The diagnostic methods in hematology

Poltava State Medical University
The Internal Medicine №1 Department

Plan of the lecture

I. Hematopoiesis

1. Hematopoietic organs

II. General Outlines. The main syndromes

1. Anemic

2. Hemorrhagic

3. Hyperplastic syndrome

4. Ulcerative-necrotic syndrome

5. Lymphadenopathy

III. Laboratory diagnostics

1. General blood analysis

2. Blood chemistry

3. Spernal puncture + counting myelogram

4. Morphological examination of bone marrow cells

5. Cytochemical study of bone marrow

6. Immunological examination of bone marrow and peripheral blood cells

7. Cytogenetic examination

8. Molecular study

9. Biopsy of target lymph node + immunohistochemical examination

I. Hematopoiesis

The volume of blood in adults is 5-5.5 liters - 1/13 of weight; 55-60% - plasma; 40-45% - cellular elements

The source of hematopoiesis - PSC - properties:

- Polypotency - ability to multilinear differentiation
- Ability to self-maintain - consistency of the pool

Hemopoiesis is a process that consists of successive divisions and differentiations, resulting in the formation of mature blood cells:

Erythrocytes - transfer of oxygen

Platelets - participation in hemostasis

Basophilic, eosinophilic, neutrophilic granulocytes, monocytes / macrophages - cells of natural nonspecific immunity

B- and T-lymphocytes are cells of humoral and cellular immunity

Central hematopoietic organs

***Bone marrow*: ripening of myelopoiesis elements,**

I stage of maturation of B-lymphocytes

- in flat bones (skull, sternum, ribs, vertebrae, pelvic bones)

- in the tubular bones epiphyses (hip, shoulder, shin)

***Thymus* - T-lymphocytes maturation**

Peripheral hematopoietic organs

Spleen

Lymphatic nodes

Mucosal associated lymphoid tissue (MALT)

Peripheral blood (PSC)

Diseases of Blood and Blood forming organs

A - Benign Hematology

- **1- Bleeding Disorders**
- **2- Thrombophilia**
- **3- Anemia**
- **4- WBC disorders/non-malignant**
- **5- BM disorders/ non-malignant (AA)**
- **6- Secondary to other diseases (reactive, pancytopenia… …etc)**

Diseases of Blood and Blood forming organs

B - Malignant Hematology

- **1- Leukemias: acute/chronic**
- **2- Lymphomas: NHL/ HL**
- **3- Plasma cell descresias: MM**
- **4- Myeloproliferative neoplasms (MPN)**

II. General Outlines

* **Symptoms and signs may not distinguish between various etiologies and syndromes**
* **Severity of symptoms may vary according to many factors**
* **Symptoms and signs may be part of the syndrome causing a hematological disease**
* **Symptoms and signs may overlap with non-hematological disease**

Signs in Hematology

* **Frequently non-specific**
* **May be characteristic**
* **Combination of the abnormalities causing the symptoms**
* **May be very apparent in advanced disease or very subtle in early disease**
* **Careful examination is needed**
* **Changing signs require caution and repeated examination**

Clinical examination of patients

The main syndromes of the hematopoiesis system diseases :

- Anemic

- Hemorrhagic

- Intoxicating

- Tumor proliferation syndrome

- Infectious complications syndrome

Features of complaints in hematological diseases

Common symptoms:

Unmotivated general weakness

Dyspnea with physical exertion

Headache

Anemic syndrome:

General weakness

Dizziness, headache

Tinnitus (noise at ears)

Dyspnea with usual physical exertion

II.1. Anemic syndrome - clinical manifestations depend on:

1. **Degree of blood oxygen saturation ↓ - degree of hypoxia**
2. **Degree of total blood volume ↓**
3. **The time of development of the first two factors**
4. **Manifestations of the underlying disease**
5. **The ability of CVS and RS to compensate anemia**

Symptoms characteristic of different anemia types

* **Dry skin, brittle nails, hair loss, perversion of taste (desire is chalk) - *sideropenic syndrome* - (IDA)**
* **Yellowing of the skin, icteric sclera, darkening of urine - *hemolytic syndrome* - (B12 deficiency anemia, hemolytic anemia)**
* ***Neurological Syndrome:***

  Paresthesia, disorder of superficial sensitivity, sometimes pareses, memories ↓ - B12 deficiency anemia
* **GI tract disorders: depends on the severity of the lesion**

Complaints

Hemorrhagic syndrome:

Bleeding gums,

Hemorrhages on the skin

Bleeding of different localizations

Intoxication syndrome(hemoblastosis, hemolytic crises, aplastic anemia):

↓ apetit, weight loss

↑ temperature

Infectious complications syndrome

↑ temperature caused by infection

Manifestations of angina, otitis, bronchitis, pneumonia

Complaints

- Ulcerative-necrotic syndrome

- Tumor proliferation syndrome (leukemia acute, chronic)

↑ lymph nodes,

↑ spleen, liver - heaviness in the right and left hypochondrium

symptoms of internal organs compression (ERW)

rashes of a purple color - leukemids

ossalgia - pain in long tubular bones, sternum

- Tumor lysis syndrome

↑ temperature,

↓ blood pressure

artralgia (secondary gout)

Kidney function disorder (ARF)

Anamnestic data:

- **Duration of symptoms**
- **Symptoms set**
- **The nature of the disease is hereditary, acquired**
- **Features of food - vegetarianism**
- **Occupational hazards - the effect of ionizing radiation, high frequency currents, chemicals**
- **Presence of oncological diseases in the anamnesis - cytostatics treatment**
- **Use of medicines - NSAIDs, glucocorticoids, mercazolyl, etc.**

Concomitent diseases

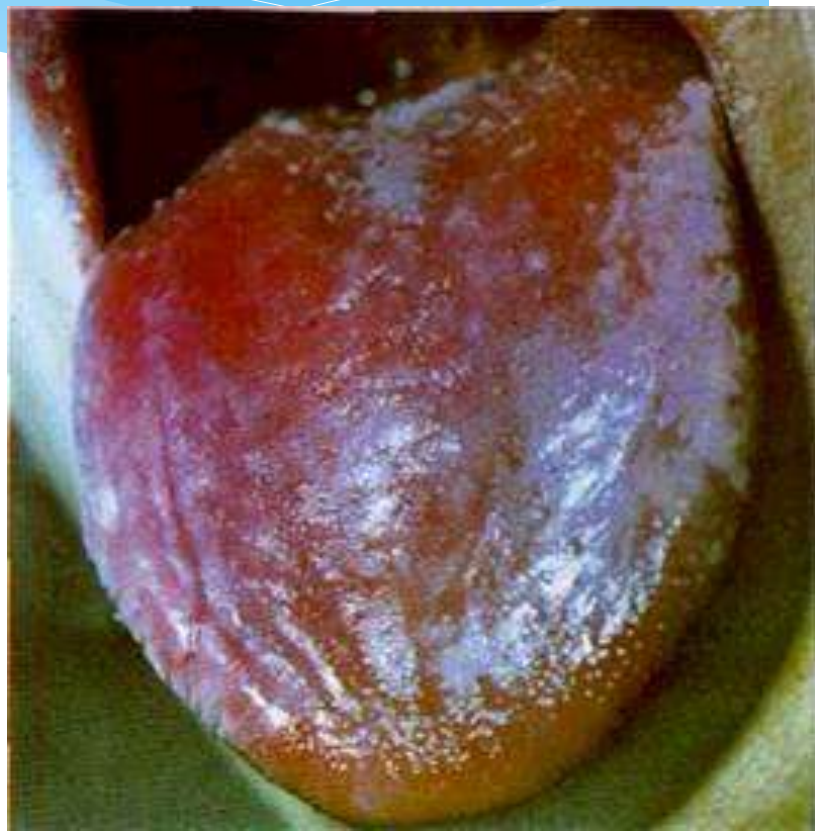
Chronic diseases of internal organs:
liver,
kidney,
diffuse connective tissue diseases
Gynecological diseases - with IDA:
duration of the menstrual cycle
duration of menstruation
number of pregnancies and births
the presence of anemia in pregnancy
presence of fibromyoma, endometriosis, hyperplasia of the uterus, malignant neoplasms

Anamnestic data

- **Gastrointestinal lesions - latent bleeding**
- **Bleeding from varicose veins of the esophagus, neoplasms of the esophagus**
- **Stomach ulcers and duodenal ulcers, anacid gastritis, neoplasm of the stomach**
- **Resection of the stomach**
- **NU**
- **Polyps of the large intestine**
- **Bleeding from the hemorrhoidal nodes**
- **Malignant colon formation**

Objective examination

Pale skin
Paleness of mucus membrans
Yellowness of the skin
“Blue sclera" symptom
"Geographical tongue"
"Lacquered tongue "



II.2. Manifestations of hemorrhagic syndrome:

Disorders of platelet hemostasis:

Bruises

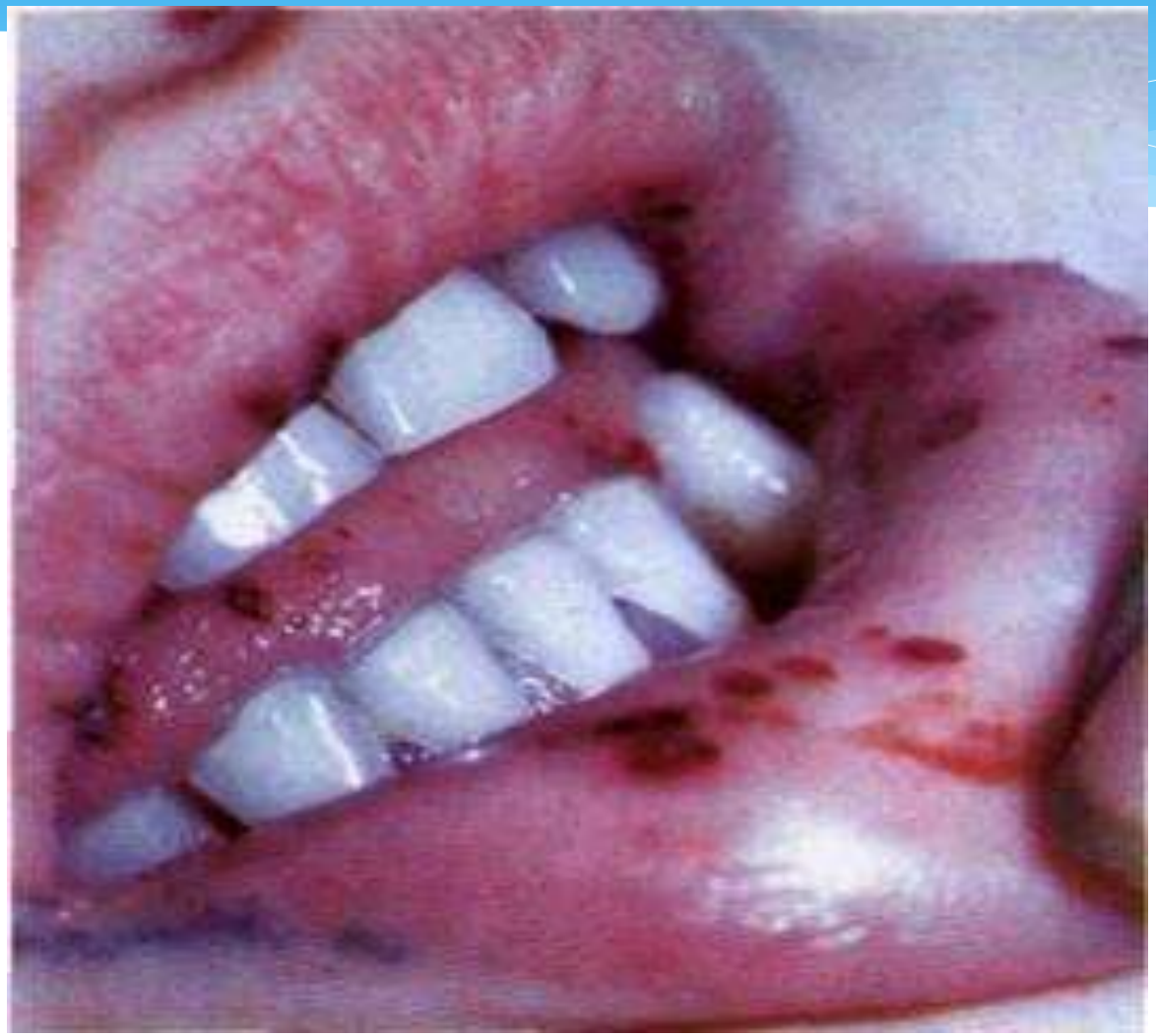
Fine petechial rash

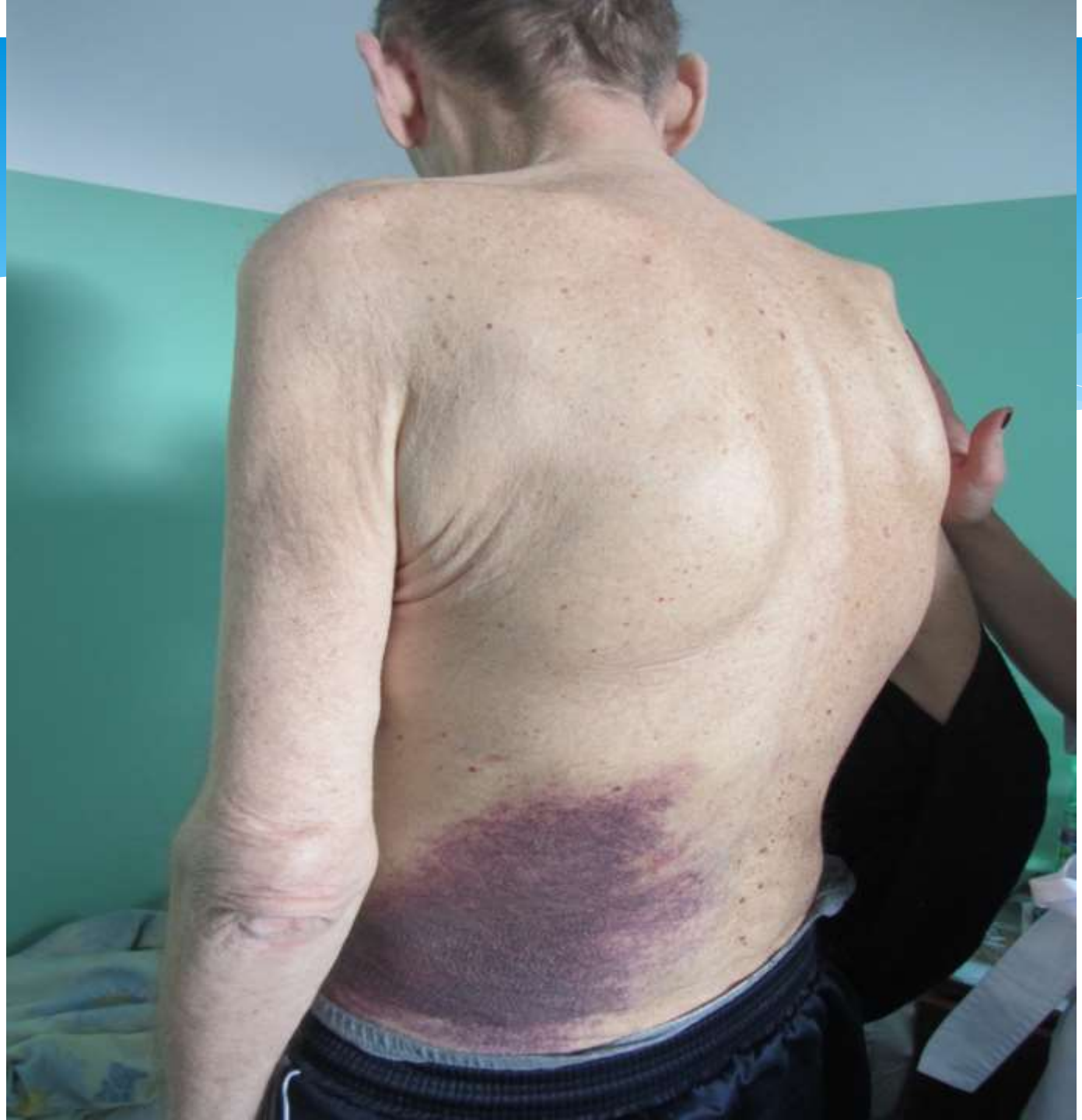
Disorders of the coagulation hemostasis:

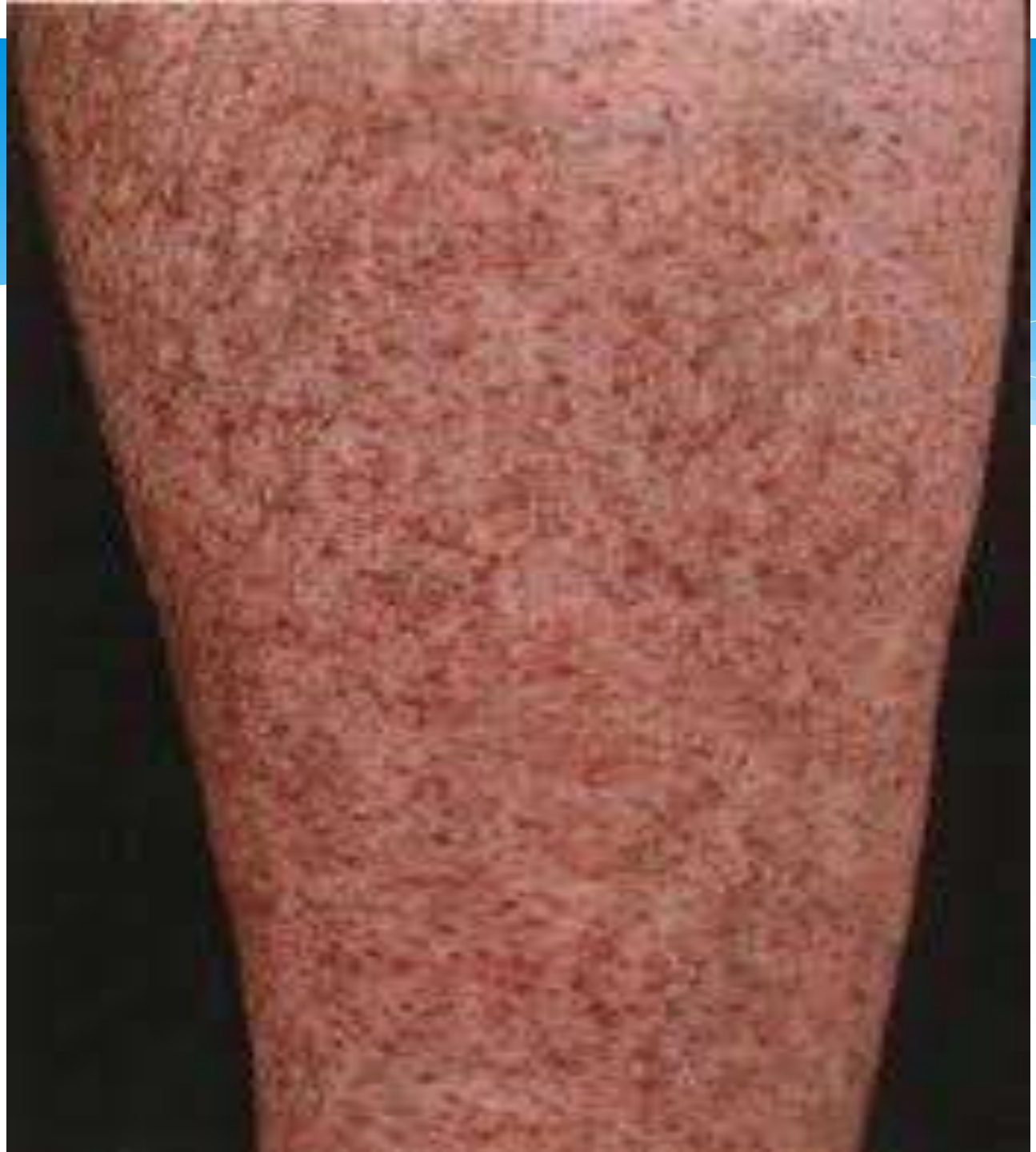
Hematomas

Hemorrhages in large joints (hemarthrosis)

Hemorrhagic syndrome with AL







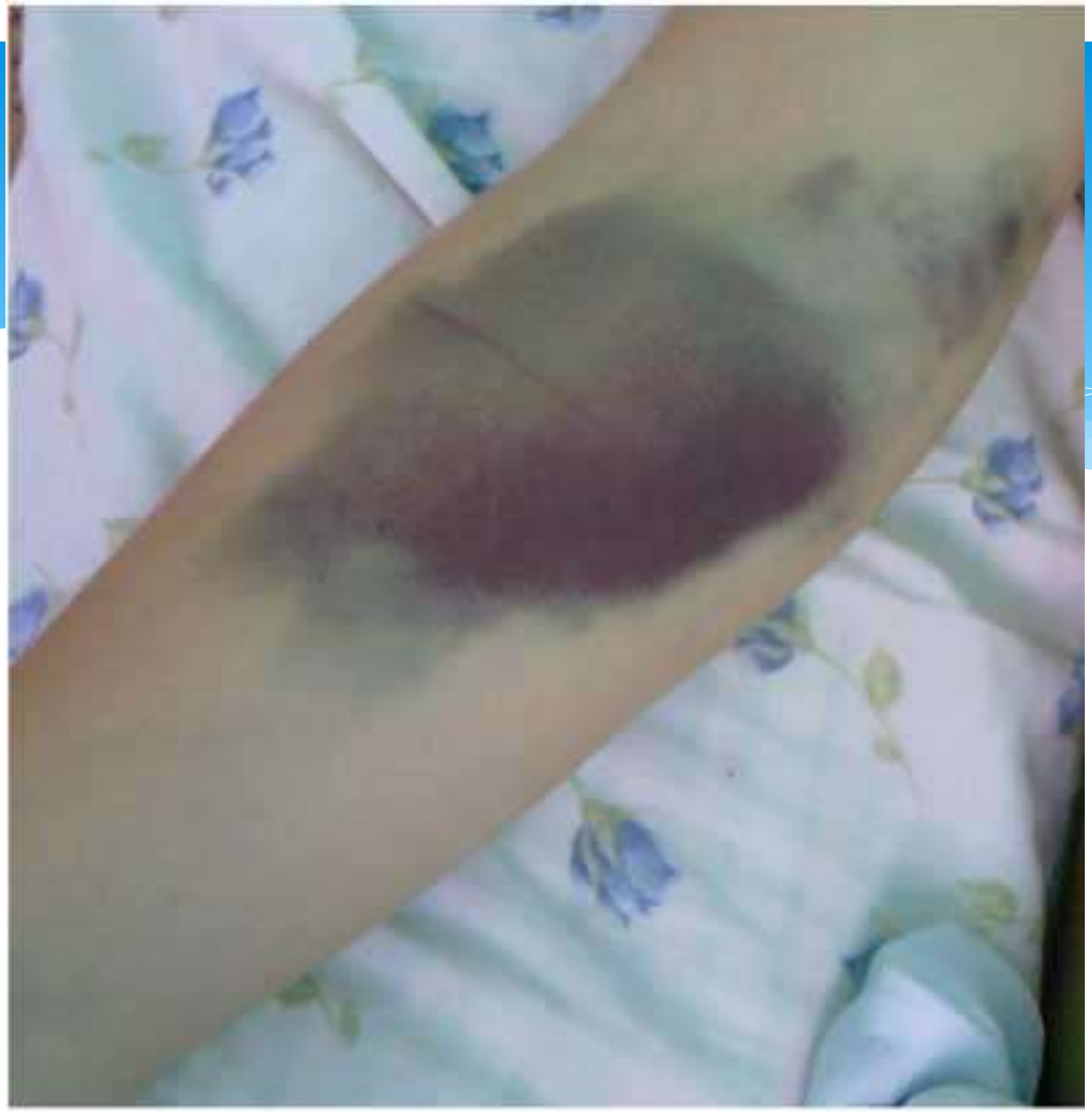


Рисунок 2. Постинъекционные экхимозы у пациента с тромбоцитопенией

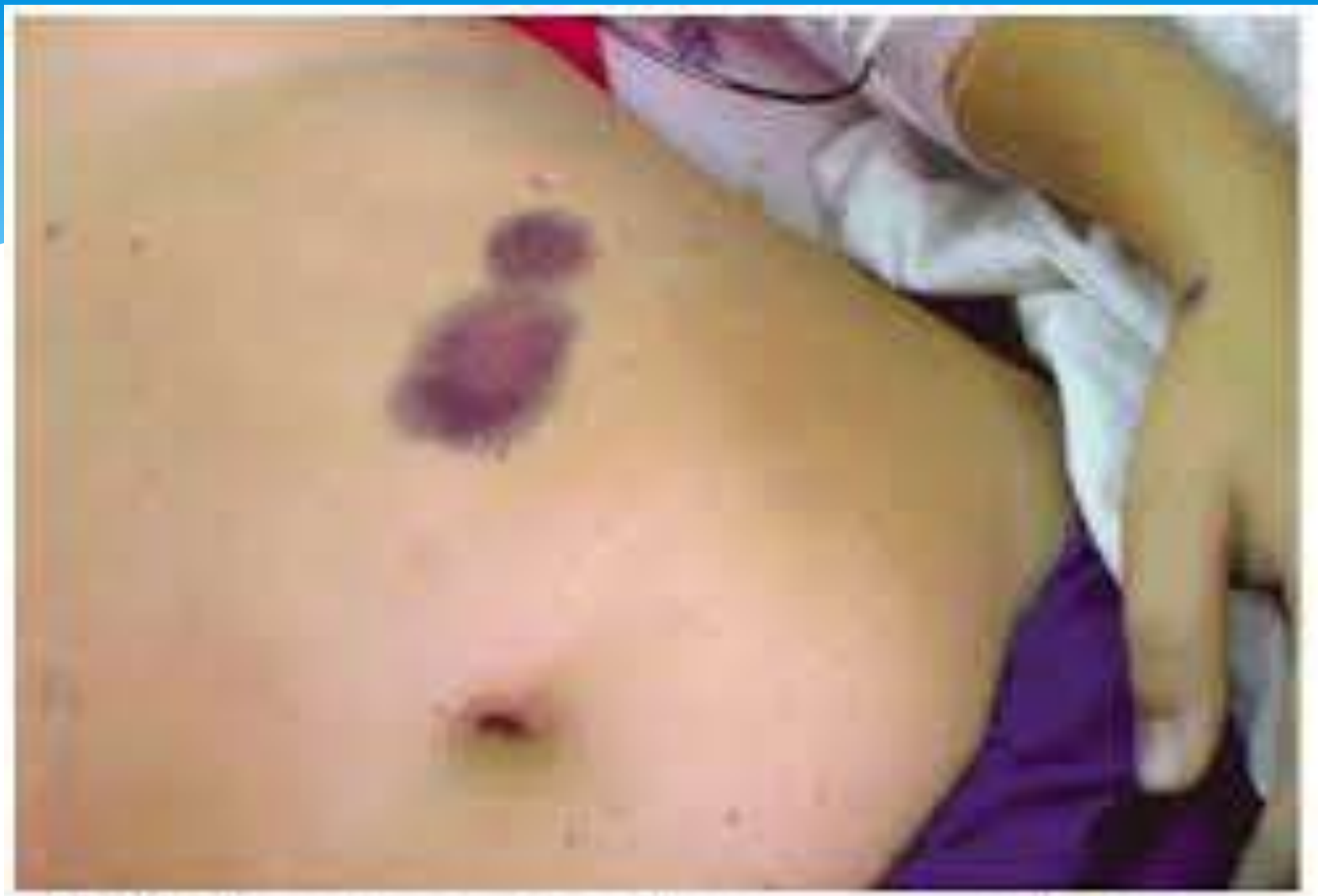
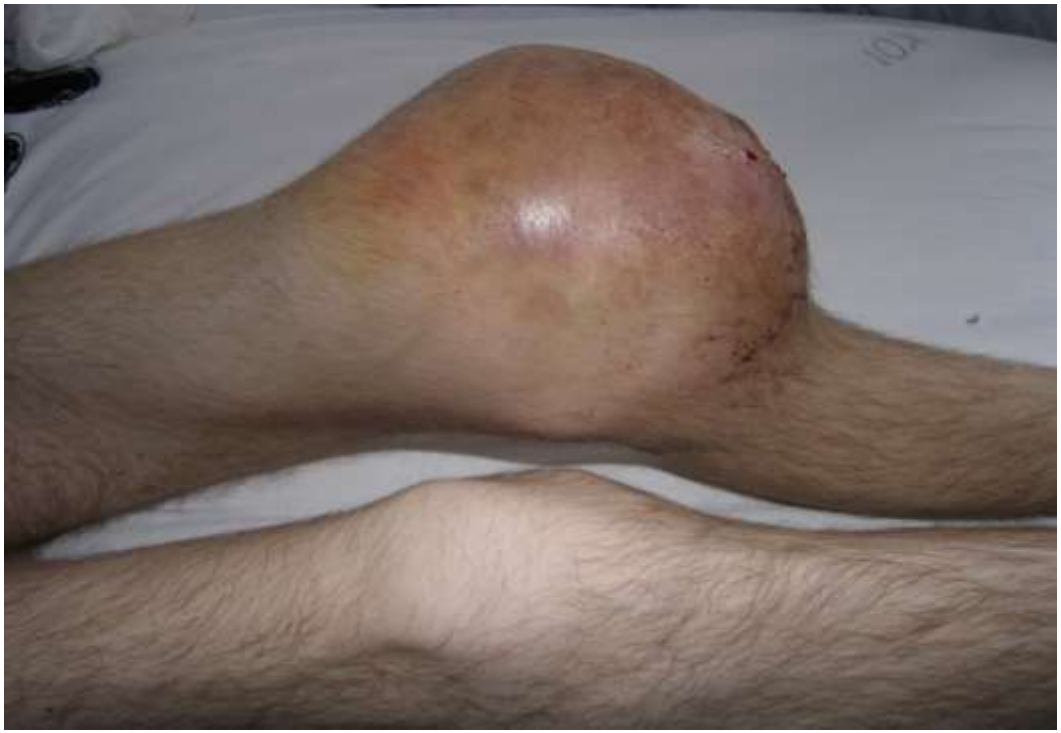
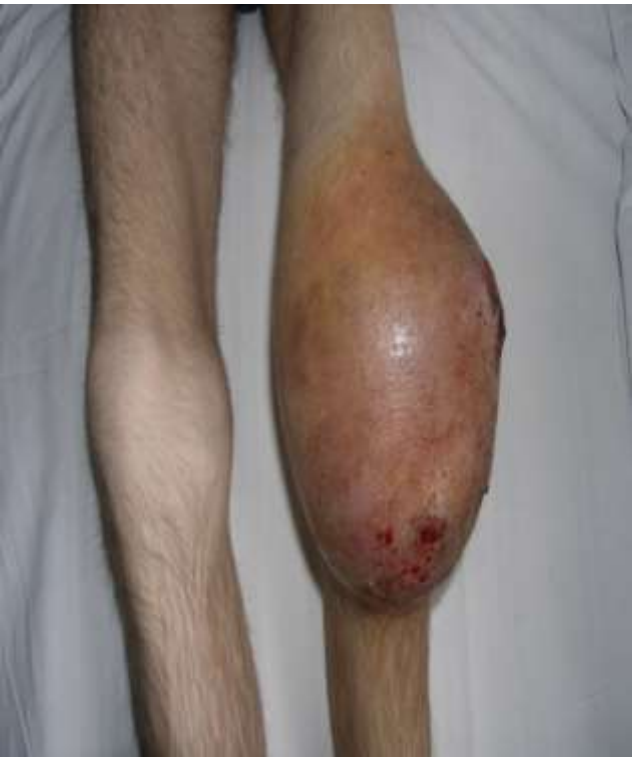


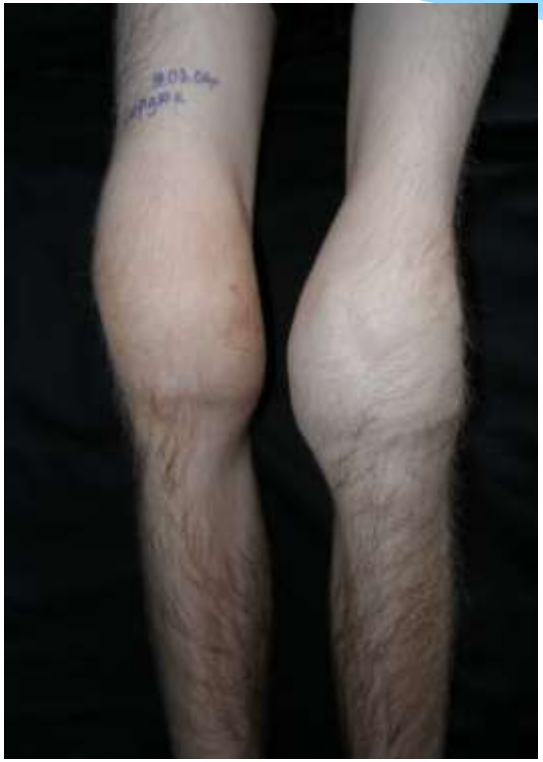
Рис. 3. Геморрагическая сыпь (петехии, экхимозы) в разных стадиях обратного развития у больного с симптоматической тромбоцитопенией (приобретенная апластическая анемия)

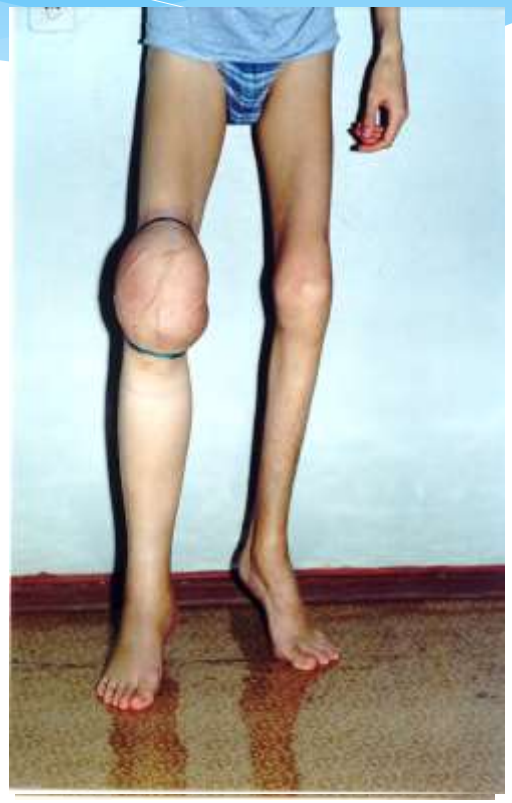
Haemarthrosis of the left knee in hemophilia

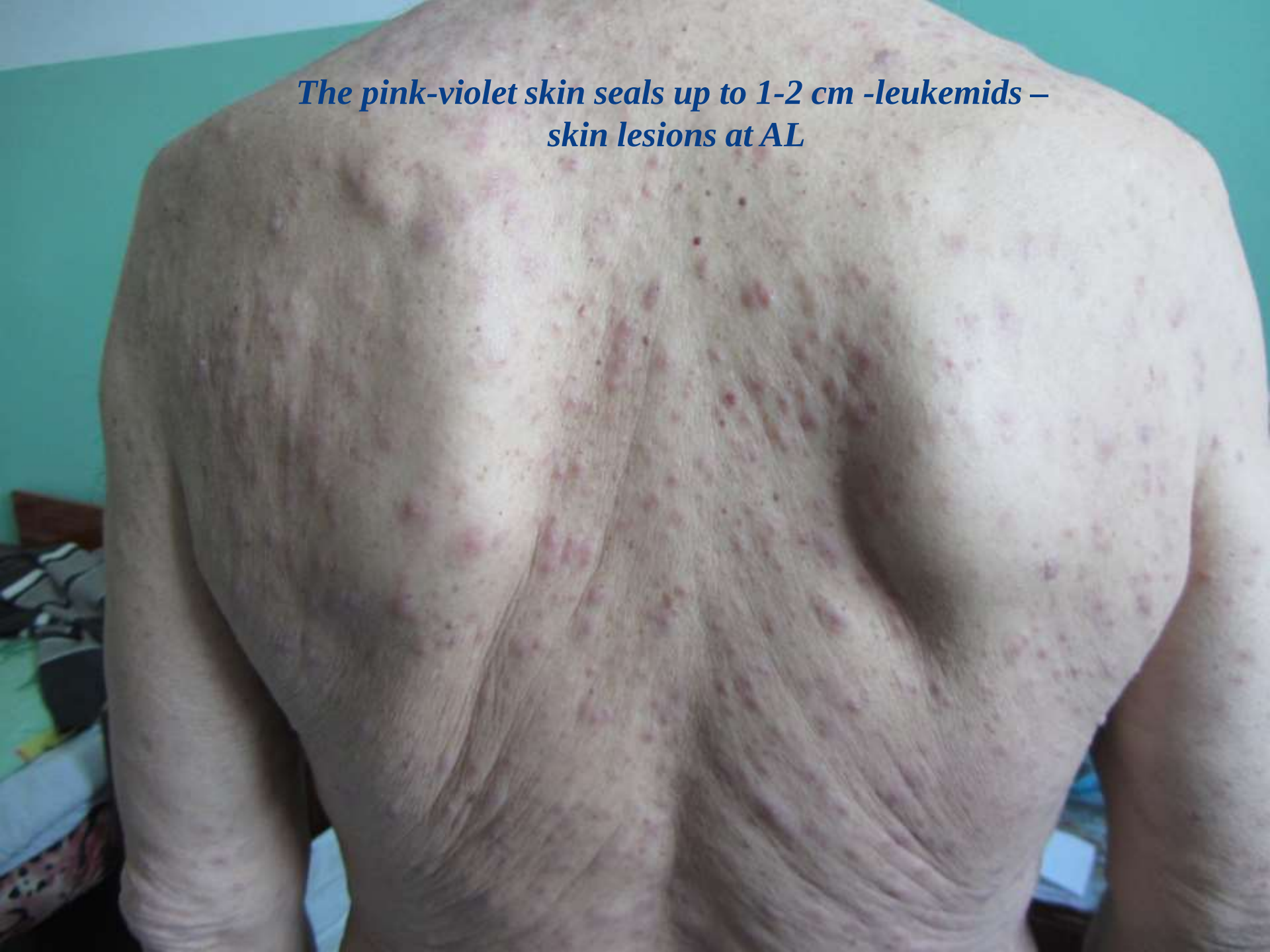


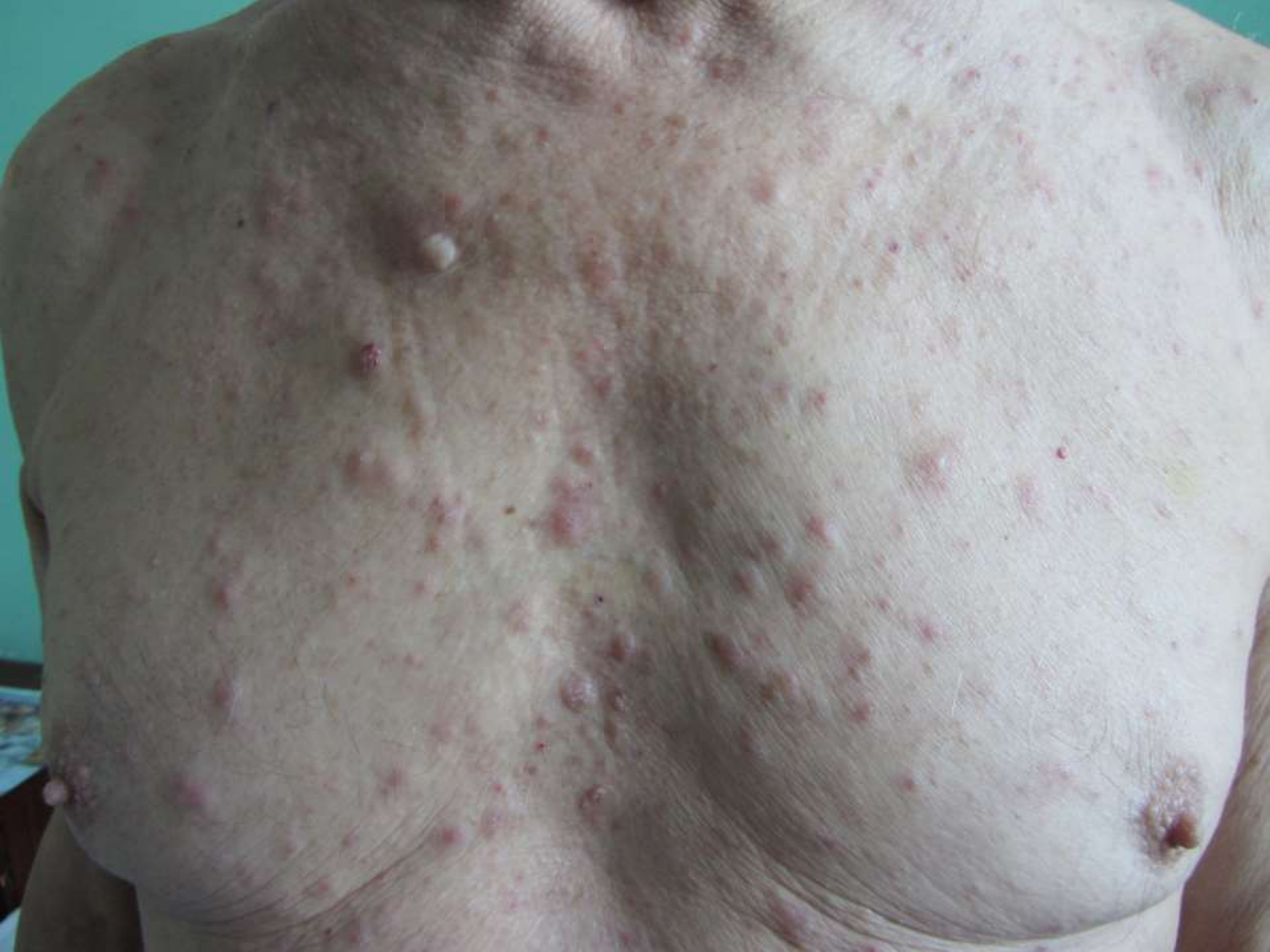
Lesion of joints with hemophilia



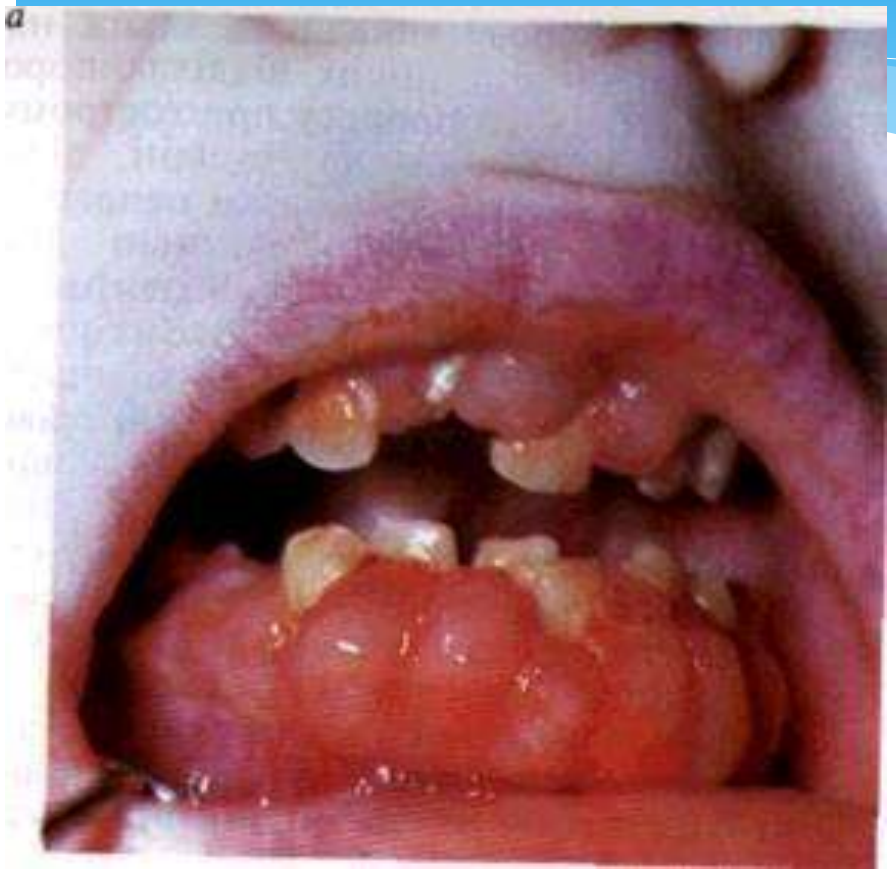


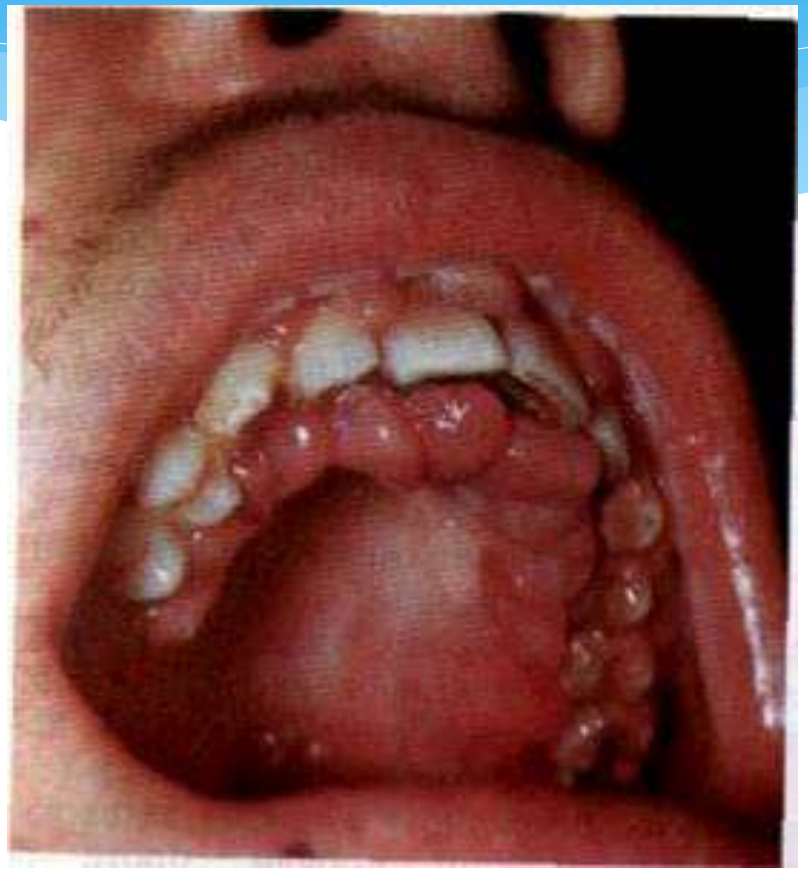



The pink-violet skin seals up to 1-2 cm -leukemids –
skin lesions at AL

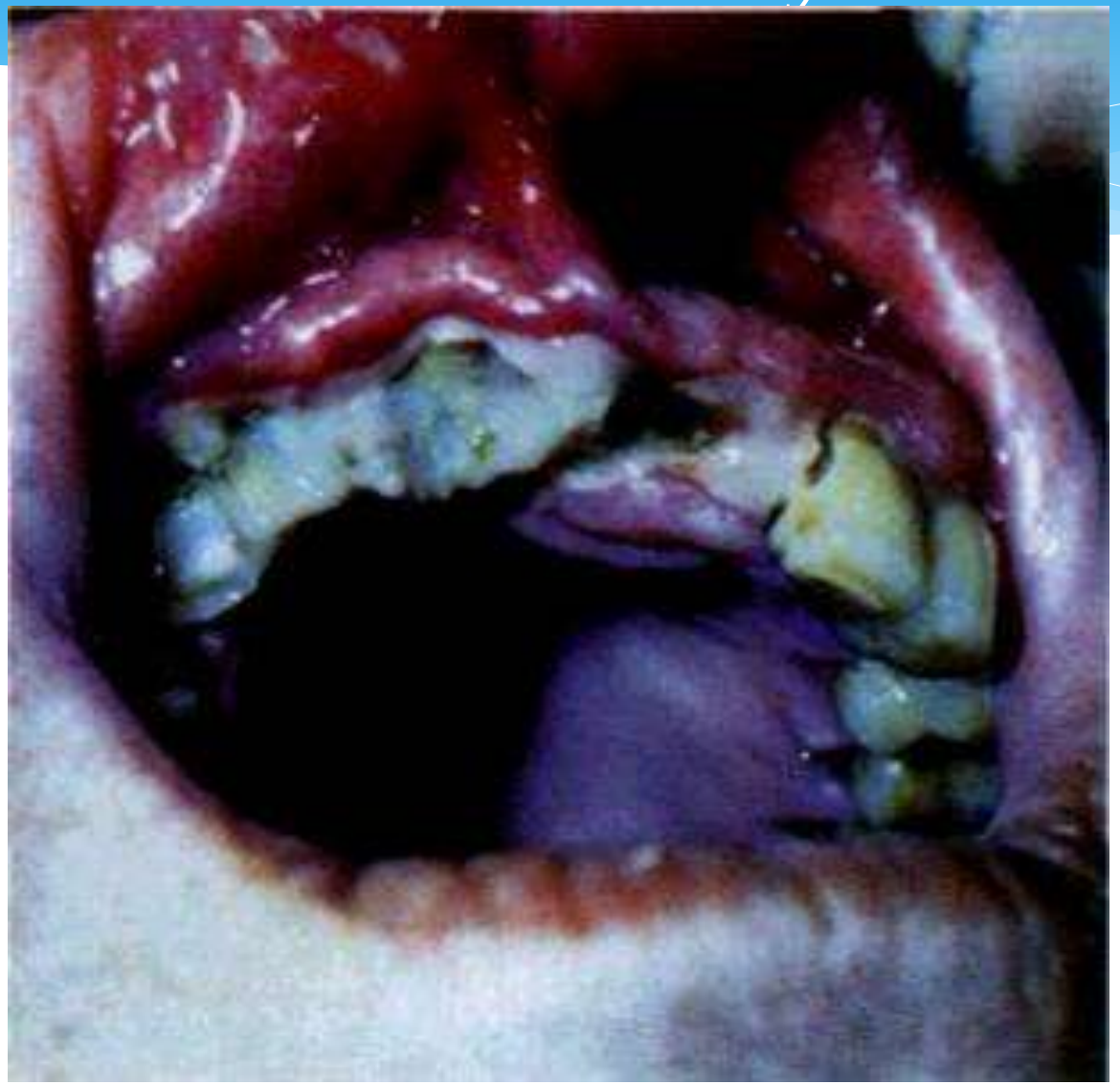


II.3. Hyperplastic syndrome with AL





II.4. Marginal necrosis of gums in patient with AL - ulcerative-necrotic syndrome



Objective examination

Palpation of peripheral lymph nodes:

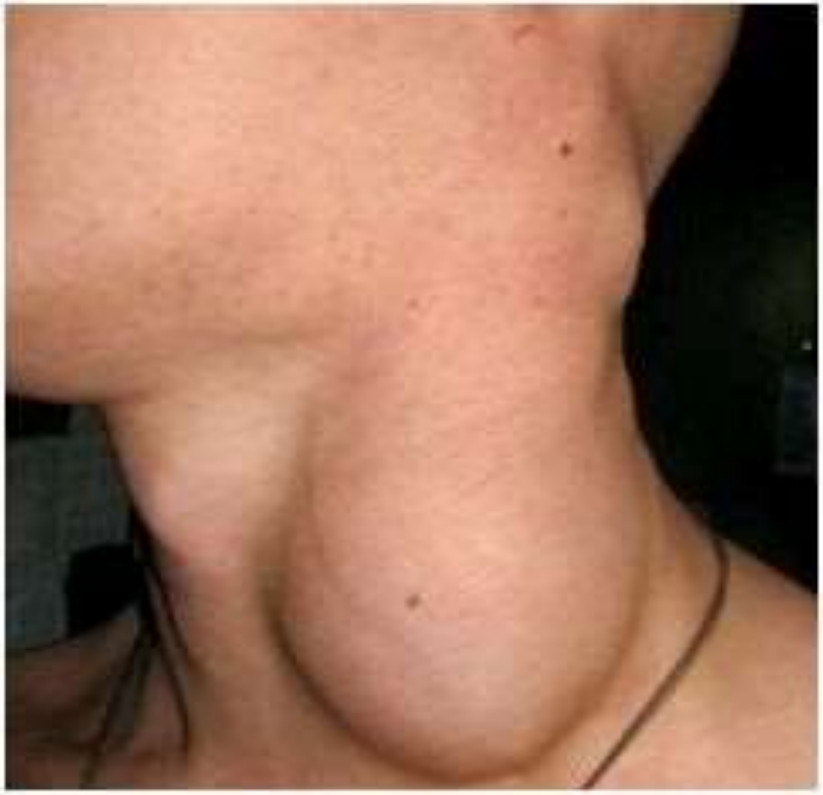
- Dimensions
- Consistency
- Confusion, mobility
- Soreness
- Skin color over an enlarged lymph node

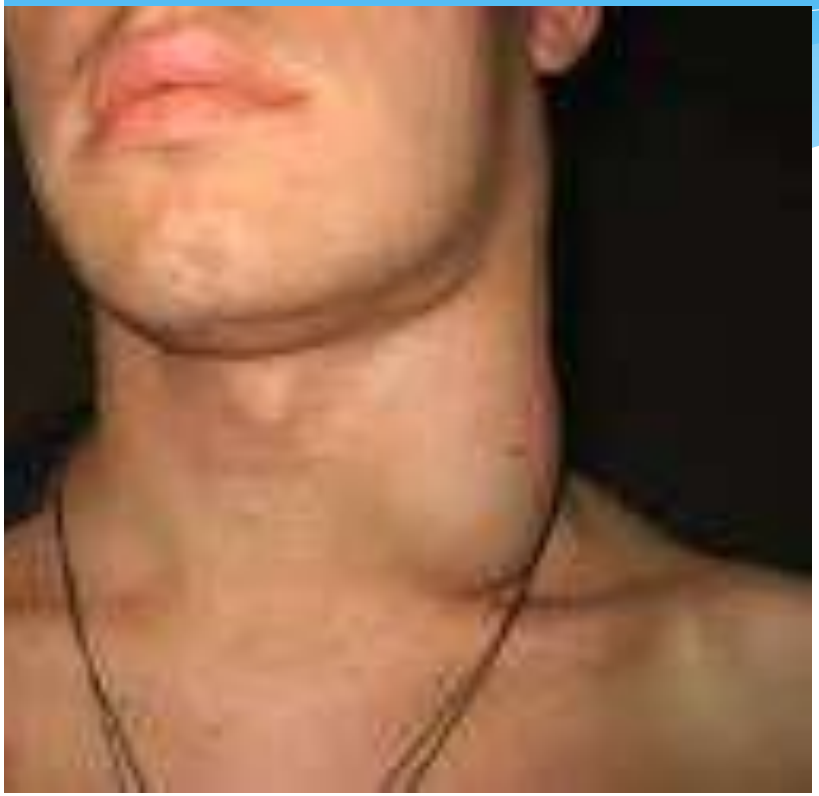
Determination of the size of the spleen - splenomegaly

Determination of liver size - hepatomegaly

Hyperplastic syndrome

II.5. Lymphadenopathy





Objective examination

Respiratory system changes :

*bronchitis, pneumonia, pleurisy, specific lung damage, symptoms of compression of the bronchial tree

Cardiovascular system changes :

*tachycardia, rhythm disturbances, weakening of the I tone on the apex, systolic murmur

Digestive system changes :

*erosive-ulcerative lesions of stomach and duodenum. Pain at palpation in the epigastric area of the abdomen

*enteropathy - painfull palpation

Secondary Nephropathy - CRF

Neurological symptoms

- disorder of superficial/deep sensitivity, paresis

III. Laboratory diagnostics in hematology

- **General blood analysis**
- **Blood chemistry**
- **Spernal puncture + counting myelogram**
- **Morphological examination of bone marrow cells**
- **Cytochemical study of bone marrow**
- **Immunological examination of bone marrow and peripheral blood cells**
- **Cytogenetic examination**
- **Molecular study**
- **Biopsy of target lymph node + immunohistochemical examination**

III.1. Hemogram indicators

Hemoglobin:

* 120 - 140 g / l for women
* 132 - 164 g / l for men

Hemoglobin concentration reduction (absolute):

* with anemia of different genesis (the degree of anemia is determined by the degree of hemoglobin decrease)

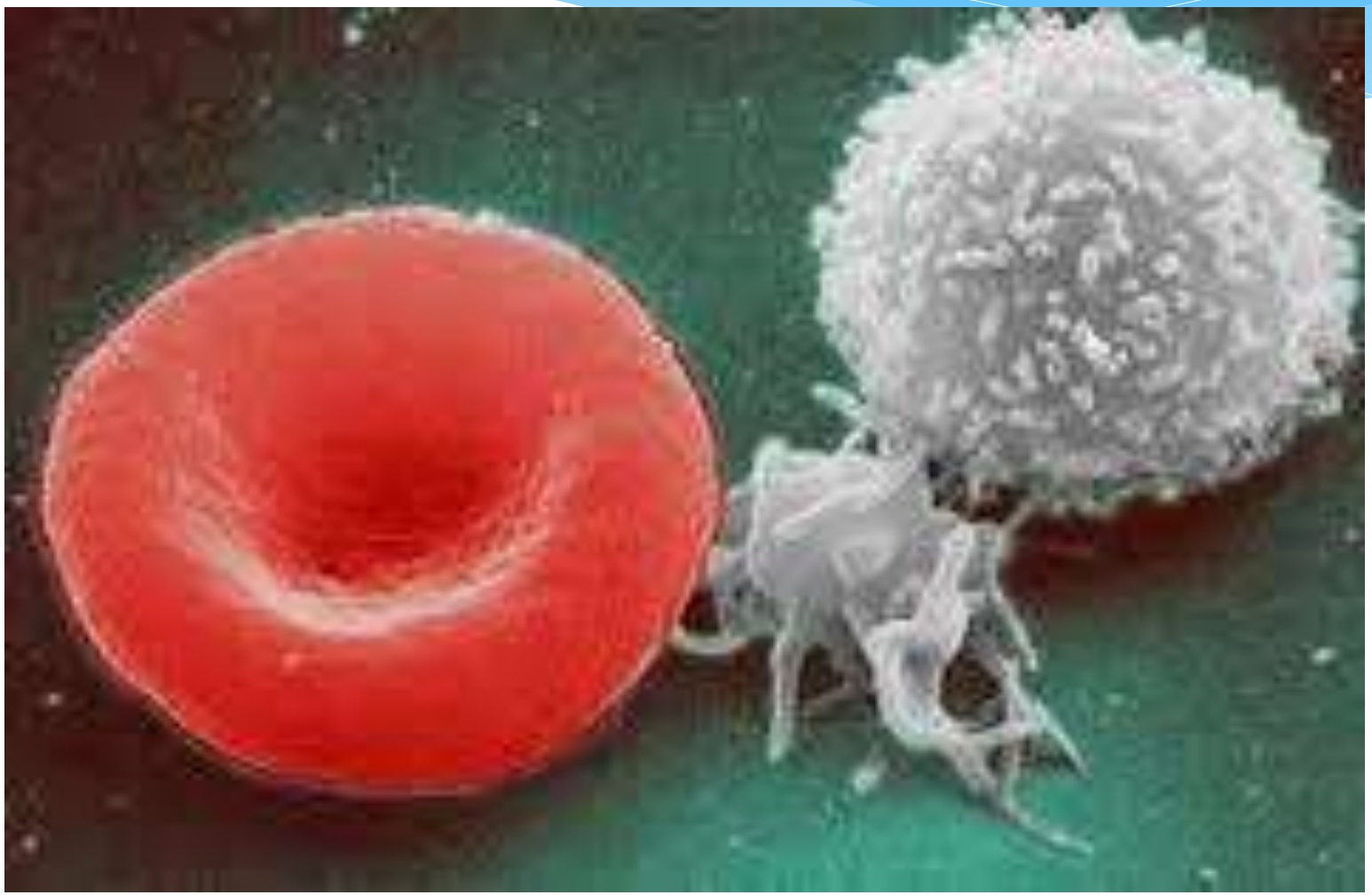
Relative hemoglobin decrease:

during pregnancy due to an increase in the volume of plasma.

Increase in hemoglobin concentration:

* with true polycythemia (PV)
* symptomatic erythrocytosis.

From left to right: erythrogyte, thrombocyte, leucocyte.



Red blood cells

* 4,5 - 5,1 × 10^{12} / l - for men
* 3,5 - 4,5 × 10^{12} / l - for women

The number of erythrocytes in anemic syndrome is decreased

Absolute erythrocytosis

* True polycythemia

Symptomatic erythrocytosis

* Relative erythrocytosis - at ↓ volume of the liquid part of the blood (with prolonged vomiting, diarrhea, burns).

Diameter of erythrocytes: 7-8 μm

* Microcytes - 5-6 ***μm***
* Macrocytes - more than 9 ***μm***

Hematologic indices

The average volume of erythrocyte MCV is 80-95 fl,

- ***MCV = 10 × hematocrit% / erythrocyte count × 10^{12} / l***

Mean hemoglobin content in one red cell MCH

- ***MCH = hemoglobin (g / l) / erythrocyte count × 10^{12} / l***
- ***MCH 26.7-33.3 pg***

The average concentration of hemoglobin in the erythrocyte MCHC

- ***MCHC = hemoglobin (g / l) × 10 / hematocrit (%)***
- ***MCHC 32-38 g / dL***

Color index

Characterizes the hemoglobin content in one erythrocyte

CI = hemoglobin × 3 / first three digits of red blood cells index

Norm 0.86 - 1.05

Hypochromic: IDA

Normochromic: hemolytic, aplastic anemia.

Hyperchromic: B-12 deficiency

Reticulocytes

- **The norm is 0.8-1.2% or 2-15 ‰ of the total number of erythrocytes**
- **Reticulocyte - young erythrocyte**
- **The number of reticulocytes reflects the regenerative capacity of the bone marrow**

Reticulocytosis is observed when:

True reticulocytosis (in both peripheral blood and BM)

- **Hemolytic anemia (hemolytic crisis)**
- **Acute posthemorrhagic anemia**

False reticulocytosis (isolated reticulocytosis in peripheral blood)

- **Metastasis of solid tumors in BM**

Leukocytes

Normal values in the adult's hemogram

4,0-9,0 × 109 / l

Physiological leukocytosis:

- **↑ physical stress - myogenic leukocytosis**
- **Pregnancy (usually in the 2nd half),**
- **Emotional stress,**
- **2-3 hours after meals, especially rich in protein - food leukocytosis**
- **After taking individual medications - glucocorticoids**

Pathological leukocytosis

* **acute and chronic inflammatory processes, sepsis**
* **infarction of different organs,**
* **massive burns**
* **intoxications,**
* **hepatic, renal failure,**
* **acute blood loss,**
* **hemolytic crisis,**
* **allergic diseases,**
* **solid tumors, especially with tumor decay.**

Leukocytosis in the diseases of the blood system is characterized by:

* **Moderate - for most AL, non-Hodgkin's malignant lymphomas, true polycythemia;**
* **Expressed (100-200 × 109 / l) - for chronic myelogenous leukemia (CML), less often for chronic lymphaticleukemia(CLL),**

  sometimes for AL

Leukopenia occurs when:

* **Acute Leukemia, NLD**
* **acute viral infections,**
* **diffuse connective tissue diseases (SLE, Felty syndrome),**
* **At a number of medications treatment (cytostatics, NSAIDs, sulfonamides, antibiotics).**

Leukocyte formula

Counted on the basis of peripheral blood smears morphological study

Stab neutrophils	**1-6%**	**0,04-0,3**
Segmented neutrophils	**47-72%**	**2,0-7,5**
Eosinophils	**0,5-5%**	**0,02-0,3**
Basophils	**0-1%**	**0-0,1**
Lymphocytes	**19-37%**	**1,5-3,5**
Monocytes	**2-10%**	**0,02-0,8**

The value of the leukocyte formula

Shift of the leukocyte formula to the left - ↑ the number of immature neutrophils in the peripheral blood (myelocytes, metamyelocytes, stab neutrophils, sometimes with the appearance of blast cells):

- ***Acute and chronic inflammatory and infectious processes***
- ***Solid tumors***
- ***Sepsis***
- ***Chronic myeloproliferative diseases***
- ***AL***

The value of the leukocyte formula

Increase in the number of segmented neutrophils with hypersegmented nuclei - a shift of the leukocyte formula to the right:

- ***Megaloblastic anemia***
- ***Myelodysplastic syndrome***
- ***Radiation sickness***
- ***Solid tumors***
- ***At the background of treatment with cytostatics***

The value of the leukocyte formula

Reduction in the number of neutrophils at less than 1.5 × 10 / L - neutropenia - with:

- ***Viral infections***
- ***Chronic inflammatory processes***
- ***Some hemoblastoses***
- ***At the backdrop of radiation therapy***
- ***At the backdrop of cytostatic therapy***
- ***Agranulocytosis - neutrophils less than 0.5 × 10 9/ L***

Platelets

Normally, the number of platelets is 150-350 × 109 / L

Thrombocytopenia is noted in hematologic diseases:

- ***immune and non-immune thrombocytopenia,***
- ***B_{12} deficiency anemia***
- ***Aplastic anemia (AA)***
- ***Hemolytic anemia***
- ***Acute leukemia (AL),***
- ***The later stages of CLL and CML,***
- ***Multiple myeloma (MM),***
- ***Myelodysplastic syndrome (MDS)***
- ***Hairy cell leukemia (HCL),***
- ***Non-Hodgkin's malignant lymphomas.***

Primary thrombocytosis:

* **Idiopathic myelofibrosis (IMF)**
* **Chronic myelogenous leukemia (CML)**
* **Polycythemia Vera (PV)**
* **Chronic myelomonocytic leukemia**
* **Myelodysplastic syndrome (MDS):**

- with the 5q deletion

- refractory anemia with ring sideroblasts and thrombocytosis

Secondary thrombocytosis (short-term)

* **Physical exertion**
* **Acute infectious diseases**
* **Therapy with vitamin B_{12}**
* **Allergic reactions**
* **Tissues injury**
* **Myocardial infarction**
* **Acute pancreatitis**
* **Acute hemorrhage or hemolysis**
* **Medication-induced (corticosteroids, adrenaline, vincristine, interleukin 1β, epinephrine, transretinic acid)**

Secondary chronic thrombocytosis

* **Iron Deficiency**
* **Surgical and functional asplenia**
* **Chronic infections**
* **Rheumatological diseases**
* **Systemic amyloidosis**
* **Chronic inflammatory diseases**
* **Celiac disease**
* **Malignant neoplasms**
* **Chronic kidney disease**

Erythrocytes sedimentation rate (ESR)

Normal 1-10 mm / h men; 2-15 mm / h women

Acceleration of ESR is noted :

- **Acute and chronic infectious and inflammatory processes,**
- **Immunosecretizing tumors (MM, Waldenstrom macroglobulinemia),**
- **Symptomatic paraproteinemia in the background of chronic lymphocytic leukemia (CLL) and non-Hodgkin's malignant lymphomas (NLL)**
- **Autoimmune diseases,**
- **Solid tumors.**

Reduction of ESR is observed at

- **PV, symptomatic erythrocytosis, viral hepatitis, mechanical jaundice.**

Міністерство охорони здоров'я України

Найменування закладу

КДЛ ПОКЛ

34

МЕДИЧНА ДОКУМЕНТАЦІЯ
Форма № 224/о
Затверджена наказом МОЗ України
04.01.2001 р. № 1

КЛІНІЧНИЙ АНАЛІЗ КРОВІ № 13186

Прізвище

Заклад _____ Відділення п-ліх

Медична карта № _____

Клінічний діагноз (профогляд) анемія

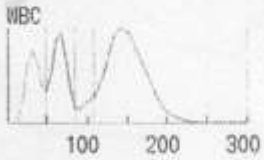
Найменування показників		Результат
Гемоглобін	ч	
	ж	
Еритроцити	ч	
	ж	
Кольоровий показник		
Ретикулоцити		
Тромбоцити		
Лейкоцити		
Швидкість осідання еритроцитів (ШОЕ)	ч	
	ж	26 мм/г
Нейтрофіли: Міелоцити		0
Нейтрофіли: Метаміелоцити		0 %
Нейтрофіли: Паличкоядерні		5 %
Нейтрофіли: Сегментоядерні		60 %
Еозинофіли		1 %
Базофіли		0
Лімфоцити		23 %
Моноцити		11 %
Плазматичні клітини		0

KDL POKL
Sysmex
XP-300

Operator

ID. 34
Date 24/07/2014
Time 12:13
Mode WB

WBC	WL*	9.0	×10³/L
RBC		3.90	×10⁶/L
HGB	-	65	g/L
HCT		-0.245	
MCV	-	62.8	fL
MCH	-	16.7	pg
MCHC	-	265	g/L
PLT	AG*	475	×10³/L



LYM%	WL*	0.254
MXD%	WL*	0.059
NEUT%	WL*	0.687
LYM#	WL*	2.3 ×10³/L
MXD#	WL*	0.5 ×10³/L

Iron metabolism study

Serum iron (9.0 - 31.3 µmol / L)

The total iron-binding capacity of blood(50-65 µmol / l)

Serum ferritin (20-160 µg / L)

Osmotic resistance of erythrocytes:

* **0,50-0,45% the beginning of hemolysis,**
* **0,35-0,40 - maximum hemolysis**
* **With microspherocytosis, the minimum osmotic resistance is 0.8-0.9% NaCl**

III.2. Biochemical studies

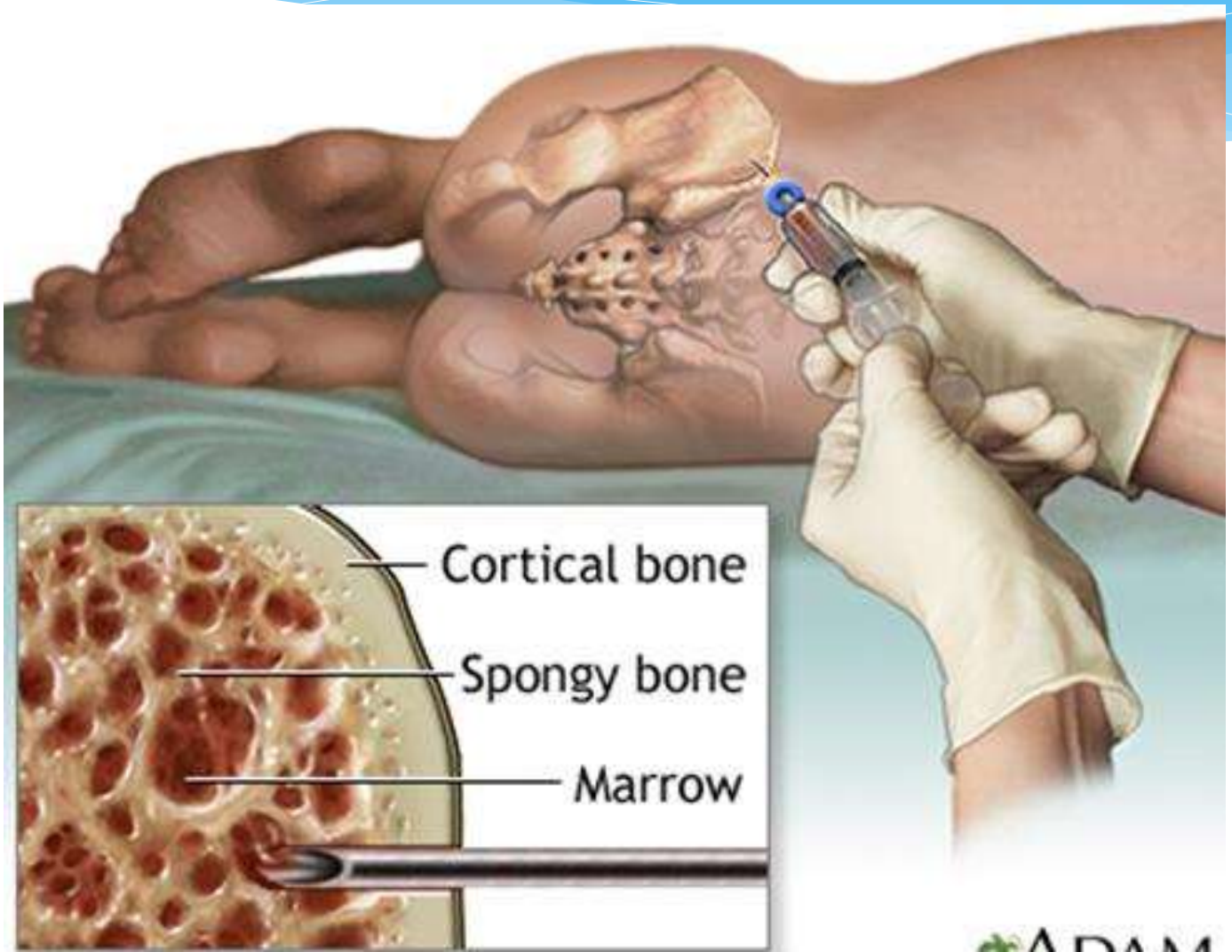
* **Bilirubin and its fractions**
* **Total protein and its fractions**
* **M-gradient - electrophoresis of plasma proteins and urine proteins**
* **Immunoelectrophoresis - a class of immunoglobulins**
* **LDH**
* **Transaminases level**
* **Creatinine level**
* **Calcium level**

III.3. Bone marrow examination (Sternal puncture, trepan biopsy of the iliac bone)

Sternal puncture - indications:

- **Anemia (except iron deficiency)**
- **Different cytopenia - the exclusion of hemoblastosis, aplasia)**
- **Suspected acute leukemia - diagnosis, control treatment**
- **Suspicion of chronic leukemia - diagnosis, treatment control**
- **Diagnosis of blood leukemoid reactions**
- **Increased ESR - the exception of MM**

Bone marrow aspiration and biopsy.



Bone marrow aspiration and biopsy.

Morphological analysis of bone marrow cells

* **Explore 500 cells**
* **Cellularity of BM**
* **The qualitative characteristic of all cell series**

Determination of the maturation indices

- Neutrophils

- Erythroid cells

Leukoerythroblastic ratio:

- Granulocytes / nucleated erythroid cells = 3-4 / 1

Determination of the hematopoiesis nature

Bone marrow

* **The number of myelokaryocytes in the norm is 50-250 × 109 /l - reflects the cellularity of the bone marrow:**

Normo-, hypo- and hypercellular marrow

Increased myelokaryocytes:

* **Acute and chronic hemoblastoses,**
* **B12-deficiency anemia,**
* **Hemolytic anemia**
* **After acute hemorrhage.**

Reduction of bone marrow cellularity:

* **Hereditary and acquired hypoplastic anemia,**
* **At toxic effect of medication,**
* **MDS (M**yelo**D**ysplastic **S**yndromes **), IMF(I**diopathic **M**yelo**F**ibrosis **),**
* **Metastases of solid tumors in the bone marrow.**

Bone marrow

Megakaryocytes 0.05-0.1 × 109 / L.

↑ megakaryocytes:

- **Myeloproliferative diseases: PV, CML, IMF,**
- **immune thrombocytopenia,**
- **metastases of solid tumors in the bone marrow**

↓ megakaryocytes:

- **Aplastic anemia,**
- **Acute leukemias,**
- **Non-Hodgkin's Malignant Lymphomas**

III.4. Cytochemical study

* **Myeloperoxidase is an accessory of myeloid cells (lymphoblasts do not contain MPO) - as the cells mature, the color intensity increases.**
* **Lipids are positive in myeloid cells: promyelocytes, myelocytes, mature neutrophils, eosinophils.**

Cytochemical study

PAS (Periodic Acid–Schiff) reaction to glycogen:

- **in myeloid variants of AL - PAS-reaction is slightly diffuse or negative,**
- **with the B-cell "common" version of AL - PAS-response positive granular, with T-cell variant AL - negative**
- **α-naphthyl esterase inhibited by sodium fluoride - positive in monoblasts, monocytes**

Blast cells cytochemical study

* **Definition of an acute leukemia variant**
* **Determination of CML blast crisis variant**
* **Allows you to clearly establish the type of cells, the stage of their maturation,**
* **Determine the characteristic enzymatic reactions based on the evaluation of a specific color of different intensity:**
* **(-) negative reaction,**
* **(+) slightly positive,**
* **(++) positive,**
* **(+++) strong positive reaction.**

III.5. Immunophenotyping

* **The method for determining on the cell surface antigens differentiation of CD "differentiation clusters" (membrane and intracellular), characteristic for determining the hemopoiesis line**

The main tasks of immunophenotyping

With AML:

* **Specification of the diagnosis: M0, M6, M7**
* **Determination of immune prognosis factors**

With tumors of the lymphoid series

* **Establishment of linearity of malignant cells**
* **Determination of the stage of differentiation**
* **Determination of the tissue equivalent of malignant tumors: NZL from cells of the mantle zone, follicular lymphomas, marginal zone lymphomas**

Determination of normal non-tumor equivalent of a malignant tumor

III.6. Immunohistochemical study

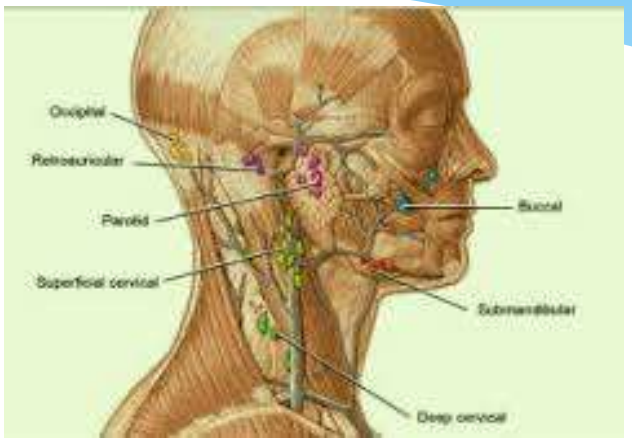
- **is a method of morphological diagnostics, based on the evaluation of antigen-antibody reactions in biopsy sections using a microscope.**

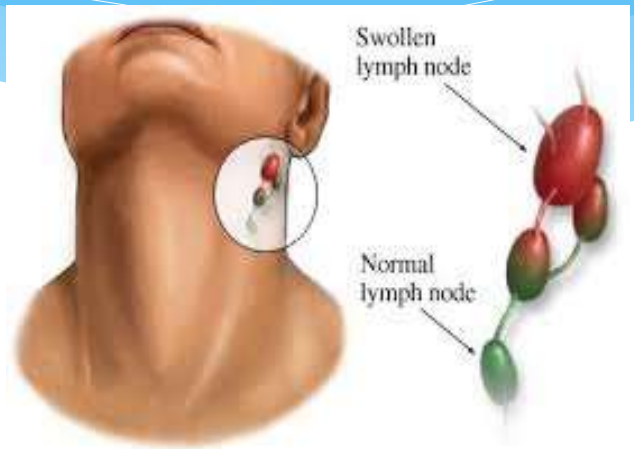
In the immunohistochemical study, the following are analyzed:

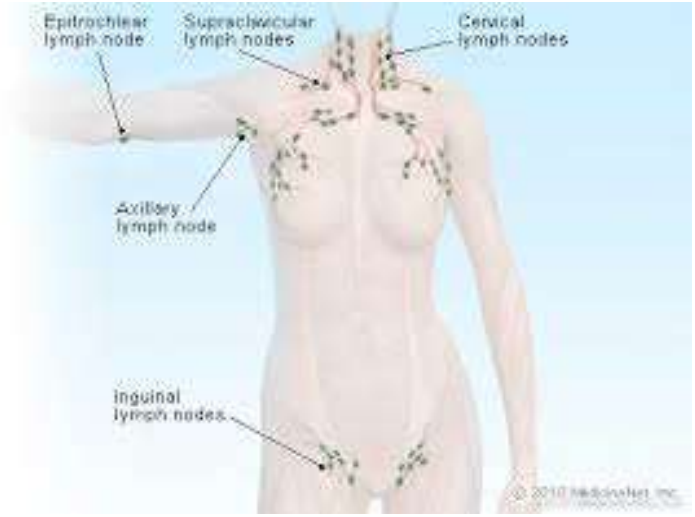
- **The presence of staining,**
- **Staining intensity**
- **Spatial staining of cytoplasm and cell membranes, nuclei, etc.**

General principles: **the use of a panel of MCA responsive to CD and subsequent evaluation of the antigen-antibody in tissue biopsy sections.**

Puncture of the lymph node







Possibilities of immunohistochemistry

* **Specification of tumor histogenesis**
* **Determination of the source of metastasis**
* **Immunophenotyping tumors of hematopoietic and lymphoid tissue**
* **Assessment of the functional state of the tumor**
* **Diagnosis of immunocomplex and autoimmune diseases**

Flow cytometry

* **is performed on blood, bone marrow, body cavity fluids (peritoneal, pleural), and aspirates of solid tissues (e.g. lymph node) placed into liquid media. This is generally used for**
* **- determination of cell lineage in lymphoma and leukemia**
- **differentiation between reactive and neoplastic expansions of lymphocytes**
- **determination of lymphocyte subsets.**

III.7. Cytogenetic study

* **Karyotyping with the determination of the quantitative composition of chromosomes and the identification of chromosomes by structure: normal or aberrant**
* **Chromosomes are examined in the stage of prometaphase and the metaphase of mitosis - the chromosome is maximally condensed, placed in one plane in the center of the cell separately from each other**

Chromosomal abnormalities can be:

* ***Quantitative*** **- karyotype with loss or addition of a chromosome**
* ***Qualitative*** **- structural, due to restructuring, loss or addition of individual chromosomal fragment**

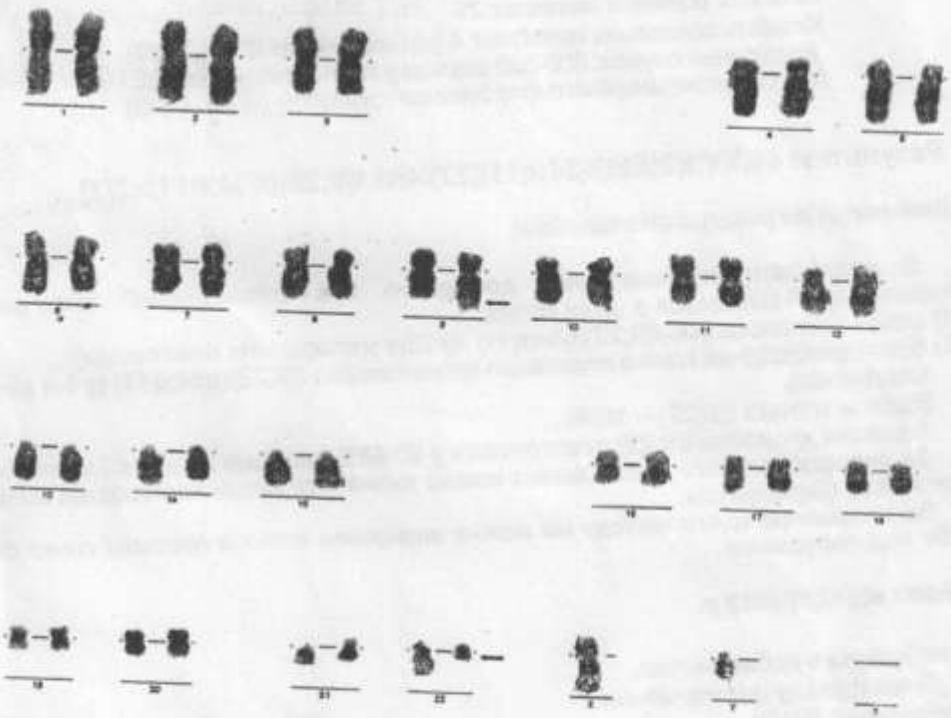
Цитогенетичний звіт

ПІБ:
Дата народження:

Код пацієнта: КМ12125
Клінічний діагноз: ?ХМЛ

Матеріал: КМ
Номер скла: ПЗ

Пофарбування: GTG
Координати: 143.0-8.0



Каріотип: 46,XY,t(9;22)(q34;q11)
Заключення:
Дата: 6/27/2012

Андреєва С.В.

III.8. Molecular genetic research

* **Genetic information is recorded on DNA as a linear sequence of 4 basic nucleotides: adenine, thymine, guanine and cytosine**
* **The principle of complementarity of DNA and RNA:**

- Adenin - Timin

- Guanine – Cytosine

Methods of molecular genetic research:

* **- FISH**
* **- PCR**

Molecular genetic research

* **FISH - (F*luorescence* I*n* S*itu* H*ybridization*) is based on the hybridization of the synthetic oligonucleotide of the DNA probe to specific sequences of the studied DNA (template DNA) that are labeled with fluorochromes followed by visualization using fluorescence microscopy**
* **The sensitivity of the DNA hybridization method makes it possible to identify one tumor cell among 100-1000 cells**

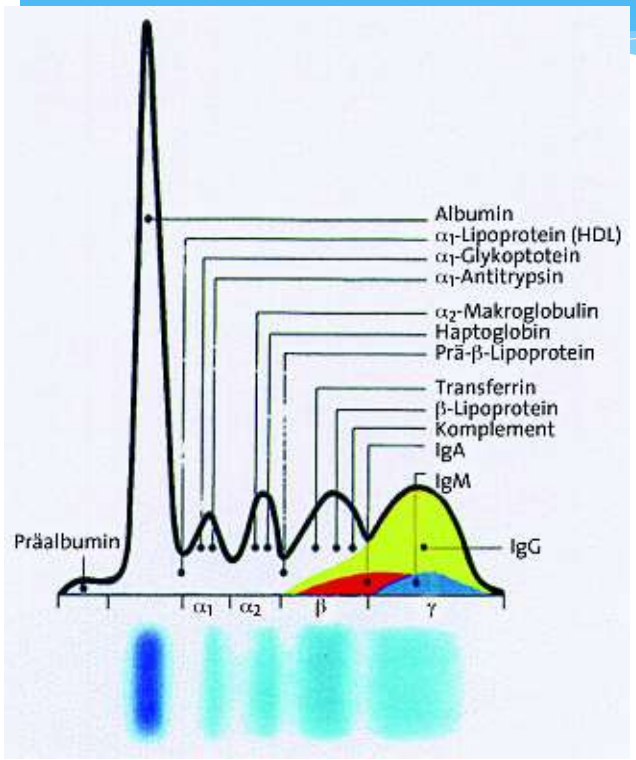
PCR:

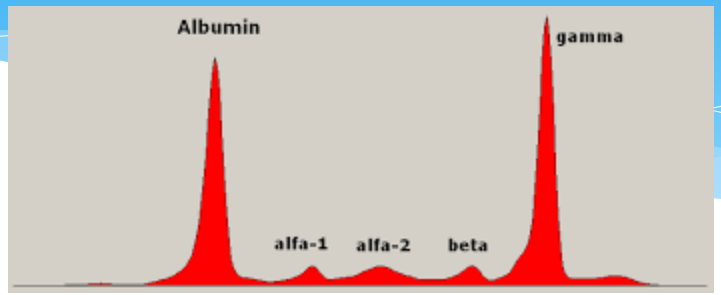
* **Performs effective amplification (doubling in each cycle of the studied DNA molecules up to 20-30 cycles) of selected DNA sequences**
* **Has a high sensitivity - it makes it possible to identify one tumor cell by 105-106 normal cells**
* **Any violation of the complementarity of DNA nucleotides - mutations**

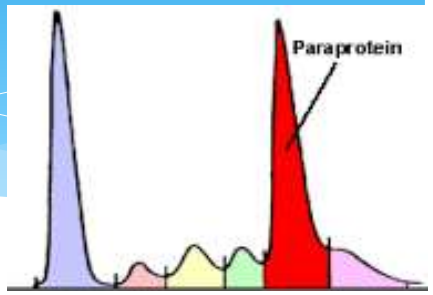
III.9. Immunochemical methods

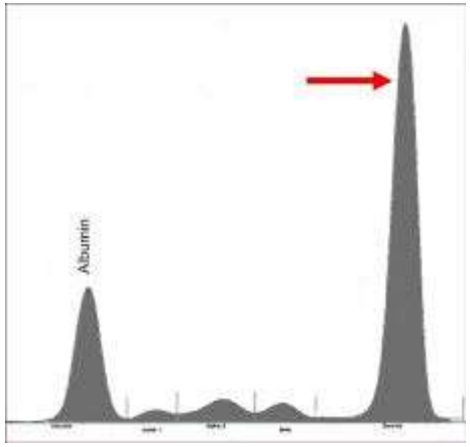
* **Diagnosis of immunoglobulinopathy**
* **Electrophoretic methods - protein electrophoresis (acetate-cellulose membrane, agarose gel):**
* **albumins**
* **α1-antitrypsin, α1-lipoprotein-α1-zone**
* **α 2-macroglobulin, haptoglobin-α2-zone**
* **Transferrin, β-lipoproteids - β-zone**
* **Fibrinogen - Y-zone**
* **Immuno-fixed electrophoresis**

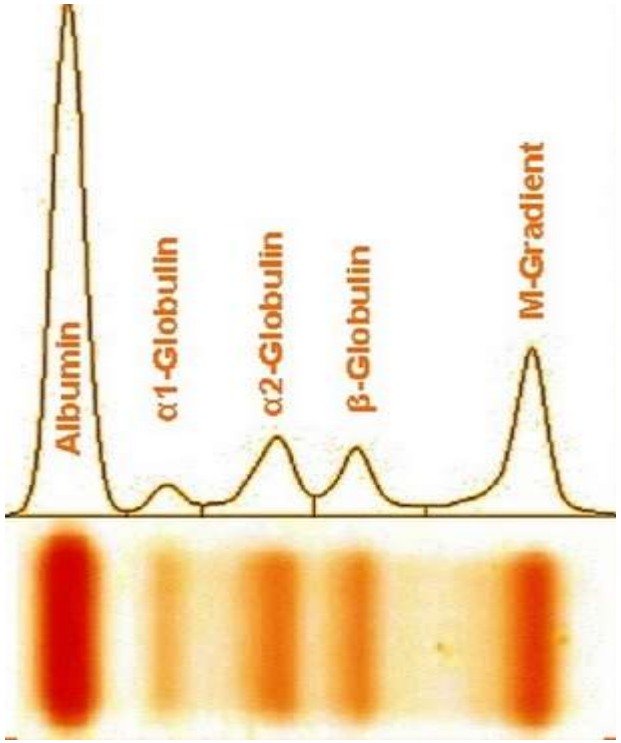
M-Gradient


Albumin
α1-Lipoprotein (HDL)
α1-Glykoptotein
α1-Antitrypsin
α2-Makroglobulin
Haptoglobin
Prä-β-Lipoprotein
Transferrin
β-Lipoprotein
Komplement
IgA
IgM
IgG
Präalbumin
α1
α2
β
γ


Albumin
gamma
alfa-1
alfa-2
beta

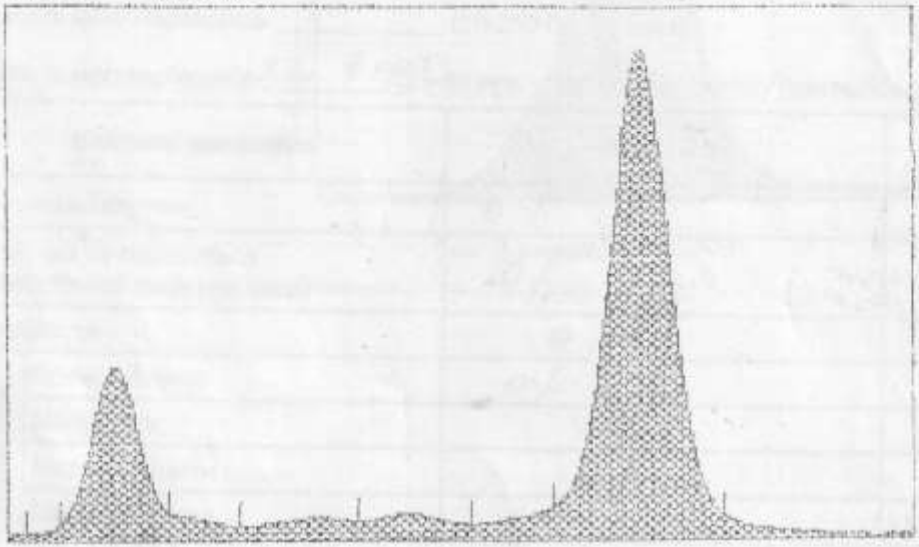

Paraprotein


Albumin


Albumin
α1-Globulin
α2-Globulin
β-Globulin
M-Gradient

KEK Electrophoresis Lab

Date : 07-12-2011 Prog.Nb: 25
Depart. : Poltava Cod.Nb :
Name : Мамыкоев.6.12.11 Age : 45
Doctor : Ljuba Sex : M



Name fr.		%	(G/L)	Normal val.
Prealbum		0.4	0.5	0.0 - 1.0
Albumin	<	19.1	25.3	55.0 - 65.0
Alpha 1		2.2	2.9	2.0 - 4.5
Alpha 2	<	5.7	7.4	10.0 - 15.0
Beta 1	<	4.0	5.3	6.0 - 13.0
Beta 2	<	2.7	3.7	6.0 - 13.0
γ-глобулин		67.7	90.2	10.0 - 20.0

Total proteins : 133.0(G/L) A/G : 0,4

Comment.: Вторичная диспротеинемия за счёт наличия в зоне фракции γ-глобулинов массивного моноклона. Для уточнения типа этого моноклона необходимо проведение дополнительного исследования сыворотки крови методом иммунофиксации.

Doct. of the lab.: Susaeb Natalie

Coagulopathy

* **No single test or combination of tests is adequate to diagnose disseminated intravascular coagulation (DIC).**
* **Perform screening tests in all patients, such as platelet count, prothrombin time (PT), activated partial thromboplastin time (aPTT), thrombin time, and fibrin degradation products or soluble fibrin monomers.**
* **Additional tests that may be useful include antithrombin III levels, protein C, D-dimer, fibrinogen, measurements of specific coagulation factors such as factor V and VIII, and plasminogen activator inhibitor type I (PAI-1).**
* **Clinical judgment in conjunction with these tests provide a means of working towards a diagnosis of DIC, although no single test results alone are confirmatory.**

Coagulopathy

* **Thrombocytopenia is an almost universal finding, and the CBC count with smear review may reveal findings suggestive of DIC, such as increased platelet size, schistocytes and helmet cells.**

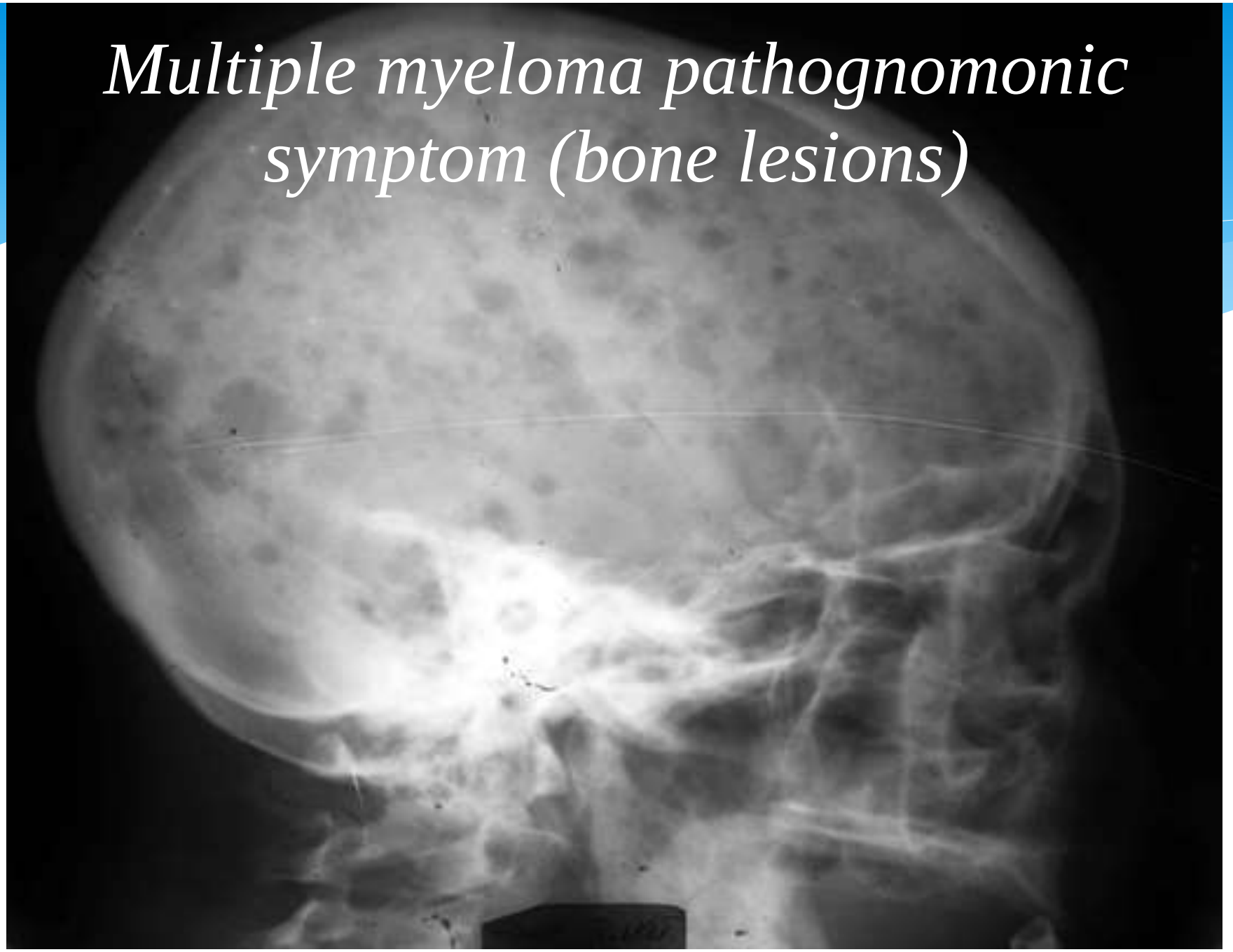
Coagulation Parameters

- **Prothrombin time (PT) 11-14 seconds**
- **Partial thromboplastin time (PTT) 25-35 seconds**
- **International normalized ratio (INR) 0.8 to 1.2**

Methods of radiation diagnostics

* **X-ray methods**
* **CT scan**
* **Magnetic Resonance Tomography**
* **Radionuclide methods: scintigraphy, positron emission tomography**
* **Ultrasonographic methods**

Multiple myeloma pathognomonic symptom (bone lesions)



Thank you
for your attention!