MYELODYSPLASTIC SYNDROMES
APLASTIC ANEMIA
DIFFERENTIAL DIAGNOSIS OF PANCYTOPENIA

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MYELODYSPLASTIC SYNDROMES

MDS – a clonal disorder of hematopoiesis.

Pathogenesis:

2 factors – mutation x host response

MUTATION of pluripotent hematopoietic stem cell leads to proliferation of an abnormal clone with growth advantage.
The clone may totally replace normal hematopoiesis.

HOST DEFENSE REACTION leads under normal circumstances to elimination of pathological clone.

An altered immunological response is unable to eliminate mutated cells and may support a proliferation of an abnormal clone.

2 groups of genes important for clonal proliferation :

ONCOGENES

not expressed in genome under normal circumstances, a mutation leads to their overexpression which is usually connected with activation of an abnormal metabolic pathway resulting in growth advantage (eg. tyrosin kinase).

ANTI-ONCOGENES (TUMOR SUPRESSOR GENES)

are expressed in genome under normal circumstances and usually have a crucial role in some regulatory mechanisms (eg. programmed cell death – apoptosis), a mutation leads to a lose of their expression, which is connected with abnormal proliferation not dependent on regulatory mechanisms.

MDS

EARLY PHASE

A mutation initiates a clonal proliferation of early progenitor cells, an abnormal immunological response leads to hyperproduction of cytokines inducing apoptosis– programmed cell death of more mature cells (TNFα, IL-2) as defense against mutated cells
A normocellular or hypercellular dysplastic bone marrow contrasting with peripheral blood cytopenia.

ADVANCED PHASE

A genome instability tending to an increased incidence of oncogenes supporting abnormal proliferation and silencing of differentiation genes, mutations of tumor suppressor genes – decreased apoptosis → a progressive increase of immature CD 34+ hematopoietic precursors belonging to the abnormal clone.
A normo – or hypercellular bone marrow with excess of myeloblasts and tendency to progression towards acute myeloid leukemia.

FAB subtype	% bone marrow blasts	% ringed sideroblasts	Further criteria
RA (refractory anemia)	< 5	< 15	< 1 % blasts in peripheral blood
RAS (refractory anemia with ringed sideroblasts)	< 5	>15	< 1 % blasts in peripheral blood
RAEB (refractory anemia with excess of blasts)	5 - 20	variable	< 5 % blasts in peripheral blood
CMML (chronic myelomonocytic leukemia)	1 - 20	variable	Peripheral blood monocytes >1x 10^{9} / l, increased % of bone marrow monocytes
RAEB-T (refractory anemia with excess of blasts in transformation)	21 - 30	variable	> 5 % blasts in peripheral blood and < 30% bone marrow blasts

FAB classification of Myelodysplastic syndromes
(Bennett et.al, Br J Haematol, 51, s.189, 1982)

MDS - WHO CLASSIFICATION 2016

refractory anemia

refractory cytopenia with multilineage dysplasia

refractory anemia with ringed sideroblasts

5q- syndrome

myelodysplastic syndrome – unclassifiable

refractory anemia with excess of blasts

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patients with > 20% bone marrow blasts -
- acute myeloid leukemia

MDS - DIAGNOSIS

PERIPHERAL BLOOD cytopenia in 1, 2 or 3 lineages.

BONE MARROW ASPIRATION

- morphology : dysplasia (nuclear abnormalities, nucleo/cytoplasmic asynchrony, hypogranulation, megaloblasts), cellularity, percentage of blasts.
- cytochemistry : ringed sideroblasts, abnormal PAS or PE,reactions.
- cytogenetics : karyotype abnormalities - chromosome 5,7,8, multiple abnormalities.
- flow cytometry : number of CD 34+ precursor cells.
- molecular biology : clonal origin of hematopoiesis, driver mutations

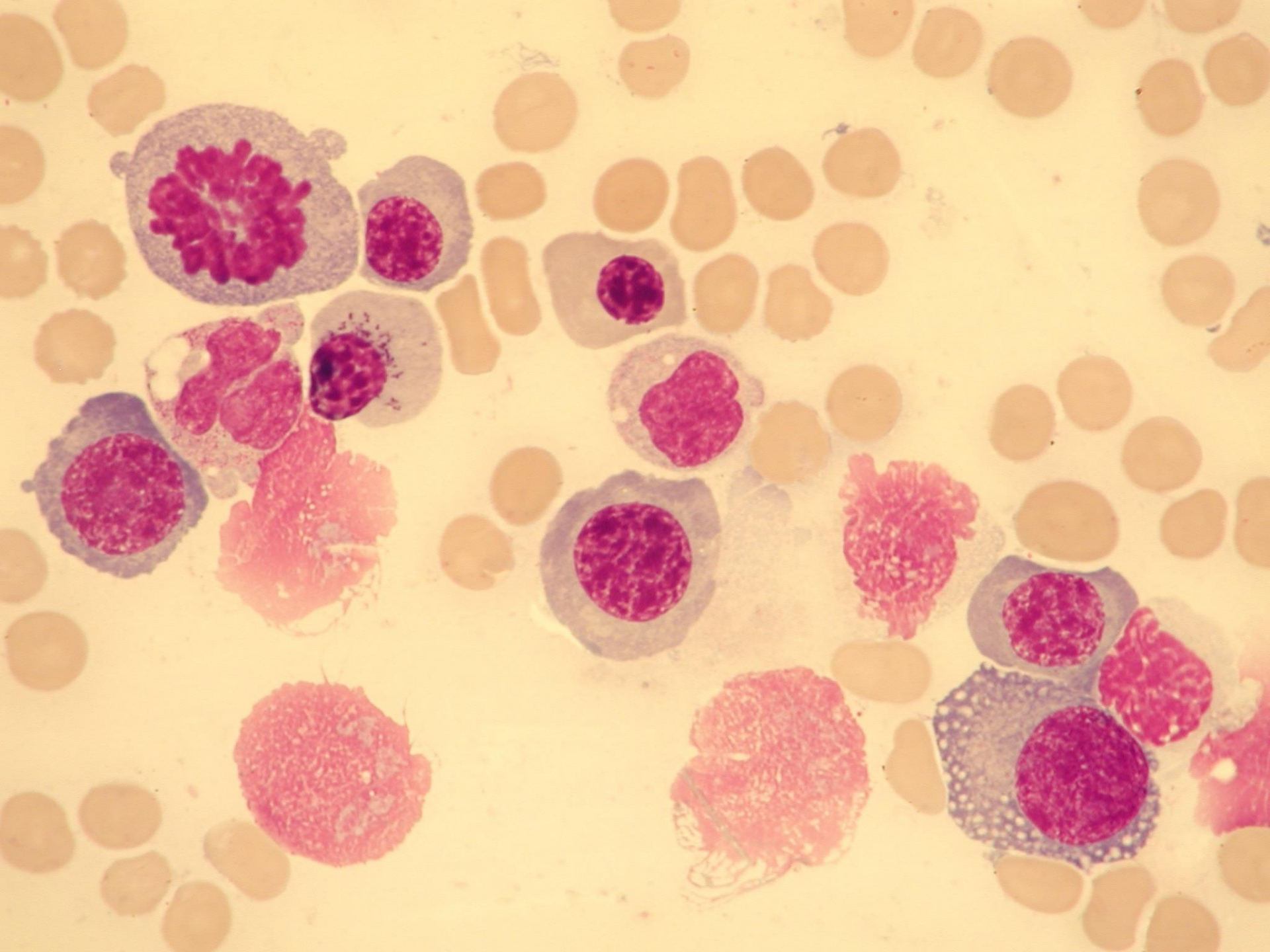
BONE MARROW BIOPSY

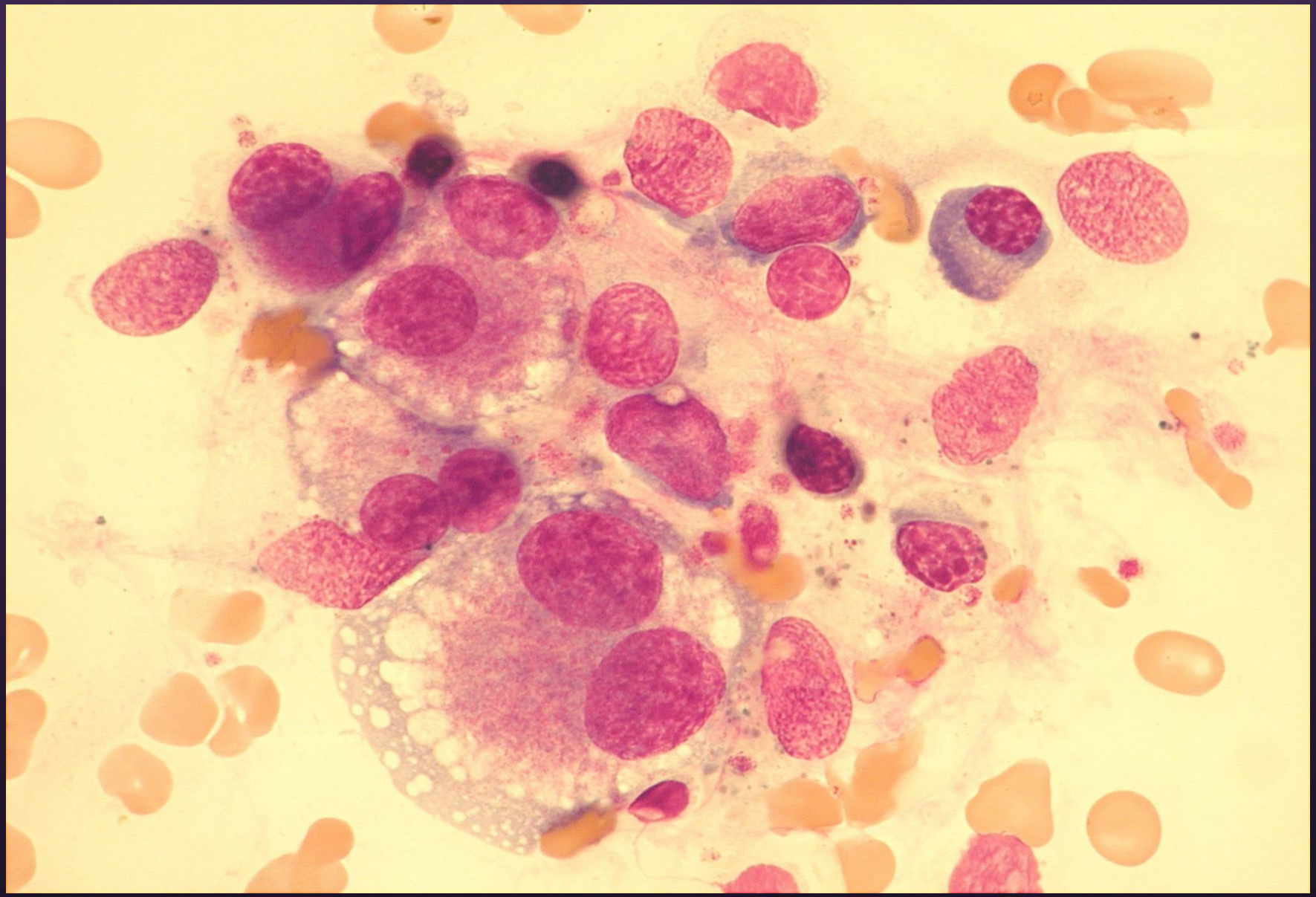
cellularity, cell distribution, immature precursor cells, fibrosis

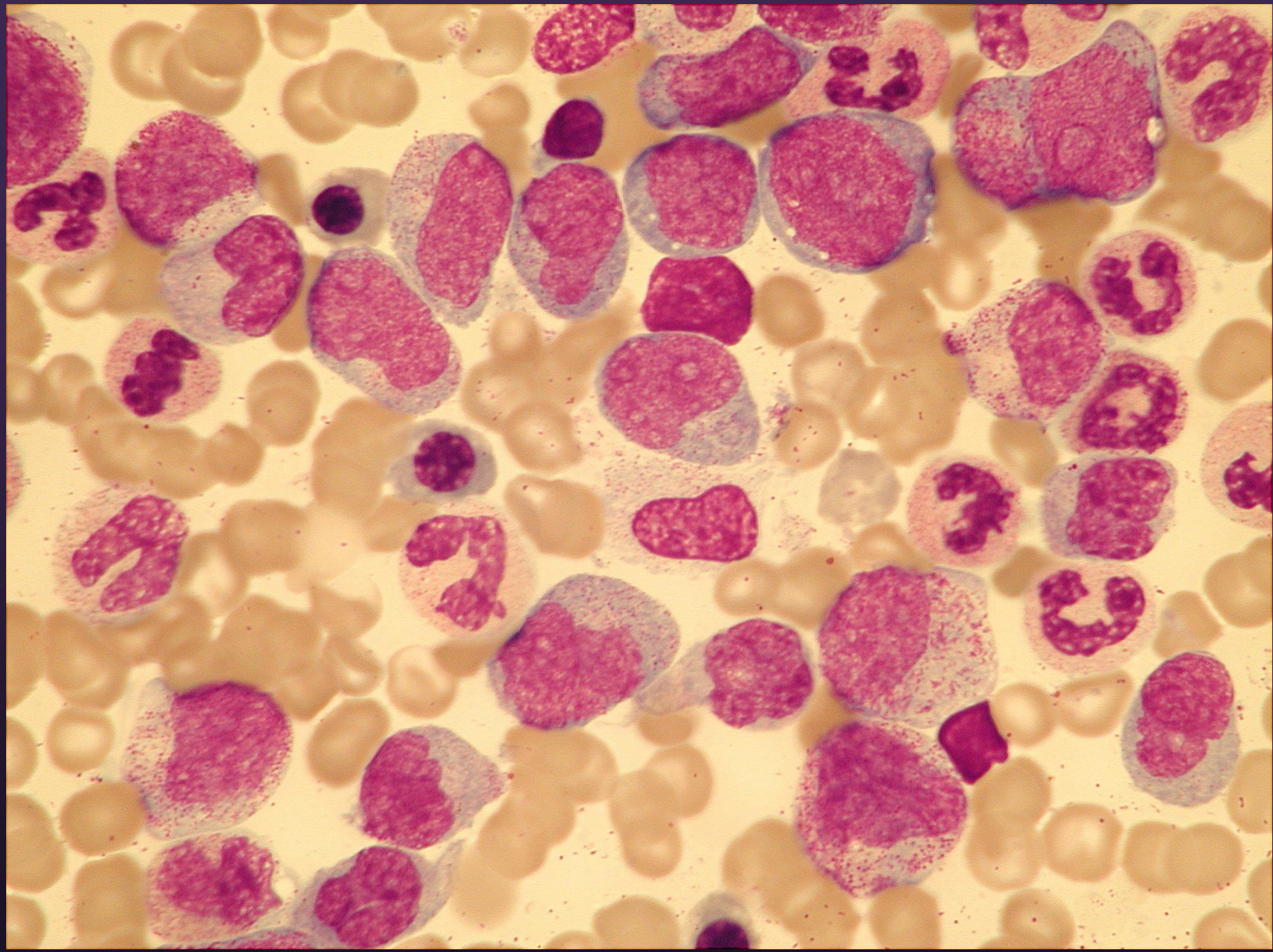
BIOCHEMISTRY

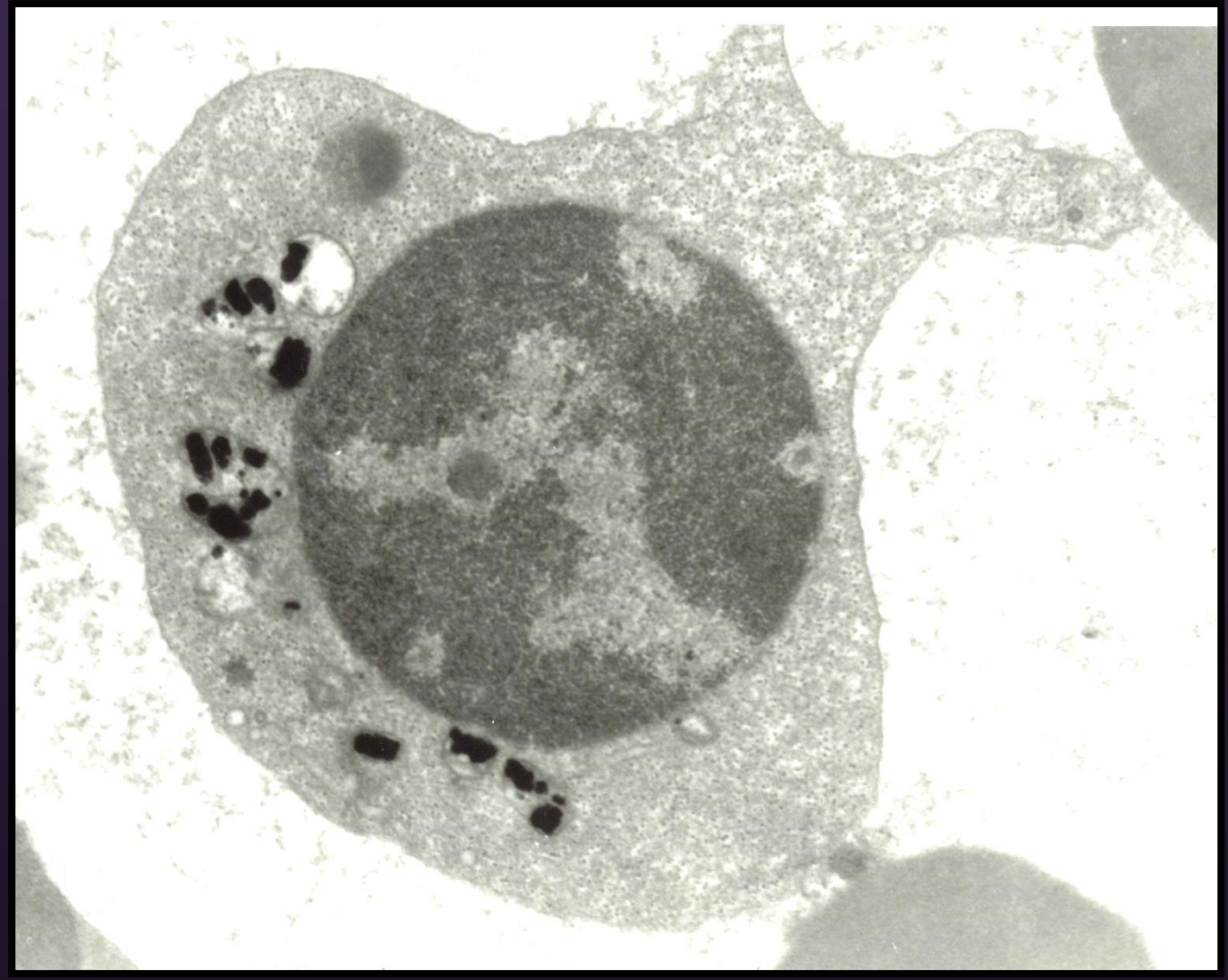
sFe, sFerritin, B12, EPO, sBilirubin, coagulation, Coombs test, CD 59-, CD 55- cells.

CLINICAL EXAMINATION exclusion of secondary MDS

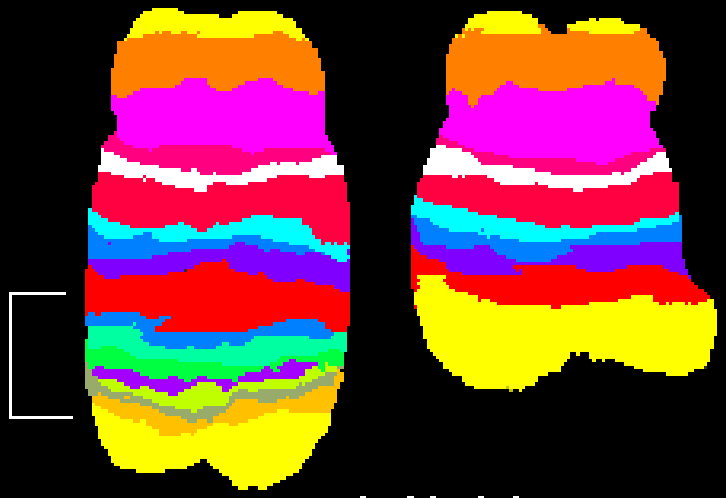








CENTER OF ONCOCYTOGENETICS, GENERAL FACULTY HOSPITAL, PRAGUE



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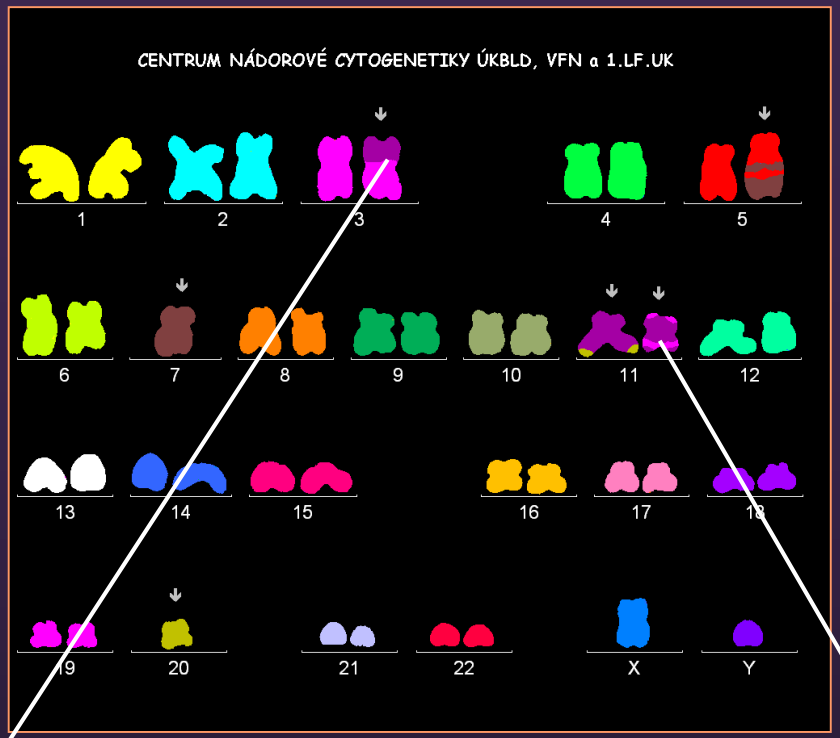
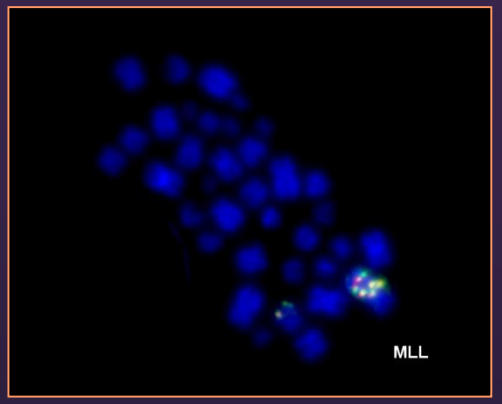




translocation involving 3q26

translocation involving 11q23

↓

abnormal expression of EVI1

↓

amplification of MLL gene

Altered proliferation and differentiation → adverse prognosis

MDS - DIFFERENTIAL DIAGNOSIS

1.APLASTIC ANEMIA

hypocellular bone marrow without dysplasia, no changes in karyotype and cytochemistry, no CD 59- cells, bone marrow blasts are not increased, no marrow fibrosis.

2.PAROXYSMAL NOCTURNAL HEMOGLOBINURIA

intravascular hemolysis with hemoglobinuria, presence of CD59- and CD55- cells, no changes in karyotype.

3.ACUTE MYELOID LEUKEMIA

>20% of bone marrow blasts according to WHO classification, no dysplastic features (if not a termination of MDS).

MDS - DIFFERENTIAL DIAGNOSIS

4. MEGALOBLASTIC ANEMIA

megaloblasts in bone marrow without additional dysplasia, no changes in karyotype and cytochemistry, ↓ sB12, ↓s folic acid, glossitis, gastritis, malabsorption.

5.SIDEROBLASTIC ANEMIA

lead poisoning, several drugs, no dysplasia, no karyotype abnormalities.

Transient dysplasia – during viral infections.
All patients with myelodysplasia should undergo a complete clinical and laboratory investigation – myelodysplasia may be secondary due to another serious illness – eg. neoplasia.

MDS – INTERNATIONAL PROGNOSTIC SCORING SYSTEM (IPSS)

			score		
Prognostic factor	**0**	**0,5**	**1**	**1,5**	**2**
Bone marrow blasts (%)	**< 5**	**5 - 10**	**---**	**11 - 20**	**21 -30**
Number of cytopenias	**0 - 1**	**2 - 3**			
Karyotype	**good**	**intermediate**	**poor**		

good = normal, -Y, del 5 (q), del 20 (q)

poor = komplex changes (≥ 3 abnormalities), abnormalities of chromosome 7

intermediate = other changes

Risk group	**score**
Low	**0**
intermediate - 1	**0,5 - 1,0**
intermediate - 2	**1,5 - 2,0**
High	**≥ 2,5**

IPSS-R – the same prognostic factors, more detailed cytogenetics,inclusion of the depth of cytopenias

MDS - TREATMENT

1.SUPPORTIVE TREATMENT

red blood cell or platelet transfusions, chelation therapy, vitamins.

2.ANTIAPOPTOTIC AGENTS

steroids

growth factors (EPO, G-CSF)

3.IMMUNOSUPPRESSIVE AGENTS

steroids + Cyclosporin A

(prednisone 20-30 mg/day + CS-A 3 mg/kg/day)

antithymocyte globulin (ATG),

immune modulation - lenalidomide

MDS - TREATMENT

4. HYPOMETHYLATING /DIFFERENTIATION INDUCING AGENTS

5-azacytidine, decitabine – reversion of silencing of differentiation genes (p15)

5. CHEMOTHERAPY

monotherapy – hydroxyurea, etoposide, cytosin arabinosid (low dose: 10-40 mg/m²), melphalan.

combination chemotherapy – anthracyclines + cytosin arabinosid (standard dose 100-200 mg/m², high dose 2-3 g/m²).

6. STEM CELL TRANSPLANTATION

allogeneic from HLA matched related or unrelated donor, haploidentical donor -the only curative treatment.

3 years survival – 40 – 45% RAEB, RAEB-T

60 – 65% RA.

High rate of transplantation related mortality (25-40%) – infections, graft vs. host disease.

APLASTIC ANEMIA / BONE MARROW FAILURE

CRITERIA

Severe aplastic anemia :

peripheral blood - at least 2 criteria :
RTC < 0,1% or < 40 x 10^9/l
NS < 0,5 x 10^9/l, PLT < 20 x 10^9/l

bone marrow - cellularity < 30 %, no fibrosis

ETIOPATHOGENESIS

Idiopathic (70%)

infectious hepatitis
other viral infections (parvovirus B19, rarely HIV)
drugs (CLMF, gold, sulphonamides)
radiation
cytotoxic drugs
infectious disease (tbc), lupus erythematodes

toxic effect on stem cells resulting in apoptosis

toxic effect on bone marrow microenvironment

immunotoxicity (activation of cytotoxic T lymphocytes,
increased production of cytokines – apoptosis of precursor cells)

production of free radicals, direct DNA damage

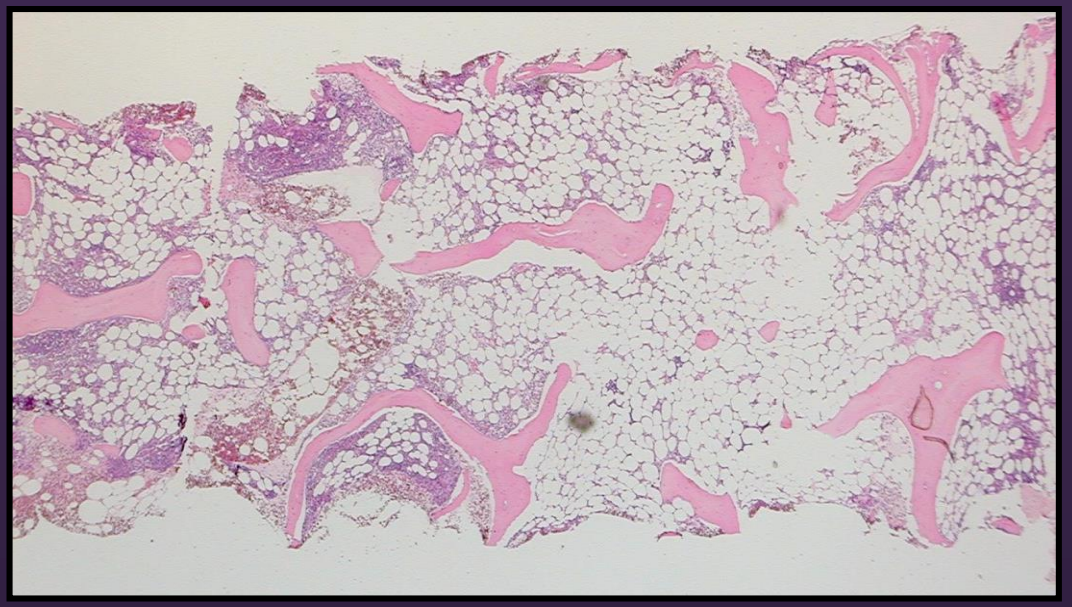
APLASTIC ANEMIA

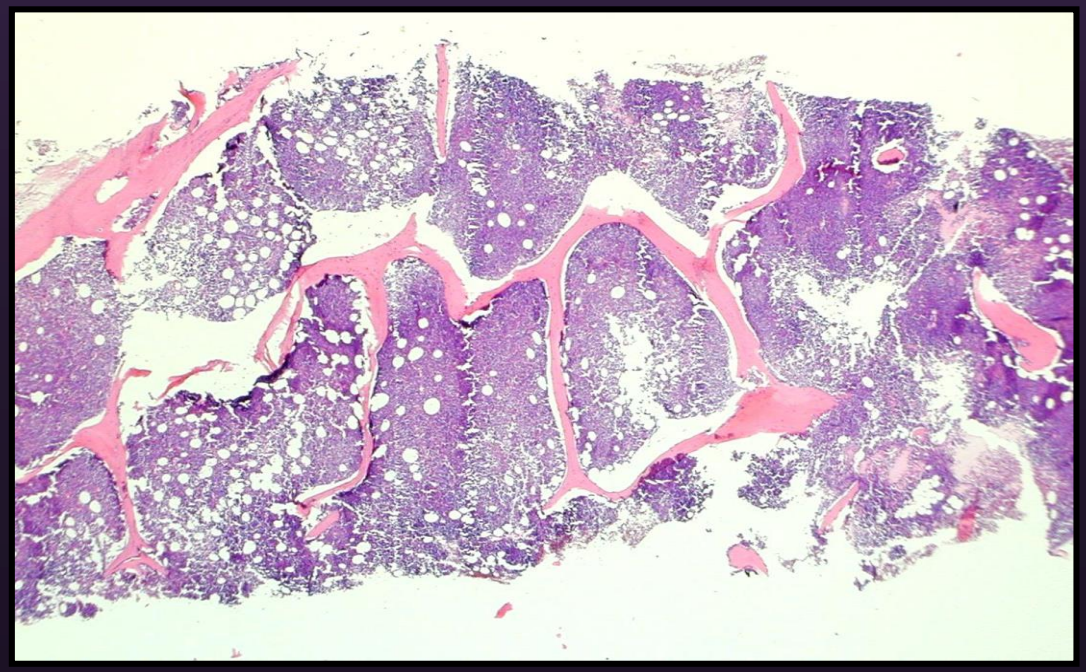
DIAGNOSIS : bone marrow aspiration
bone marrow biopsy
cytogenetics, CFU-GM growth in vitro
flow cytometry

Hypoplastic bone marrow without fibrosis and dysplasia, without karyotype abnormalities, without excess of blasts, decreased in vitro growth of hematopoietic precursors.

DIF. DG.: myelodysplastic syndrome (dysplasia, karyotype abnormalities, excess of blasts)
paroxysmal noctnurnal hemoglobinuria (i.v. hemolysis, CD 59-, CD55-cells)
myelofibrosis (marrow fibrosis,↑% of normoblasts in peripheral blood, splenomegaly)
hypoplastic acute leukemia (increased %of bone marrow blasts)
hairy cell leukemia (fibrosis, hairy cell lymphocytes, splenomegaly)

APLASTIC ANEMIA





MDS

APLASTIC ANEMIA - TREATMENT

a) **ALLOGENEIC STEM CELL TRANSPLANTATION**

serious aplastic anemia, patients aged < 55 years.

efficiency – HLA matched related donor – 3 years survival – 70-90%

unrelated donor or partially matcheddonor – 3 years survival – 60%

b) **COMBINATION IMMUNOSUPRESSION**

ATG – 4 mg/kg/4-5 days + Prednisone - 1 mg/kg/day 14 days +

Cyclosporin A – 3 mg/kg/days 6 months.

efficiency – 3 years survival - 70% patients.

c) **ELTROMBOPAG + EPO**

stimulation of early progenitors in less severe AA , 60-70% efficiency

d) **SUPPORTIVE TREATMENT**

red blood cell + platelet transfusions

antibiotics, growth factors (G-CSF, GM-CSF)

PURE RED CELL APLASIA

normochromic normocytic anemia, ↓ RTC < 0.1%,
↓ bone marrow erythroblasts < 0.5% + normal WBC and PLT counts

CONGENITAL FORM : syndrome Diamond Blackfan

ACQUIRED FORM :

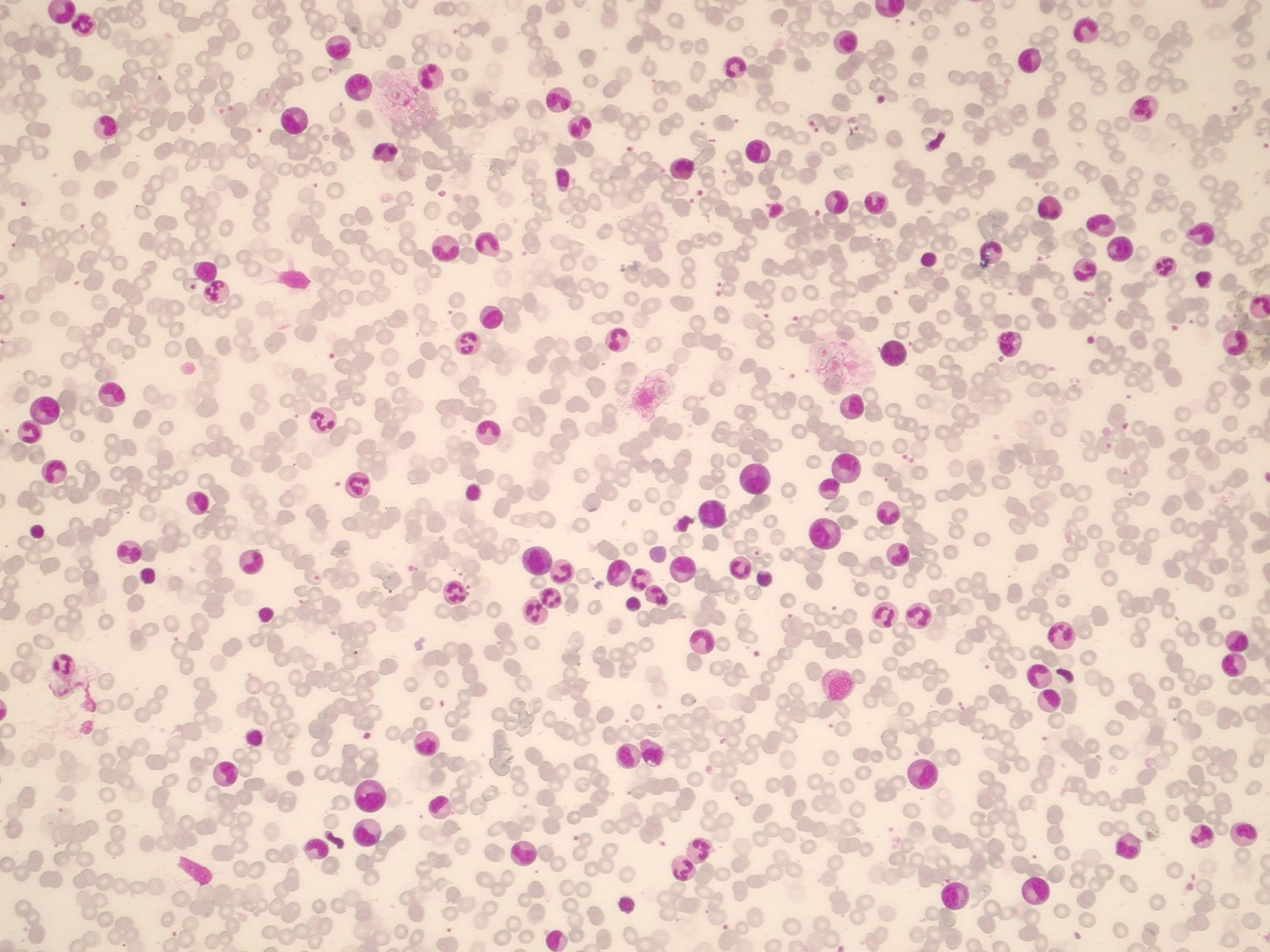
idiopatic
secondary - 1. thymoma

2. hematological malignancies (chronic lymfoproliferative disorders, rarely preceeding AL)

3. infections (parvovirus B19 ,hepatitis, infectious mononucleosis)

4. solid tumors

5. systemic disorders, chronic hemolytic anemias



PATHOGENESIS

IgG plasmatic inhibitor of erythropoiesis,
antibodies against erythropoietin
cytotoxic lymphocytes directed against erythroid precursor cells

DIAGNOSIS

peripheral blood – normocytic normochromic anemia,
RTC < 0,1 %, normal WBC and PLT counts
bone marrow - absent RBC precursors (< 0.5%)
otherwise normal
normal sFe, sFerritin, sBilirubin, sB12, s folic acid, ↑ sEPO
normal karyotype

TREATMENT

Prednison 20-30 mg/day + Cyclophosphamide 50-150 mg/d
+ Cyclosporin A 5-15 mg/d
effective in 80% of patients
ATG, i.v. IgG, plasmapheresis, thymectomy, splenectomy

PAROXYSMAL NOCTURNAL HEMOGLOBINURIA

PATHOGENESIS

mutation of PIG-A gen that is producing glykosylphosphatidyl inositol – a critical component for attachment of some proteins to cell membrane, eg. CD 59 /MIRL/ or CD 55 /DAF/ - inhibitors of activated C3b and C8b parts of complement.
Consequence: an abnormal sensitivity of cells to complement mediated lysis.

CLINICAL MANIFESTATION

hemoglobinuria (in the morning), freqeuntly provoked by stress, infection,
Released Hb → NO consumption – prolonged vasoconstricton → ischemia - abdominal pain,
increased risk of thrombosis (activation of coagulation by free Hb)

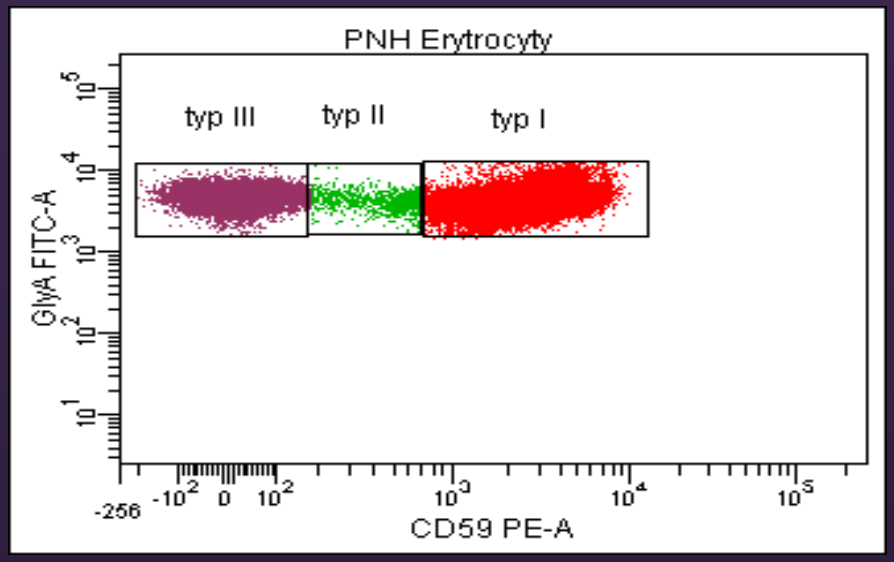
PAROXYSMAL NOCTURNAL HEMOGLOBINURIA

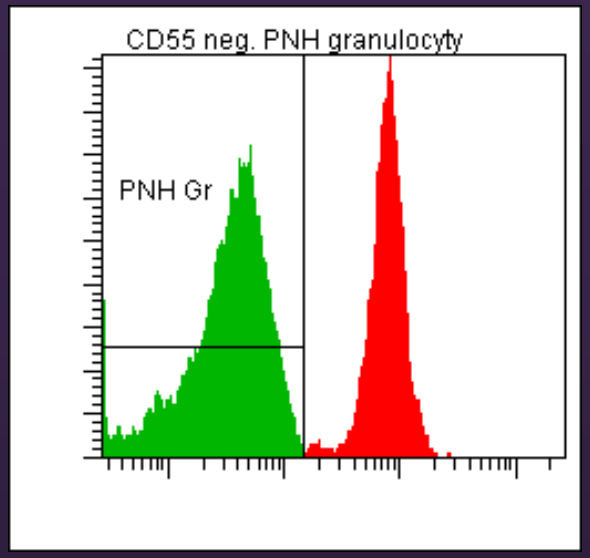
DIAGNOSIS

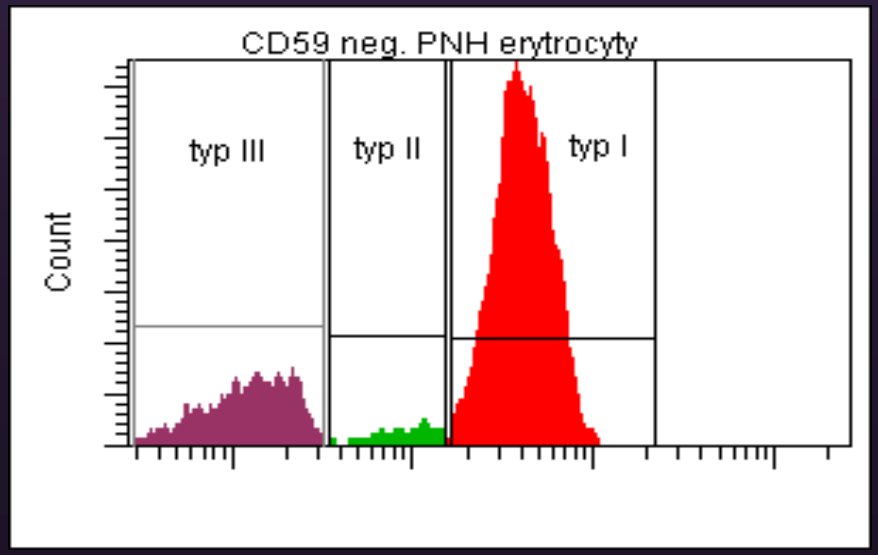
normocytic normochromic anemia, leukopenia, thrombocytopenia
hemoglobinuria, ↑ RTC
↑ sBilirubin, ↑ free plasmatic Hb, ↑ LD but : negative Coombs test, normal osmotic resistance and autohemolysis
Ham/Dacie test – hemolysis of patient,s red blood cells by acidified serum containing complement
Flow cytometry – deficiency of CD 59 and CD 55 on RBC, granulocytes and monocytes

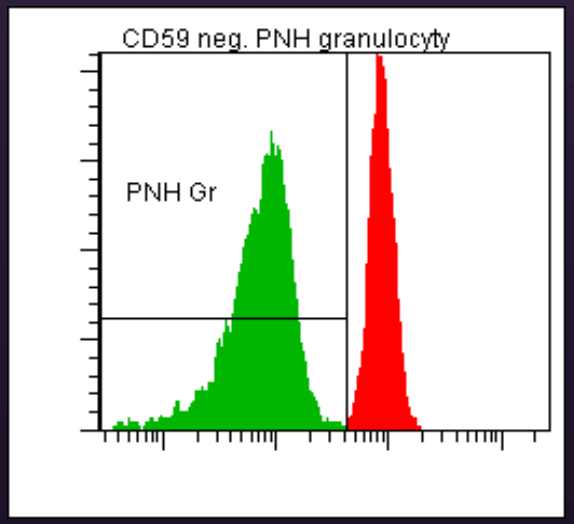
TREATMENT

red blood cell transfusions (leukocyte depleted, washed)
steroids in combination with imunosupressives (CS-A, ATG)
prevention of thrombosis - anticoagulants
stem cell transplantation
inhibition of coagulation cascade (eculizumab)


PNH Erytrocyty
typ III
typ II
typ I
GlyA FITC-A
CD59 PE-A


CD55 neg. PNH granulocyty
PNH Gr

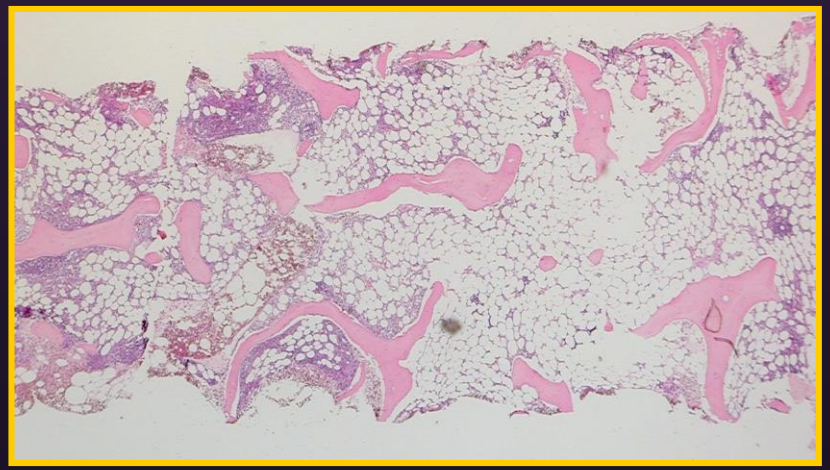

CD59 neg. PNH erytrocyty
typ III
typ II
typ I
Count


CD59 neg. PNH granulocyty
PNH Gr

PERIPHERAL BLOOD CYTOPENIA – DIFFERENTIAL DIAGNOSIS

CYTOPENIA WITH HYPOCELLULAR BONE MARROW

acquired aplastic anemia
congenital aplastic anemia (eg. Fanconi anemia)
hypoplastic myelodysplastic syndrome
hypoplastic acute myeloid leukemia de novo
lymfoproliferative disorders (hairy cell leukemia)
infections (tbc, legionelosis)
hypothyreoidism



PERIPHERAL BLOOD CYTOPENIA – DIFFERENTIAL DIAGNOSIS

CYTOPENIA WITH NORMOCELLULAR OR HYPERCELLULAR BONE MARROW

Primary disorders of bone marrow :

- myelodysplastic syndrome
- some forms of acute leukemias – acute promyelocytic leukemia
- paroxysmal nocturnal hemoglobinuria
- some lymfoproliferative disorders
- myelofibrosis – early stages

Secondary affection of bone marrow caused by systemic disorders :

- lupus erytematodes, sarkoidosis
- infections (tbc, brucelosis, sepsis)