced serum iron and ferritin level is seen in:
   A. Sideroblastic anemia
   B. Thalassemia
   C. Anemia of chronic disease
   D. Iron deficiency anemia
   E. Hemolytic anemia
6. The surface CD cell markers can be found by:
   A. Direct Coomb’s test
   B. Flow cytometry
   C. BM aspiration
   D. Polymerase chain reaction
   E. Karyotyping
7. Spherocytosis is best diagnosed by:
   A. Splenic puncture
   B. BM aspiration
   C. Plasma
   D. Peripheral blood smear
   E. Phenotyping
8. Direct Coomb's test is used to diagnose:
   A. Autoimmune haemolytic anemia
   B. Thrombotic thrombocytopenic purpura
   C. Haemophilia
   D. Aplastic anemia
   E. Idiopathic thrombocytopenic purpura

## Recommended literature:

**I. Main:**

1. Internal Medicine: in 2 books. Book 1. Diseases of the Cardiovascular and Respiratory Systems: textbook / N.M. Seredyuk, I.P. Vakaliuk, R.I. Yatsyshyn et al. Київ, Медицина., 2019. - 664 + 48 кольор. вкл.).
2. Internal medicine: Part 1 (cardiology, rheumatology, haematology): textbook for English-speaking students of higher medical schools / edited by Professor M.A. Stanislavchuk and Professor V.A. Serkova. - Vinnytsia: Nova Knyha, 2019. - 392 p.

3. Медицина за Девідсоном: принципи і практика / Навчальний посібник: пер. 23-го англ. вид.: у3 т. Т.3 С. Ралстона, Я. Пенмана, М. Стрекена, Р. Гобсона; К.: ВСВ «Медицина», 2021. – 642 с.
4. CURRENT Medical Diagnosis and Treatment 2012, Fifty-First Edition (LANGE CURRENT Series) by Stephen McPhee, Maxine Papadakis and Michael W. Rabow (Paperback - Sep 12, 2011)/
5. Побічнадіяліків – SideEffectsofMedications: навчальнийпосібнику 2 т. / зазаг.ред. В.М. Бобирьова, М.М. Потяженка. – Вінниця:
6. Cardiovascular diseases. Classification, standards of diagnosis and treatment / Edited by Academician Kovalenko V.M., Prof. Lutaia M.I., Prof. Sirenko Yu.M., Prof. Sychova O.S. – Kyiv. – 2020.
7. Perederii V.H., Tkach S.M. Principles of internal medicine. – Vol.2 / Textbook for students of higher educational institutions. – Vinnytsia: Nova knyha. – 2018.
8. Internal diseases. The textbook based on the principles of evidentiary medicine, 2018.

**II. Additional literature:**

1. Recommendations of the Association of Cardiologists of Ukraine for the diagnosis and treatment of chronic heart failure / Voronkov L.H. – moderator, working group of the Ukrainian Association of Heart Failure Specialists. – 2017.
2. Respiratory diseases / Ghanei M. - In Tech, 2012. - 242 p.
3. Clinical respiratory medicine / Spiro S., Silvestri G., Agusti A. - Saunders, 2012. - 1000 p.
4. Principles and practice of interventional pulmonology / Ernst A., Herth F. -Springer, 2012. - 757 p.
5. Clinical respiratory medicine / Spiro S., Silvestri G., Agusti A. - Saunders, 2012. - 1000 p.
6. Petrov Y. The chief symptoms and syndromes in patients with cardiovascular pathology : The practical handbook fur medical students / Ye.

Petrov, Yu. Goldenberg, N. Chekalina; UMSA. - Poltava : TexcepBic, 2010. - 143 .

7. Gastroenterology and Hepatology Board Review: Pearls of Wisdom, Third Edition (Pearls of Wisdom Medicine) by John K. DiBaise (May 11, 2012)
8. Clinical Pulmonology 2012 (The Clinical Medicine Series) by M.D., C. G. Weber (Oct 30, 2011) - Kindle eBook
9. Clinical Nephrology 2012 (The Clinical Medicine Series) by M.D., C. G. Weber (Sep 19, 2011) - Kindle eBook
10. Clinical Nephrology 2012 (The Clinical Medicine Series) by M.D., C. G. Weber (Sep 19, 2011) - Kindle eBook
11. Hematology: Clinical Principles and Applications, 4e by Bernadette F. Rodak MS MLS (Feb 18, 2017)
12. Rheumatology, 2-Volume Set: EXPERT CONSULT - ENHANCED ONLINE FEATURES AND PRINT, 5e by Marc C. Hochberg MD MPH, Alan J. Silman MD, Josef S. Smolen MD and Michael E. Weinblatt MD (Oct 19, 2019)
13. Endocrine Pathology: Differential Diagnosis and Molecular Advances by Ricardo V. Lloyd (Nov 5, 2018)
14. Clinical Endocrinology 2012 (The Clinical Medicine Series) by M.D., C. G. Weber (Sep 19, 2017) - Kindle eBook
15. Williams Textbook of Endocrinology: Expert Consult-Online and Print, 12e by Shlomo Melmed, Kenneth S. Polonsky MD, P. Reed MD Larsen and Henry M. Kronenberg MD (May 27, 2016)
16. Electrocardiography, 3e with Student CD (Booth, Electrocardiography for Health Care Personnel) by Kathryn A. Booth (Jan 27, 2017)
17. Echocardiography Review Guide: Companion to the Textbook of Clinical Echocardiography: Expert Consult: Online and Print, 2e (Expert Consult Title: Online + Print) by Catherine M. Otto (Mar 7, 2017).

**Composed by** **Lymanets T.V.**