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HEMOLYTIC ANEMIAS

CHISINAU - 2020

HEMOLYTIC ANEMIAS **are the erythrocyte disorders predominantly caused by the increased red cell destruction. Hemolytic anemias comprise approximately 11% of the total anemia population. Persons of all ages may be affected.**

HEMOLYSIS **may occur predominantly within the circulation (intravascular) or within the tissue macrophages (extravascular). The clinico-laboratory picture of hemolytic anemia includes anemic and hemolytic syndromes.**

HEMOLYTIC SYNDROME **enrolls the following features:**

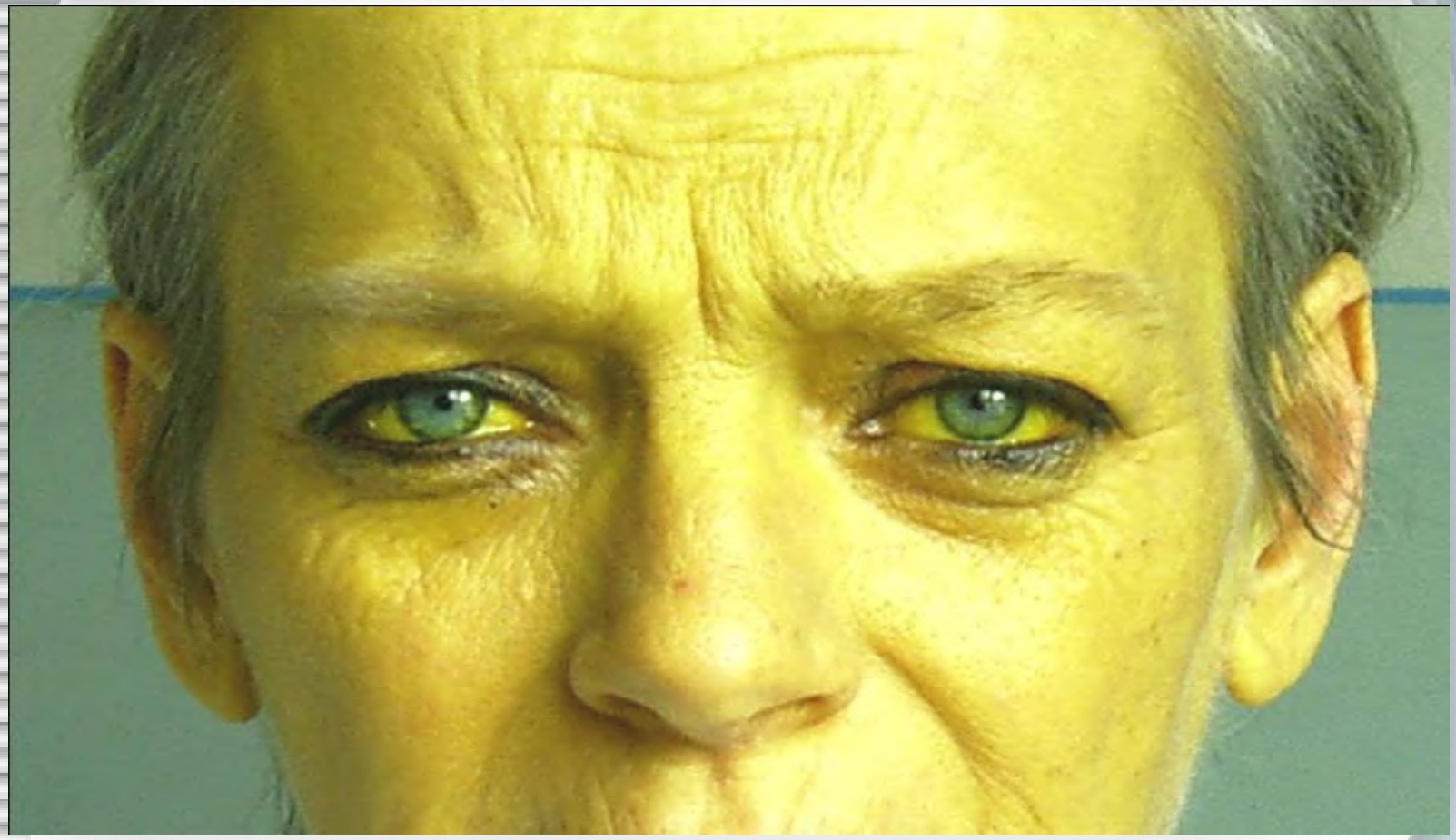
1) **Clinical – pallor, jaundice, splenomegaly, and**

2) **Laboratory, which can be divided conveniently into two groups:**

those related to the increase of erythrocyte destruction: increased unconjugated serum bilirubin, urobilinuria, decreased serum haptoglobin;

those related to the compensatory increase in the rate of erythropoiesis: elevated reticulocyte count (reticulocytosis), erythroid hyperplasia of the bone marrow.

CLINICAL FEATURES OF HEMOLYTIC SYNDROME: JAUNDICE



CLASSIFICATION OF HEMOLYTIC ANEMIAS:

I. HEREDITARY HEMOLYTIC ANEMIAS

1. Defects in the erythrocyte membrane (hereditary spherocytosis, hereditary elliptocytosis, hereditarystomatocytosis, hereditary acanthocytosis)

2. Deficiency of erythrocyte enzymes

a) Anemias associated with the abnormalities of erythrocyte anaerobic glycolysis;

b) Anemias associated with the abnormalities of nucleotide metabolism;

c) Anemias associated with the deficiences involving the pentose phosphate pathway: glucose-6-phosphate dehydrogenase deficiency;

d) Anemias associated with the deficiencies of glutathione metabolism;

e) Anemias related to the deficiencies of ATP metabolism;

3. Defects in globin structure (sickle cell anemia) and synthesis (thalassemias)

II. ACQUIRED HEMOLYTIC ANEMIAS

1. Immune hemolytic anemias (antibody-mediated anemias)

a) Isoimmune hemolytic anemias;

b) Autoimmune hemolytic anemias;

c) Heteroimmune hemolytic anemias;

d) Transimmune hemolytic anemias;

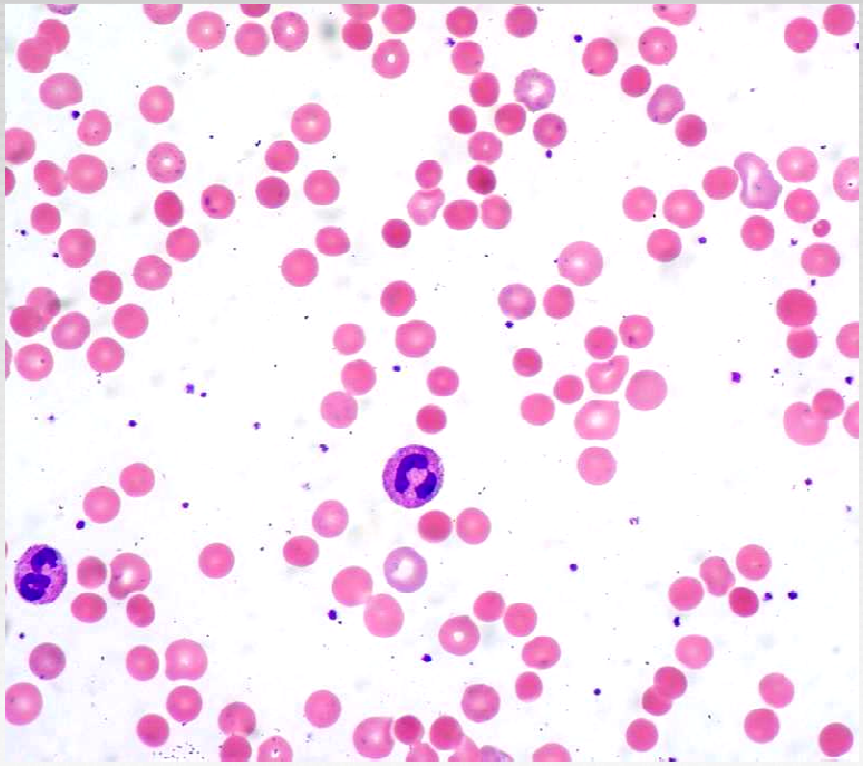
2. Hemolytic anemias due to the mechanical damage: microangiopathic, traumatic cardiac, march hemoglobinuria, etc.

3. Hemolytic anemias due to the chemical or physical agents

4. Hemolytic anemias due to the infections with microorganisms: malaria, toxoplasmosis, etc.

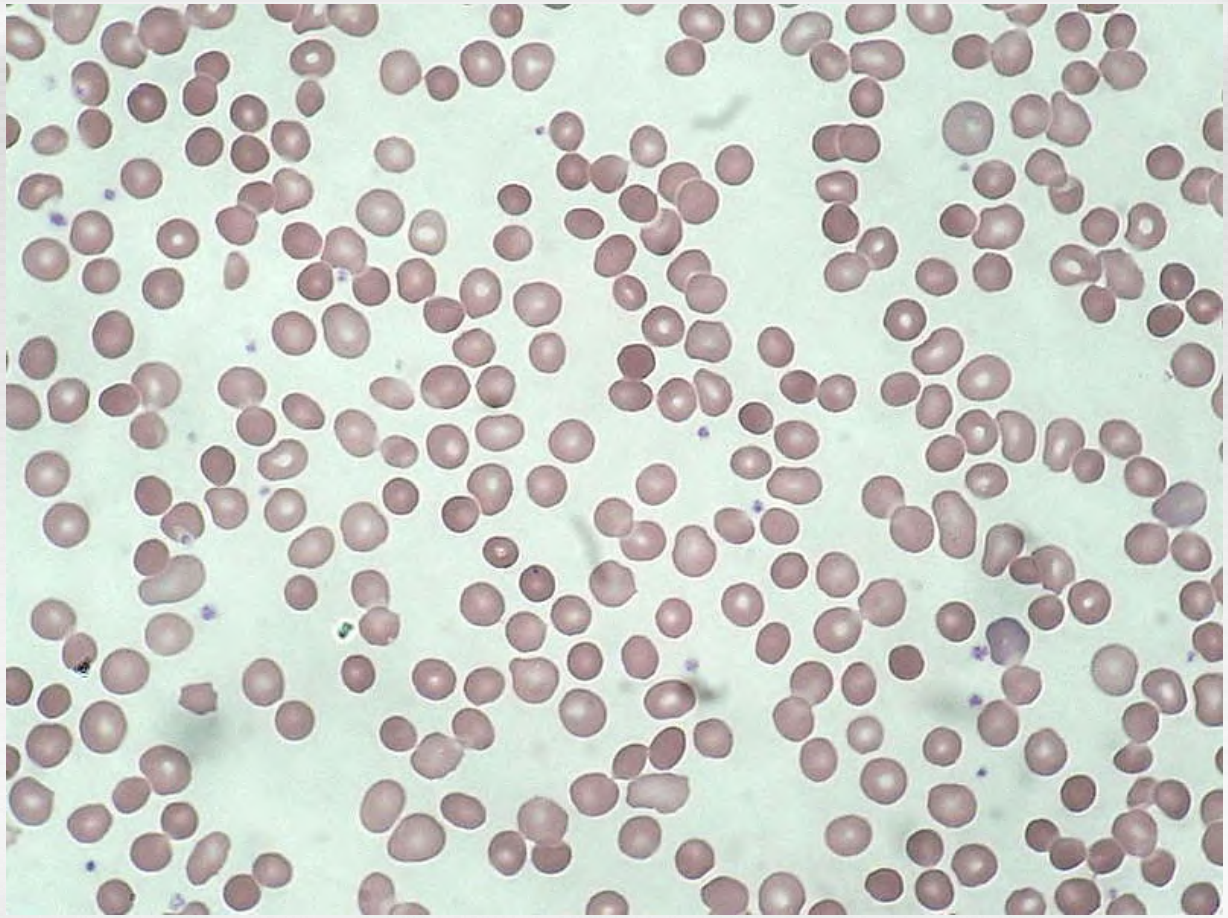
5. Hemolytic anemias due to the somatic mutation: paroxysmal nocturnal hemoglobinuria

BLOOD SMEAR IN HEREDITARY SPHEROCYTOSIS



Wright-Giemsa staining. Magnification: x 400.

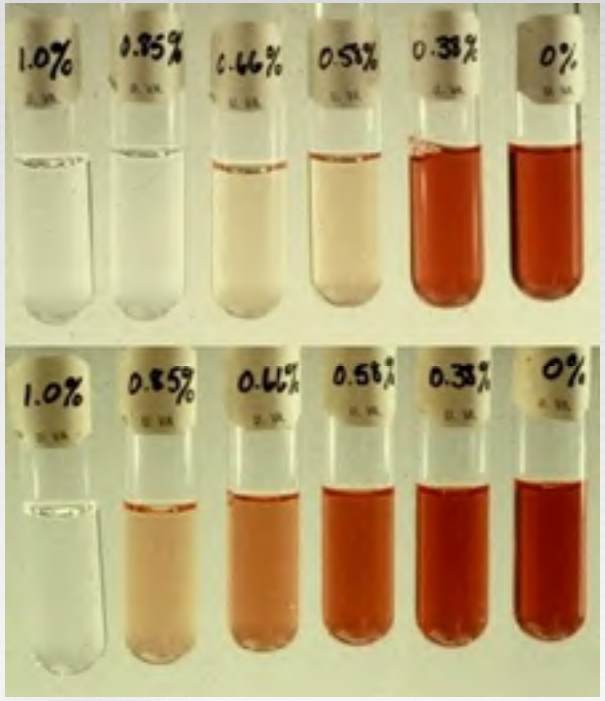
BLOOD SMEAR IN HEREDITARY SPHEROCYTOSIS

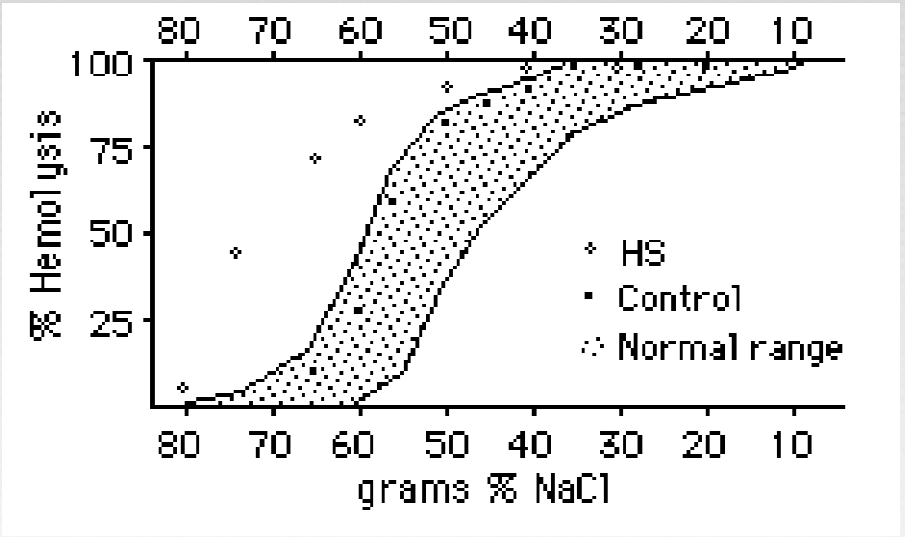


Staining: MGG. Magnification: x 500.

OSMOTIC FRAGILITY OF ERYTHROCYTES IN HEREDITARY SPHEROCYTOSIS

Normal osmotic fragility is seen at the top.

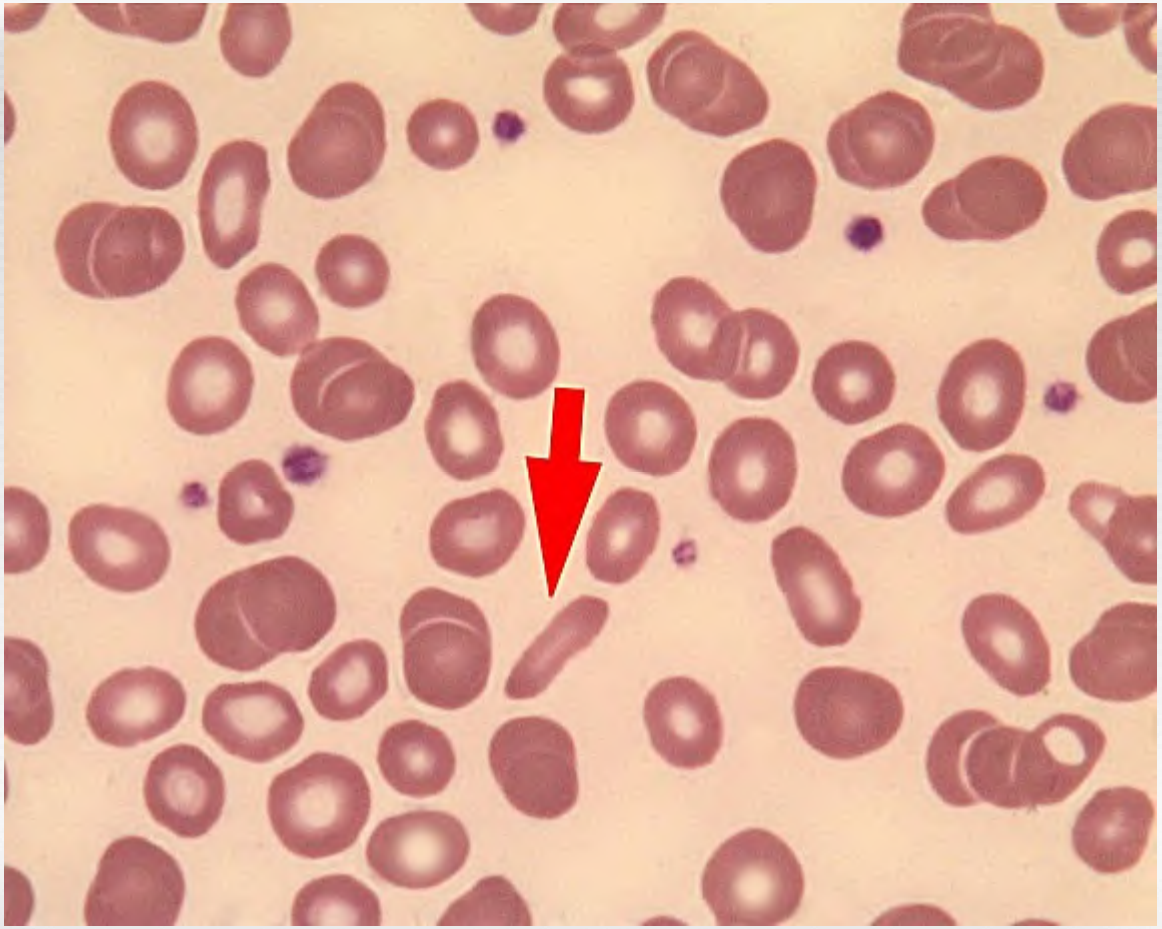




Osmotic fragility test results.

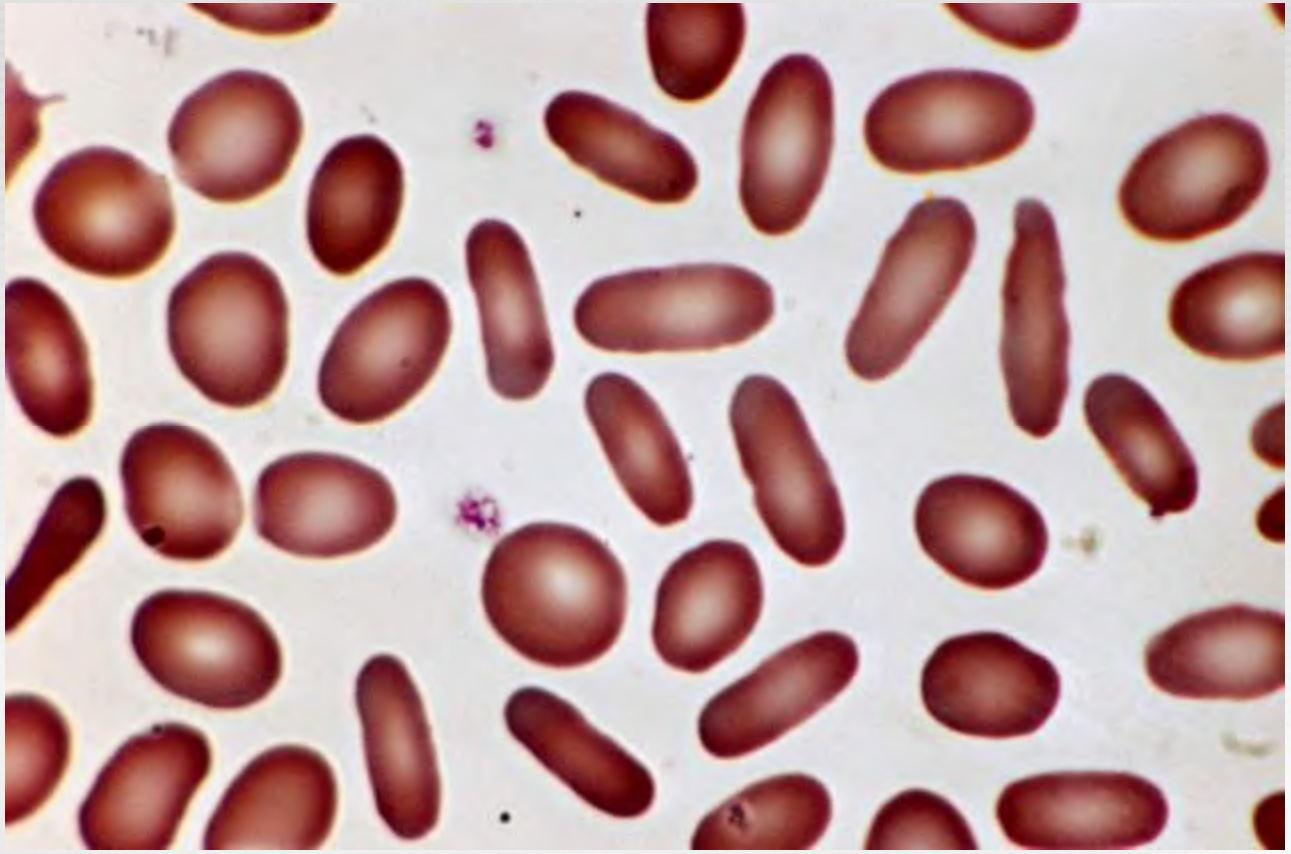
Abnormal lysis of RBCs in mildly hypotonic solutions is seen at the bottom (hereditary spherocytosis).

BLOOD SMEAR IN HEREDITARY ELLIPTOCYTOSIS



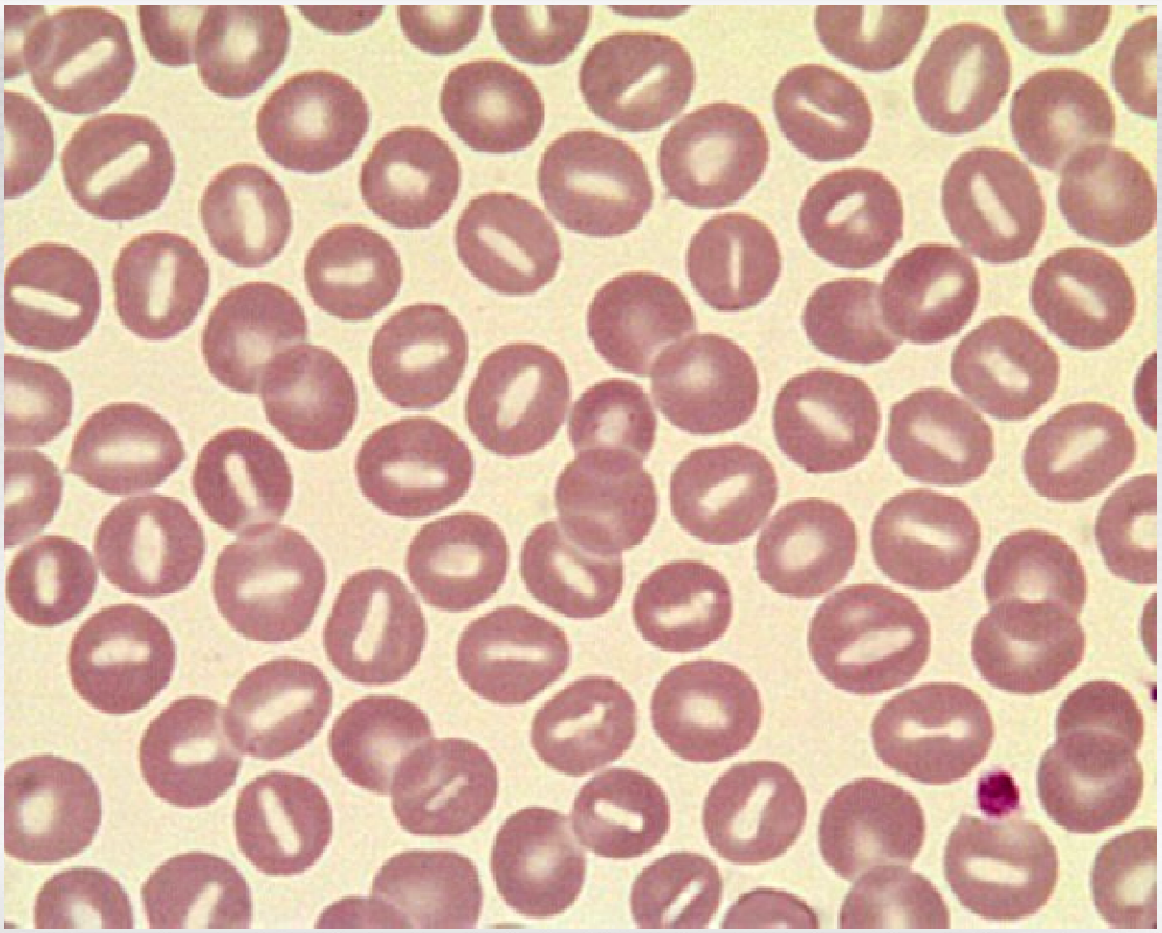
Oval or elliptical erythrocytes. Indicated by the arrow extremely elongated ovalocyte is sometimes called pencil-like cell. Besides it other six ovalocytes less elongated are seen.
Also distinct anisocytosis is present. Normal platelets are present.
Staining: MGG. Magnification: x 1000.

BLOOD SMEAR IN HEREDITARY ELLIPTOCYTOSIS



Staining: MGG. Magnification: x 1000.

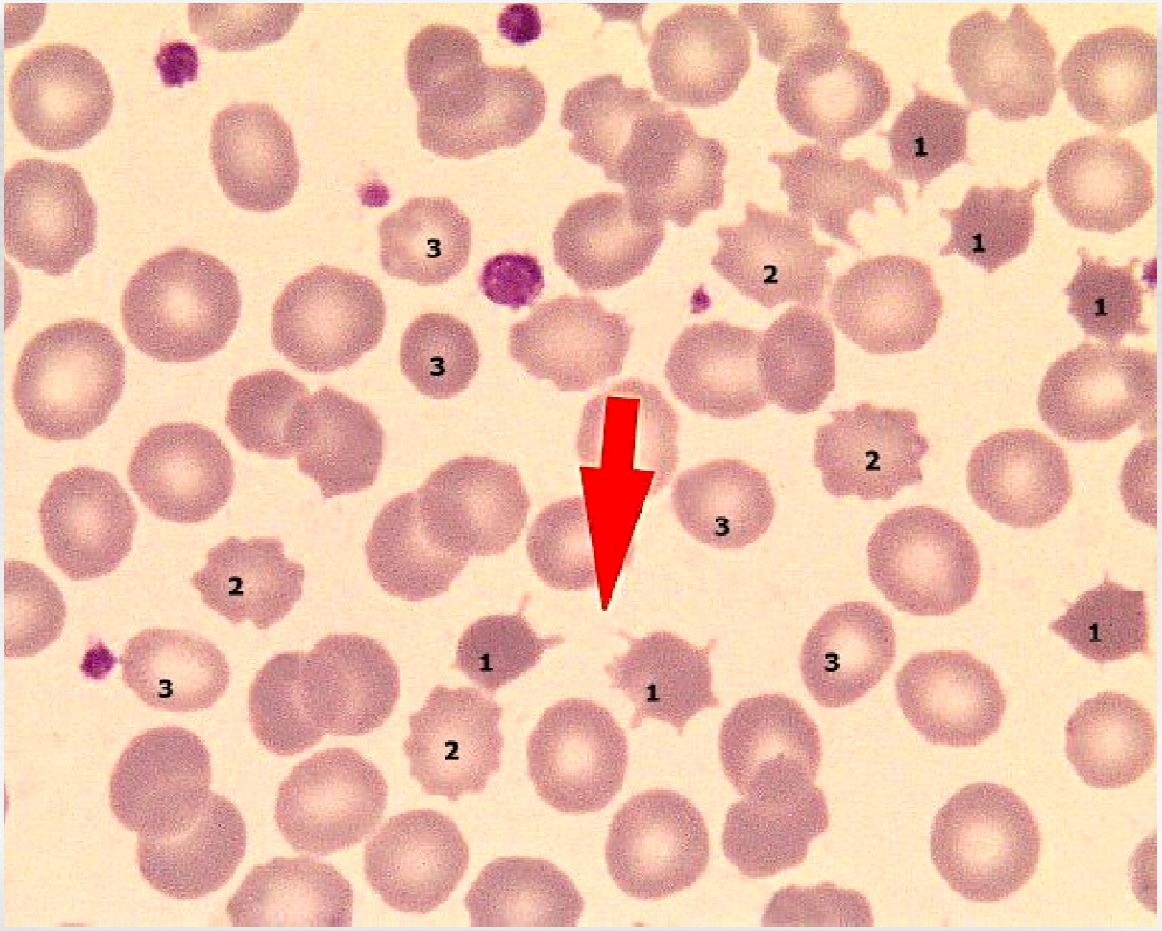
BLOOD SMEAR IN HEREDITARY STOMATOCYTOSIS



The erythrocyte with elongated central palor. Very numerous stomatocytes in the course of inherited stomatocytosis.

Staining: MGG. Magnification: x 1000.

BLOOD SMEAR IN HEREDITARY ACANTHOCYTOSIS



Erythrocytes with irregular, long, sharply pointed and bent spicules of cytoplasm.
In the picture six acanthocytes are shown (two of them indicated by the arrow) and several echinocytes.

Also slight microcytosis can be seen. 1. acanthocyte 2. burr-cell 3. microcyte

Staining: MGG. Magnification: x 1000.

CLASSIFICATION OF β-THALASSEMIAS:

I. Homozygous form:

1. Thalassemia major, or Cooley' anemia.

II. Heterozygous form:

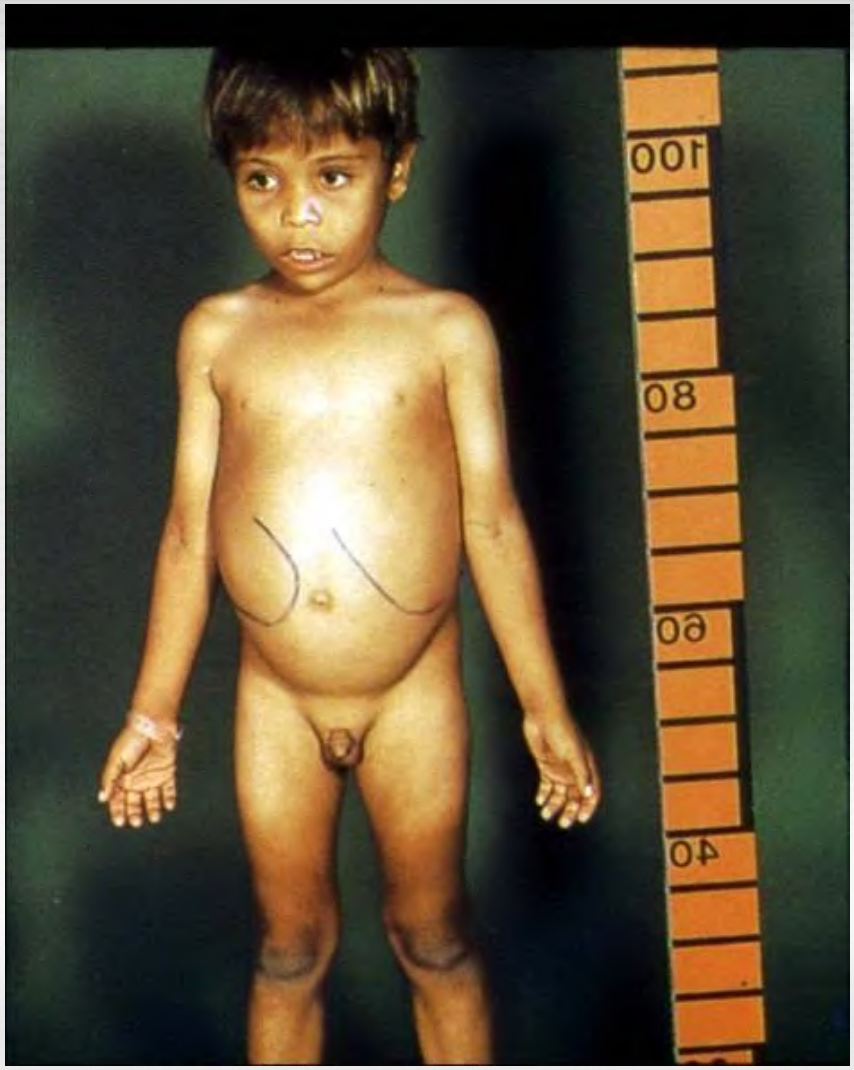
1. Severe hemolytic anemia, with chronic hemolysis, jaundice, splenomegaly;

2. Thalassemia Minor, with a mild to moderate hypochromic anemia, moderate splenomegaly (in 50% of cases), and minimal skeletal changes;

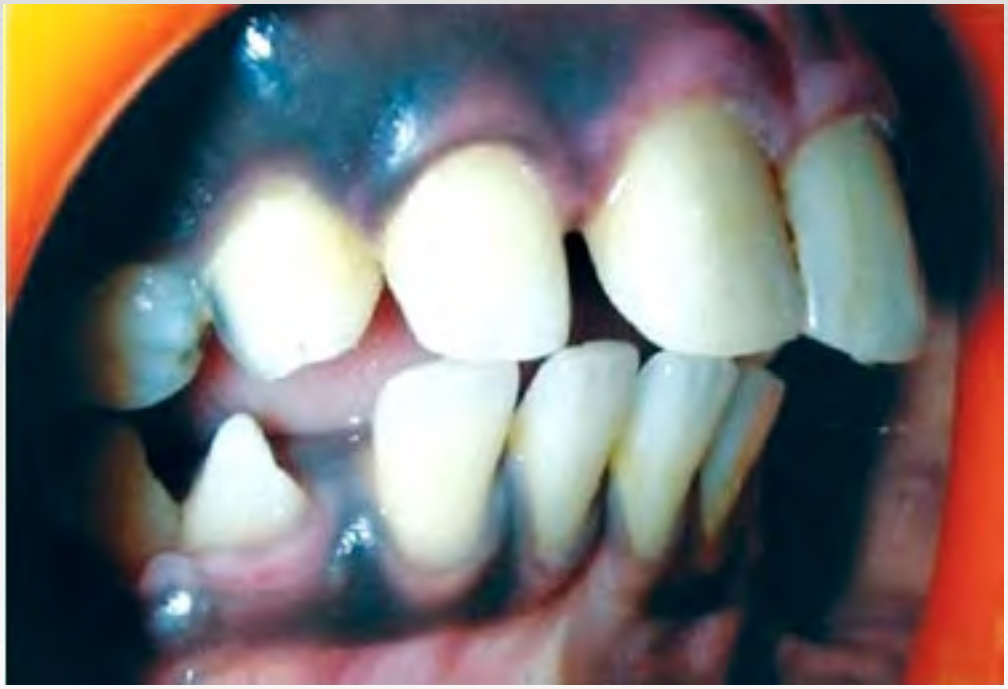
3. Thalassemia Minima – an asymptomatic form, with normal or slightly subnormal hemoglobin level;

4. Asymptomathc β–thalassemias.

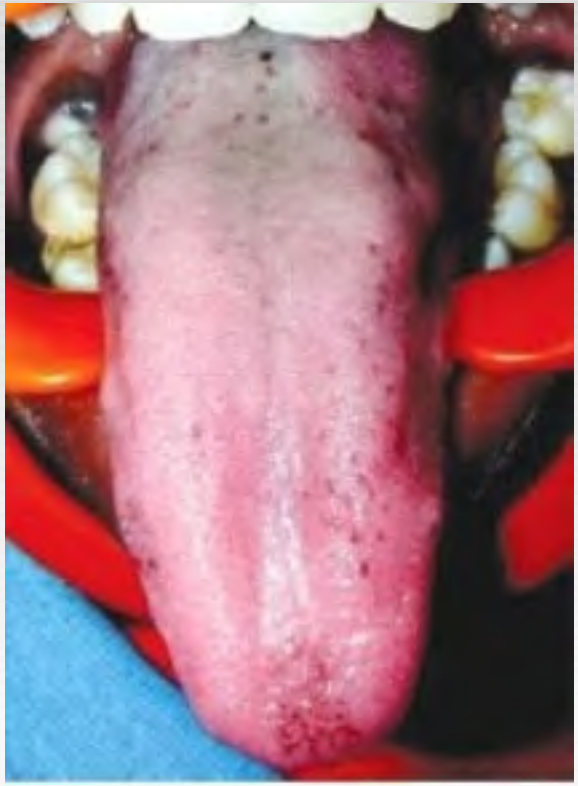
CLINICAL FEATURES OF THALASSEMIA: JAUNDICE, "THALASSEMIC FACIES", SPLENOMEGALY, HEPATOMEGALY, DELAY OF SKELETAL AND SEXUAL MATURATION



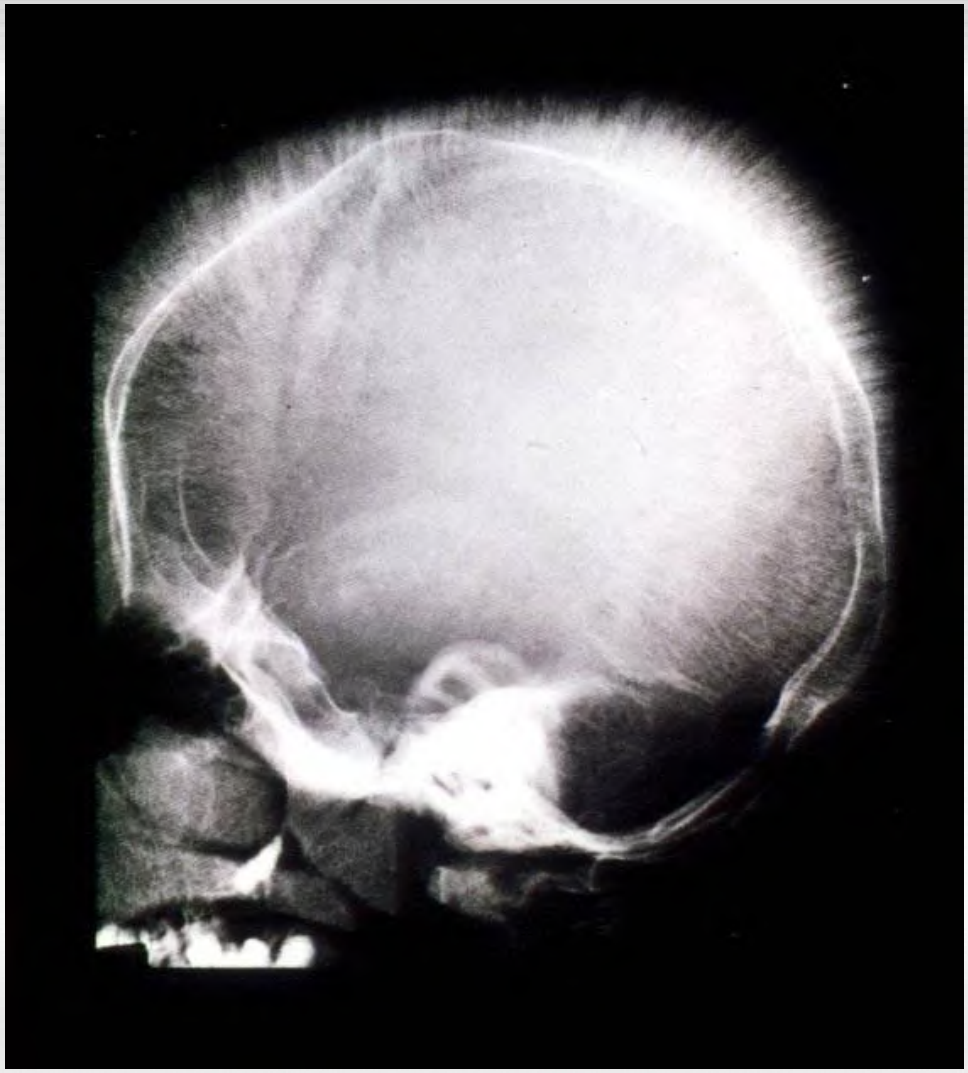
ORO-FACIAL MANIFESTATIONS OF THALASSEMIA: GINGIVAL PIGMENTATION, PROCLINATION OF TEETH AND SPACING



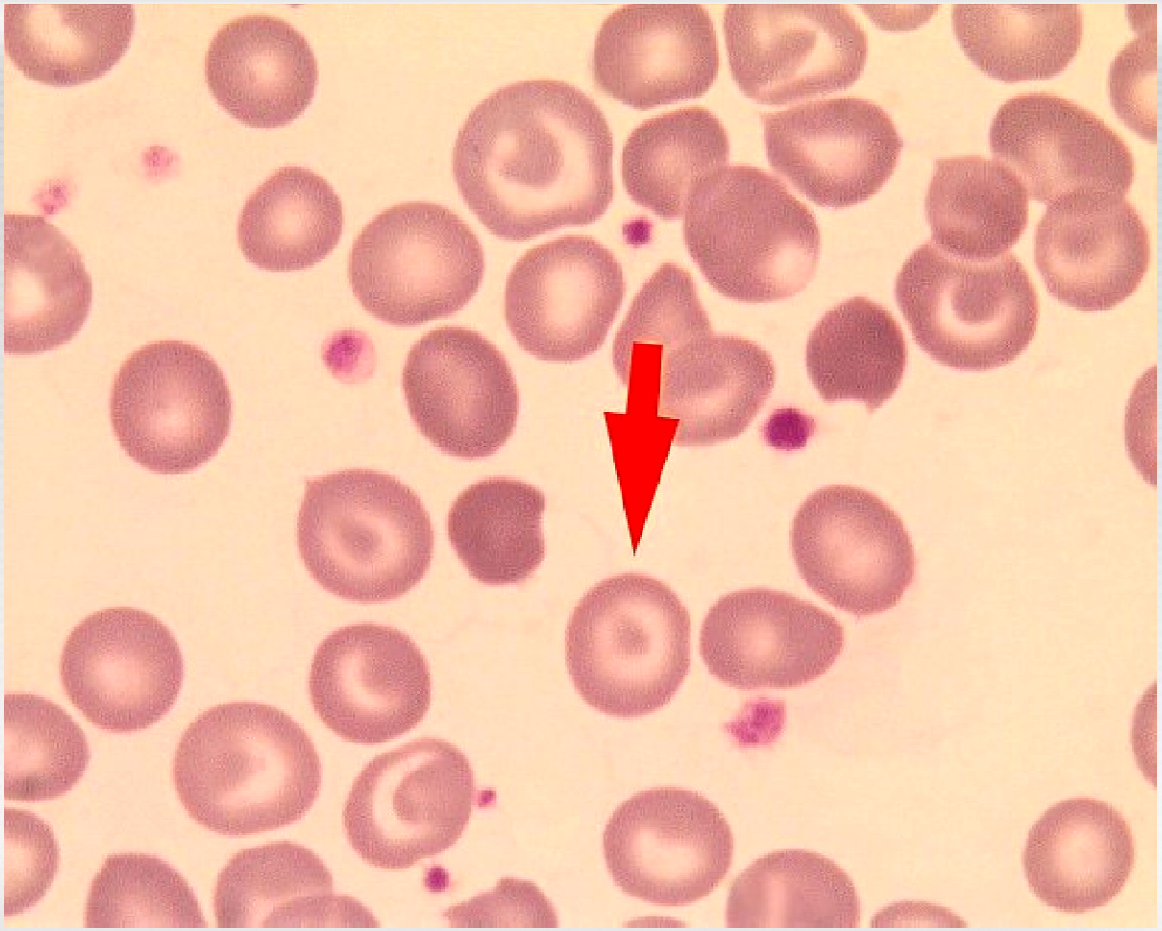
ORO-FACIAL MANIFESTATIONS OF THALASSEMIA: PIGMENTATION OF TONGUE



X-RAY FEATURES OF THE CRANIAL BONES IN THALASSEMIA: THINNING OF THE CORTICES, SPARSENESS OF THE TRABECULAR PATTERN, FRONTAL BOSSING



BLOOD SMEAR IN THALASSEMIA

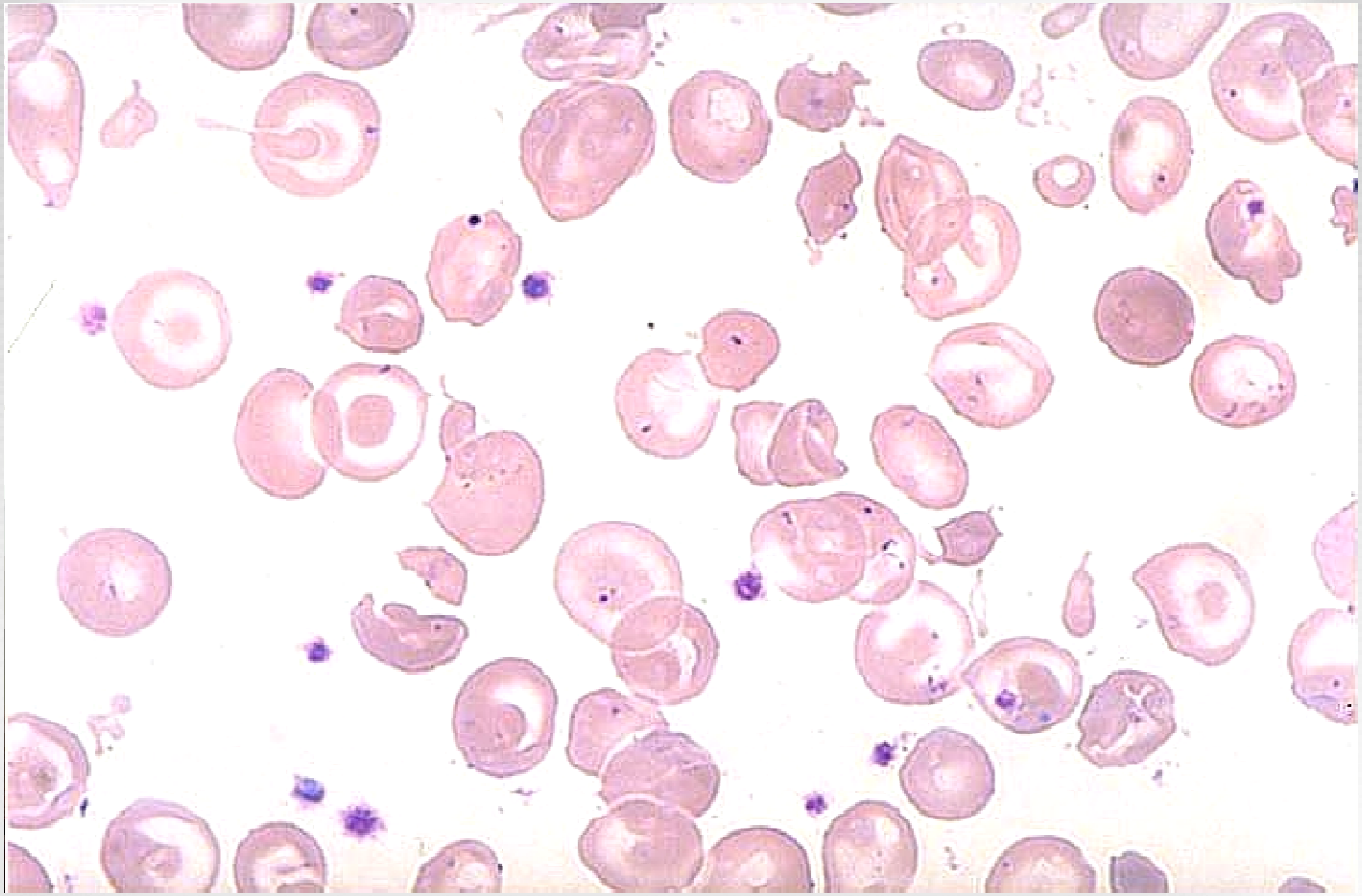


Erythrocyte containing dark stained central area surrounded by lightly stained ring of cytoplasm without hemoglobin. In the picture seven target cells are present.

Also slight anisocytosis of the erythrocytes and platelets.

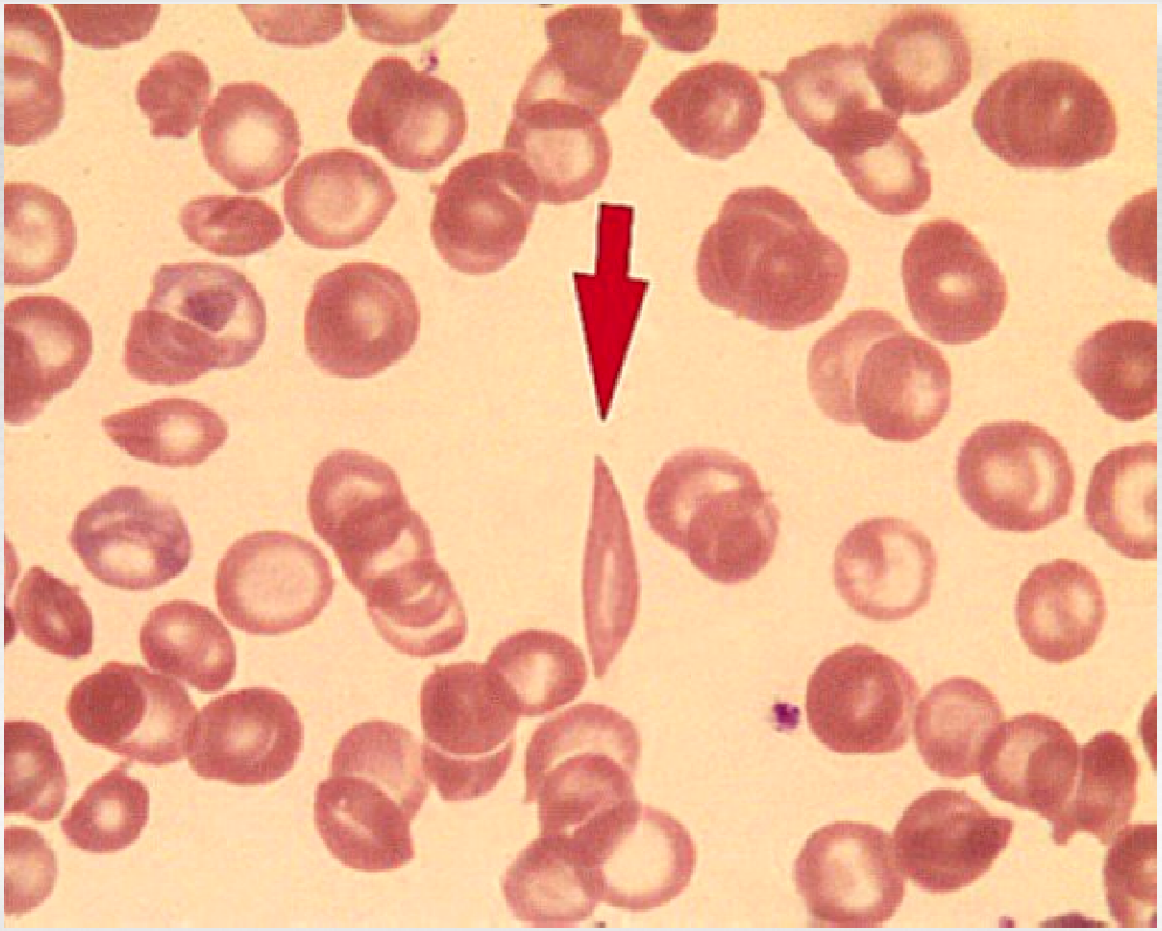
Staining: MGG. Magnification: x 1000.

BLOOD SMEAR IN THALASSEMIA



May-Giemsa staining. Magnification: x 1000.

BLOOD SMEAR IN sickle cell anemia



Elongated erythrocytes, usually curved with sharply pointed one or two poles. Single sickle cell. Also distinct anisopoikilocytosis and staining disturbance of erythrocytes.

Staining: MGG. Magnification: x 1000.

CLASSIFICATION OF AUTOIMMUNE HEMOLYTIC ANEMIAS:

I. On the basis of serologic characteristics:

1. Warm autoantibody type;

2. Cold autoantibody type;

3. Biphasic hemolysin type;

4. Complete cold agglutinin type

II. On the basis of presence or absence of underlying or associated disorder:

1. Primary or idiopathic;

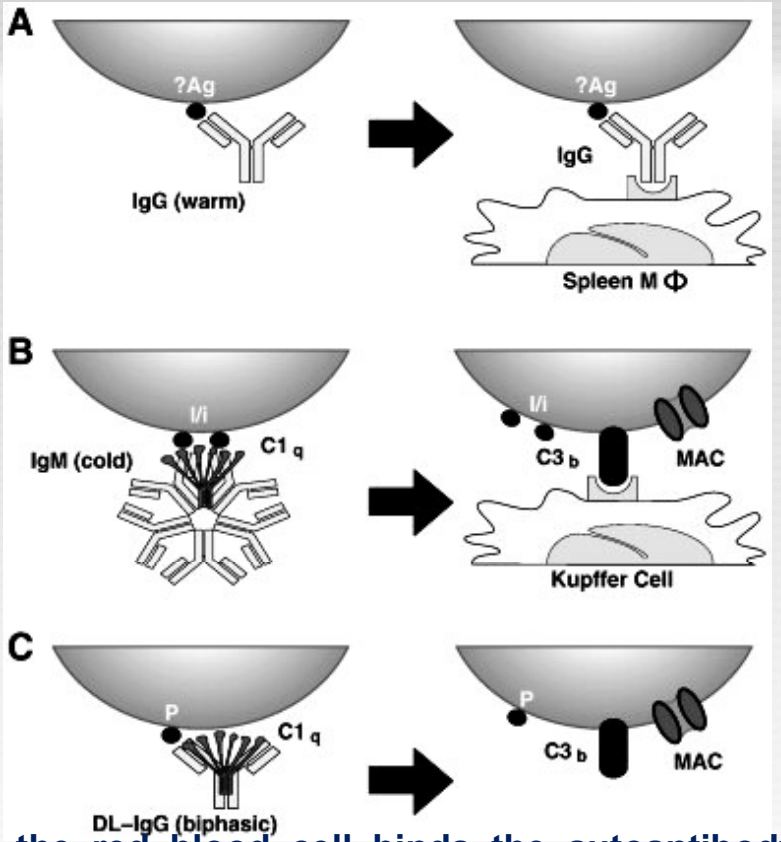
2.Secondary or symptomatic (in lymphoproliferative, rheumatic, chronic inflammatory diseases, in certain infections, etc.)

III. On the basis of the affinity to the type of red cell antigen:

1. Autoantibodies with affinity for the antigens of the peripheral red cells;

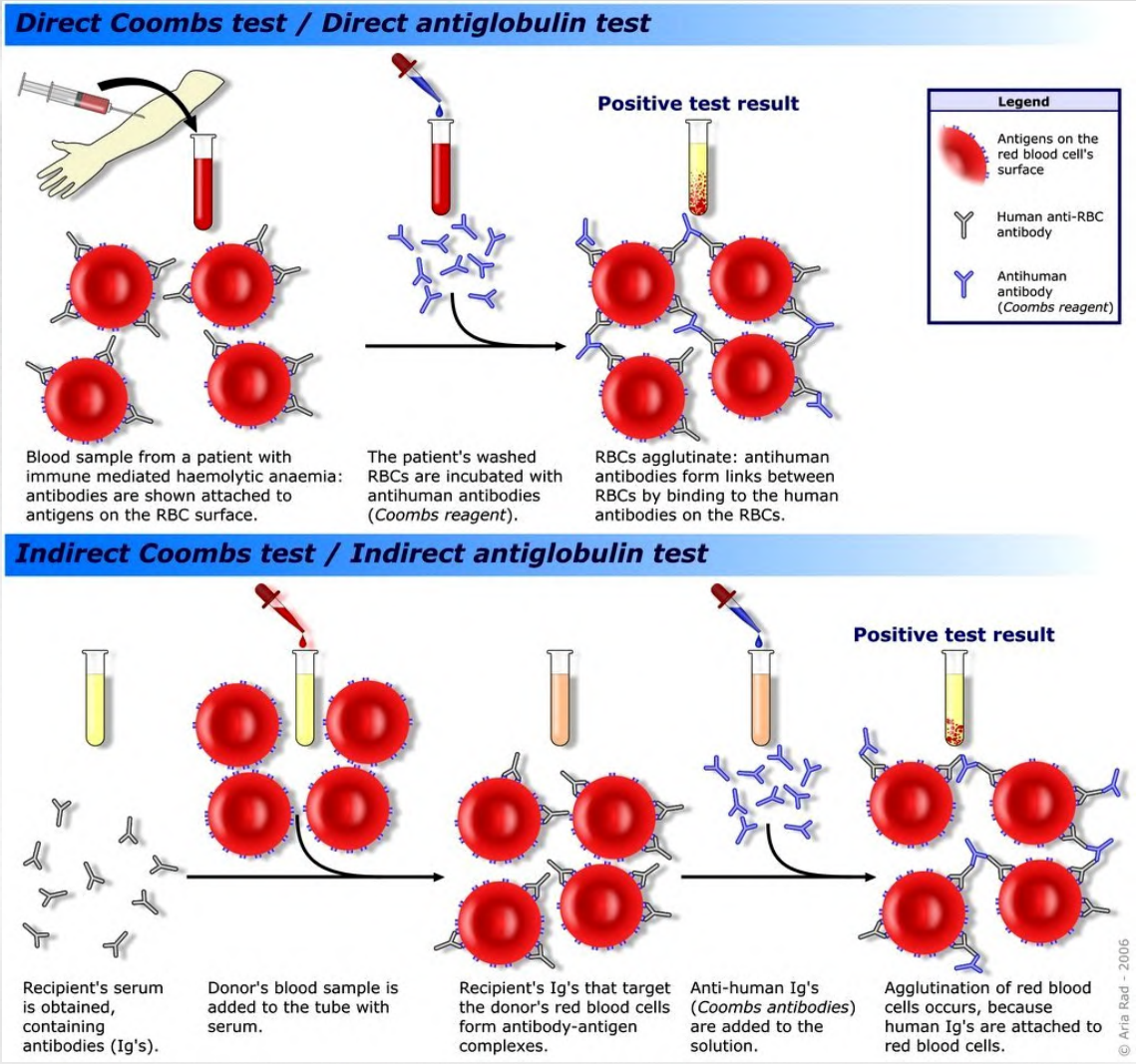
2. Autoantibodies with affinity for the antigens of the bone marrow nucleated red cells.

MECHANISTIC BACKGROUND OF RED BLOOD CELL HEMOLYSIS IN AUTOIMMUNE HEMOLYTIC ANEMIAS



A. **In warm reactive IgG AIHA, the red blood cell binds the autoantibody to a membrane expressed cognate autoantigen of elusive specificity. Thereafter (right panel), the RBC is trapped by spleen macrophages through Fc receptor interaction.** ***B.*** **In cold reactive IgM AIHA, the I/i antigens on RBCs become covered with IgM class antibodies simultaneously fixing C1q whereupon the IgM leaves the cell at higher temperature leaving opsonising complement (C3b) and MAC back on the cell. Thereafter, RBC is trapped by liver macrophages (Kupffer cells) through complement receptor interaction.** ***C.*** **In paroxysmal cold hemoglobinuria, the biphasic Donath–Landsteiner IgG interacts with P antigen carrying red blood cells at cold temperature, fixes complement, and leaves the cell surface at warm temperature. Thereafter, complement dependent hemolysis is triggered mainly through membrane attack complex (MAC) perforation.**

COOMBS TESTS



TREATMENT OF *AUTOIMMUNE HEMOLYTIC ANEMIAS:*

Steroids inhibit the antibody production by lymphocytes and the function of macrophages. Treatment is initiated with doses of oral prednisolone 1 mg/kg daily, that constitutes approximately 60 mg/day. Critically ill patients with rapid hemolysis should receive prednisone therapy equivalent to at least 80 – 150 mg/day. In patients with concurrent underlying disorders, such as coronary artery disease with angina, or in patients who suddenly develop severe anemia and exhibit signs of circulatory failure, red cell transfusions may prove lifesaving. Red cells for transfusion should be selected by indirect Coombs test. Relief from anemia can be expected in 77.5% of cases within 4 – 6 weeks of treatment. With continued improvement in hematocrit , the prednisone dose may be further tapered at a rate of 5 mg per day every week, to a dose of 15 – 20 mg daily. Patients will require these maintenance doses for 3 – 4 months. Stable complete remission may be achieved by prednisone therapy in 5% of cases.

The relapses may serve as the indication for splenectomy, which ensures stable complete remission in 74% of cases (И.Л. Идельсон et al.). Patients, who fail to respond to splenectomy, require immunosuppression with vincristine, cyclophsphamide, etc. Immunosuppressive agents (antilymphocyte or antithymocyte globulin, cyclosporine A) may be also administered prior to splenectomy, or if the latter is contraindicated.

Patients with mild cold antibody AIHA require no specific therapy. In such patients the most important principle of management is the maintenance of an ambient temperature above the maximum temperature at which the antibodies react. Steroids are used in severe hemolytic crises. Splenectomy is not efficient.

The treatment of erythroblastopenia is carried out according to the principles of treatment of the warm antibody AIHA.

CLASSIFICATION OF COLD ANTIBODY AUTOIMMUNE HEMOLYTIC ANEMIAS:

I. Cold agglutinin disease;

II. Paroxysmal cold hemoglobinuria.

COLD AGGLUTININ DISEASE **is traditionally considered to occur in primary (idiopathic) and secondary forms. The most common settings for the secondary form are (1) an acute, self-limited hemolytic process occasionally complicating infections (Mycoplasma pneumoniae, infectious mononucleosis, etc.) and occurring mainly in adolescents or young adults; and (2) a chronic disorder occurring in older patients with malignant lymphoproliferative diseases.**

The clinical picture may be that of moderate hemolytic anemia without splenomegaly. In other patients, the principal feature is episodic, acute hemolysis, induced by chilling. Acrocyanosis and other cold-mediated, vaso-occlusive phenomena affecting the fingers, toes, nose, and ears are associated with sludging of red cells in the cutaneous microvasculature.

Often cold hemagglutination is observed, that makes difficult the determination of red cell count and blood groups. The autoantibodies are of IgM type.

PAROXYSMAL COLD HEMOGLUBINURIA **is a very rare (1 - 2% of total cases) AIHA. This form of AIHA is mediated by biphasic Donath-Landsteiner autoantibodies of Ig G type. Donath-Landsteiner autoantibodies and early-acting complement components apparently bind to red cell P antigen. The hemolysis is intravascular. Constitutional symptoms are prominent a few minutes to several hours after cold exposure. The patient develops aching pains in the back or legs, abdominal cramps. Chills and fever usually follow. Raynoud's phenomenon and jaundice sometimes occur. Spleen and liver are not enlarged. Laboratory findings: anemia, reticulocytosis, hemoglobinemia, hyper-bilirubinemia, and hemoglobinuria may be present. Donath-Landsteiner test is the major test procedure for establishing the immune nature of this form of AIHA.**

Another rare form of AIHA is that mediated by antibodies with affinity for the antigens of the bone marrow nucleated red cells. It is characterized by a decrease or absence of nucleated red cells in the bone marrow. Anemia is severe and chronic. Reticulocytosis is absent. Jaundice is not observed. Red cells are normochromic. Leukocyte and thrombocyte counts are within the normal limits. Recovery of the nucleated red cells in the bone marrow and reticulocytosis certify the efficiency of treatment.

PAROXYSMAL NOCTURNAL HEMOGLOBINURIA

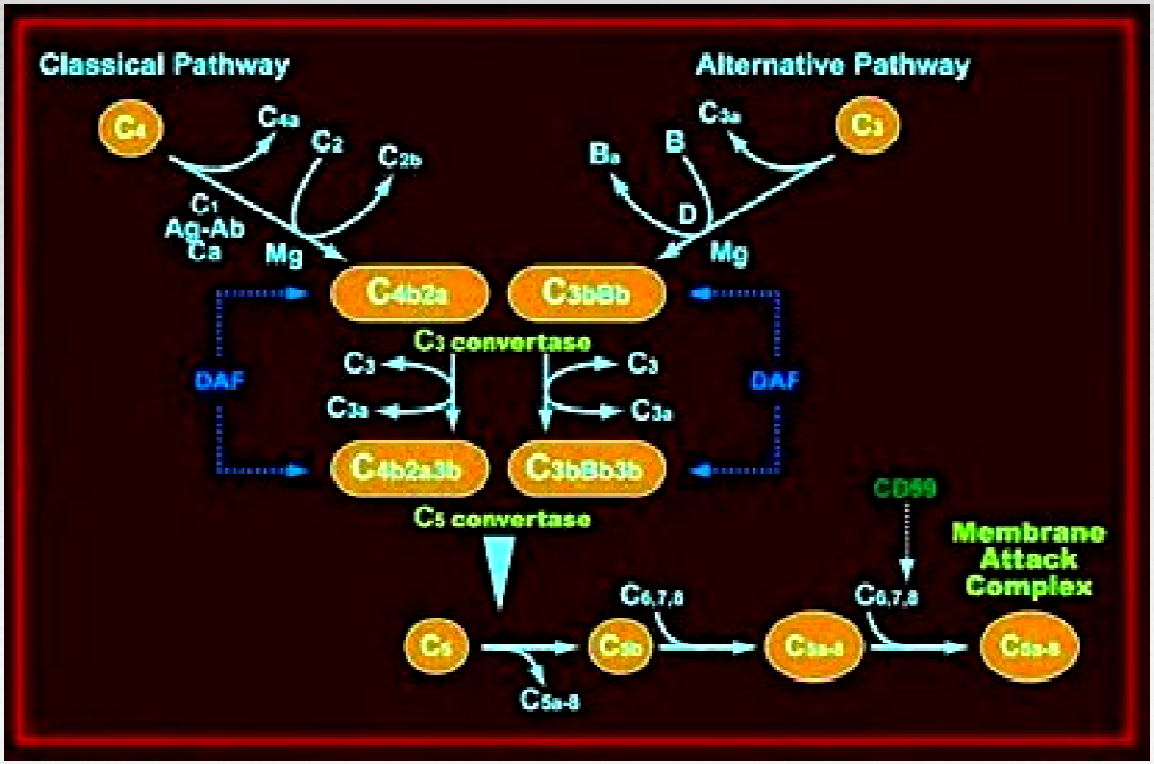
PAROXYSMAL NOCTURNAL HEMOGLOBINURIA (PNH), **also known as the Marchiafava – Micheli anemia is an uncommon disorder, which affects both males and females of all ages. The disease has been described in many racial groups.**

ETIOLOGY. **PNH is an acquired hemolytic anemia. PNH is due to a structural or biochemical defect of the red cell membrane, but the nature of the defect remains elusive. The membrane glycoproteins are supposed to be affected. The abnormal cells in PNH appear to be products of an abnormal clone of myeloid precursor cells originated as the result of a somatic mutation.**

PATHOGENESIS. **Cross-transfusion studies have established that PNH is due to an intrinsic abnormality of the red cells, as normal cells survive normally in patients with PNH, while PNH erythrocytes have a shortened life-span within the patient or in a normal recipient. Erythrocyte lysis is mediated by C_3 component of complement. Excessive lysis in acidified human serum is a characteristic feature of PNH cells. PNH may be manifested by intravascular hemolysis, or by pancytopenia, iron deficiency. PNH granulocytes and platelets appear to share the membrane defect of PNH red cells, since they also are much more sensitive to lysis by complement. That's why leukopenia and thrombocytopenia may occur. Nocturnal hemoglobinuria, when present, occurs as the result of an increase in hemolysis during sleep due to a slight fall in plasma pH. In normal conditions the renal threshold for free hemoglobin constitutes 120 – 130 mg%. In cases with PNH, the haptoglobin level is reduced and the renal threshold for free hemoglobin is below 120 mg%.**

COMPLEMENT ACTIVATION AND REGULATION IN PAROXYSMAL NOCTURNAL HEMOGLOBINURIA

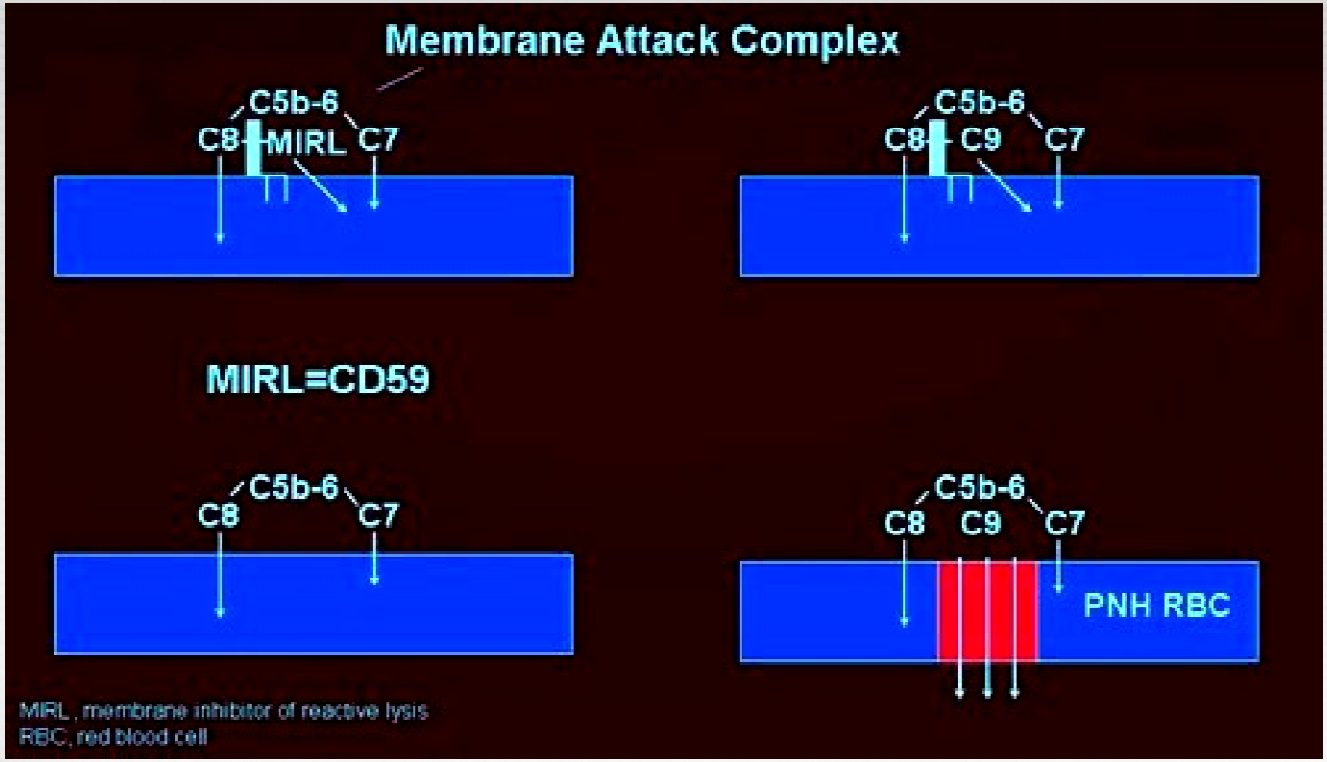
(Nakakuma H., Kawaguchi T., Crit. Rev. Oncol. Hematol., 1996; 24: 213 – 229)



DEFICIENT INACTIVATION OF COMPLEMENT

IN PAROXYSMAL NOCTURNAL HEMOGLOBINURIA

(Nakakuma H., Kawaguchi T., Crit. Rev. Oncol. Hematol., 1996; 24: 213 – 229)



PATHOGENESIS

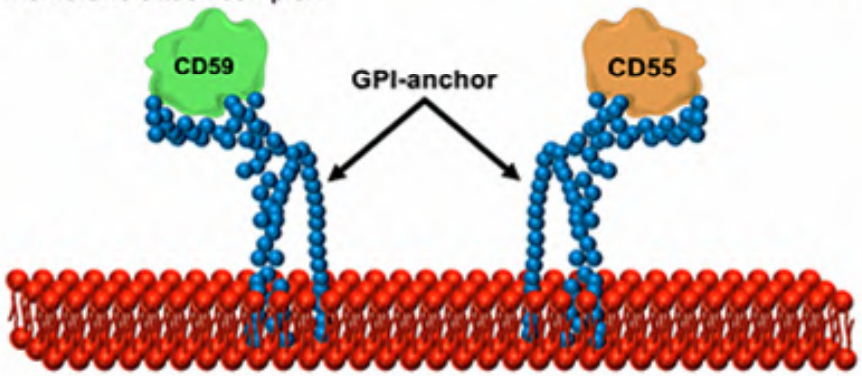
- PIG-A gene is one of >20 genes involved in GPI formation.
- A somatic mutation in the PIG-A gene prevents all GPI-anchored proteins from binding to cell surface

CD59

- Forms a defensive shield for RBCs from complement-mediated lysis
- Inhibits the assembly of the membrane attack complex

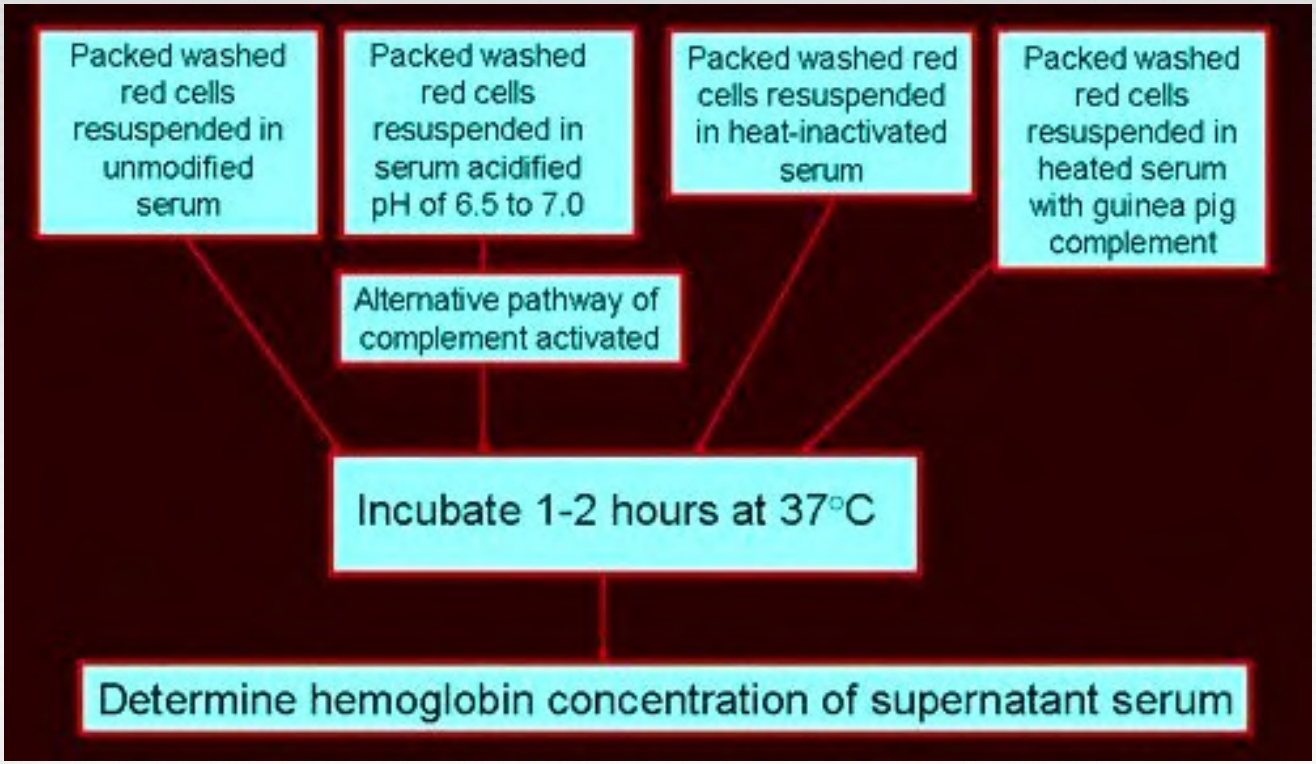
CD55

- Prevents formation and augments instability of the C3 convertases, attenuating the complement cascade

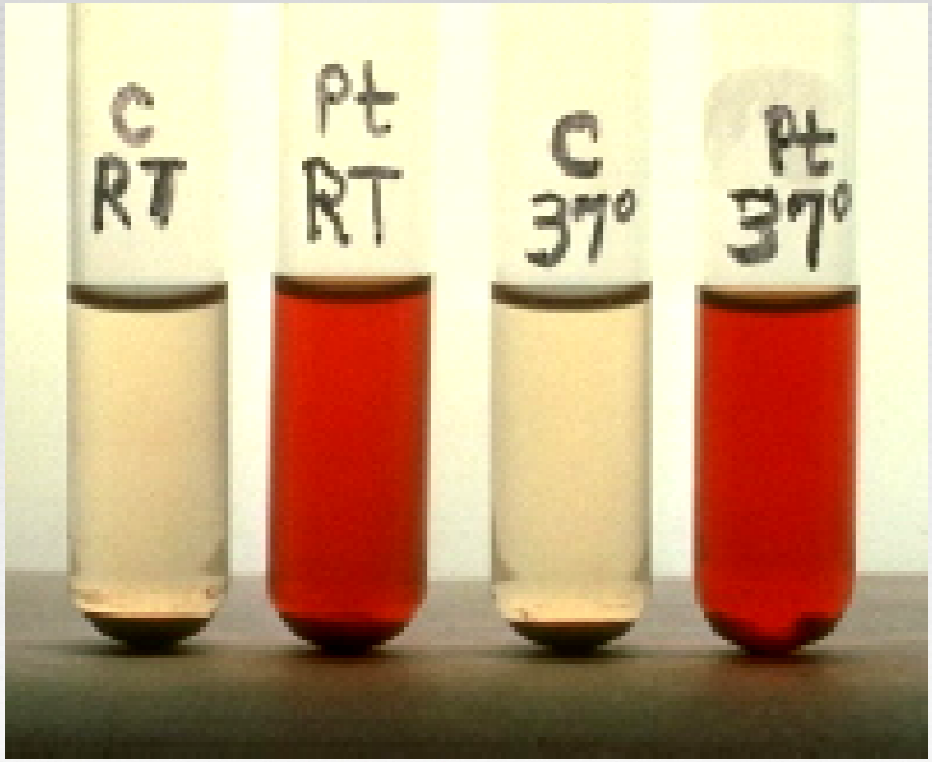


Johnson RJ et al. *Mol Pathol*. 2002;55:145-152.[1]
Adapted from Brodsky R. *Paroxysmal Nocturnal Hemoglobinuria*. 2005;419-427.[2]

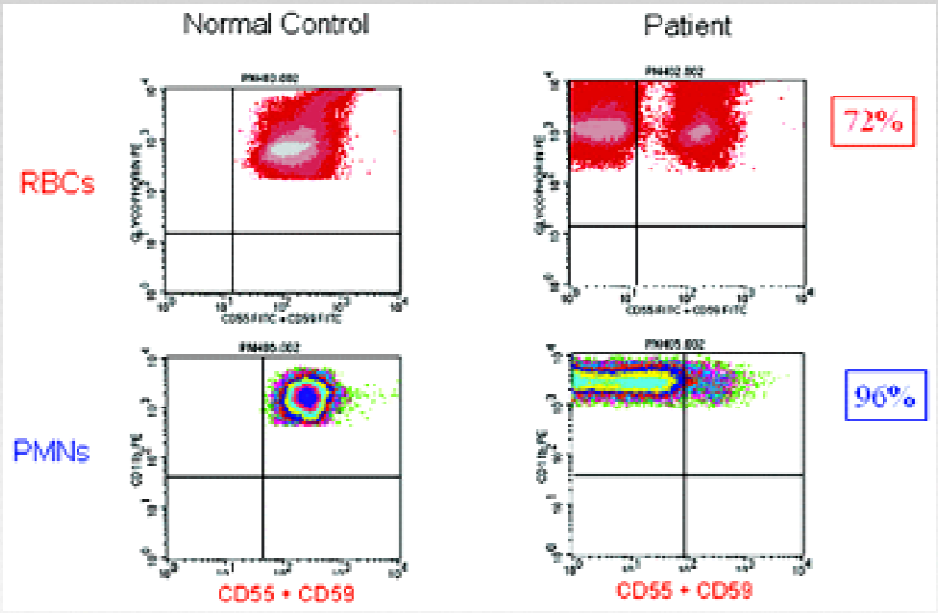
HAM TEST


Packed washed red cells resuspended in unmodified serum
Packed washed red cells resuspended in serum acidified pH of 6.5 to 7.0
Packed washed red cells resuspended in heat-inactivated serum
Packed washed red cells resuspended in heated serum with guinea pig complement
Alternative pathway of complement activated
Incubate 1-2 hours at 37°C
Determine hemoglobin concentration of supernatant serum

HAM TEST

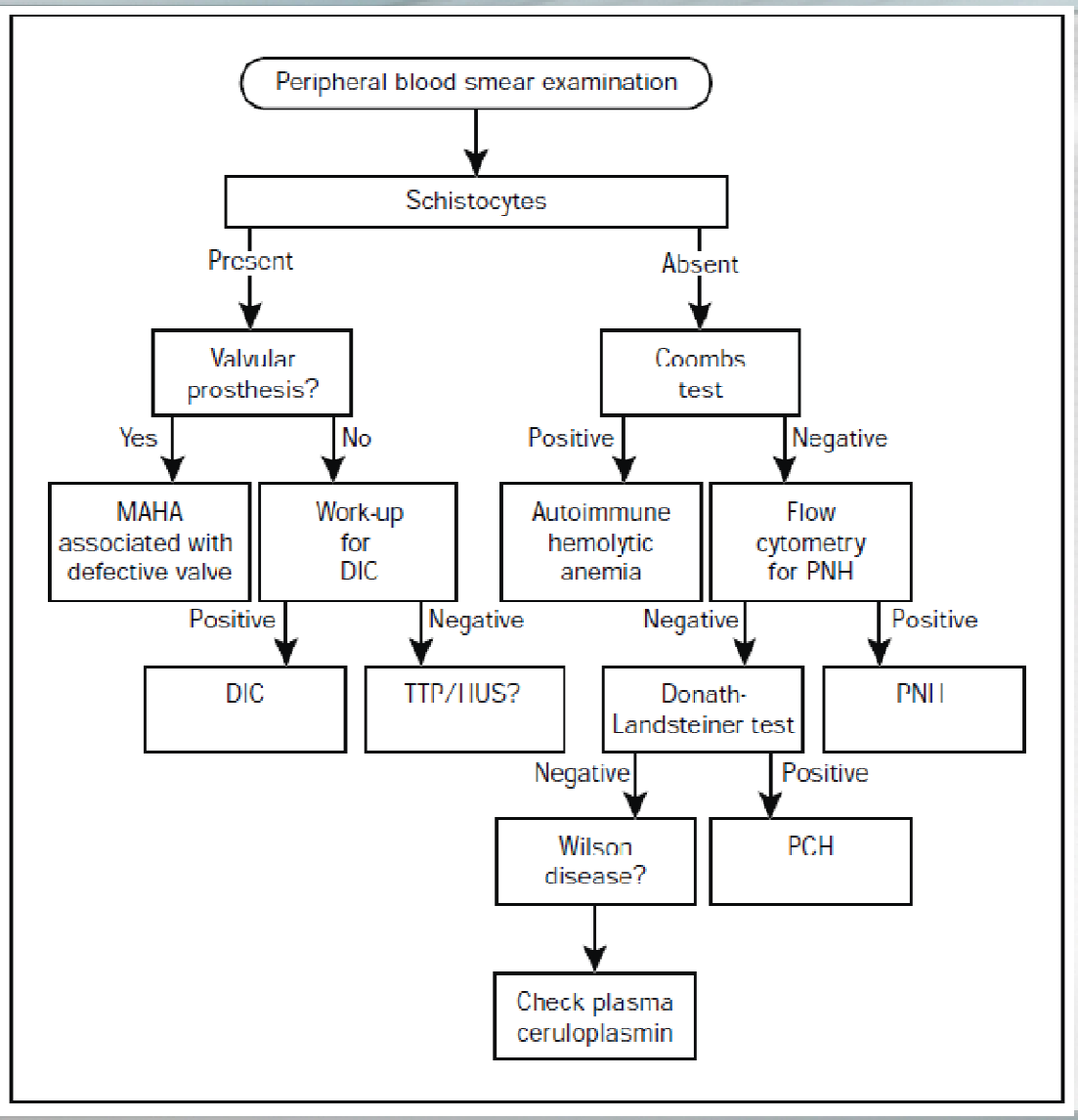


FLOWCYTOMETRY IN PAROXYSMAL NOCTURNAL HEMOGLOBINURIA



EVALUATION OF INTRAVASCULAR HEMOLYSIS

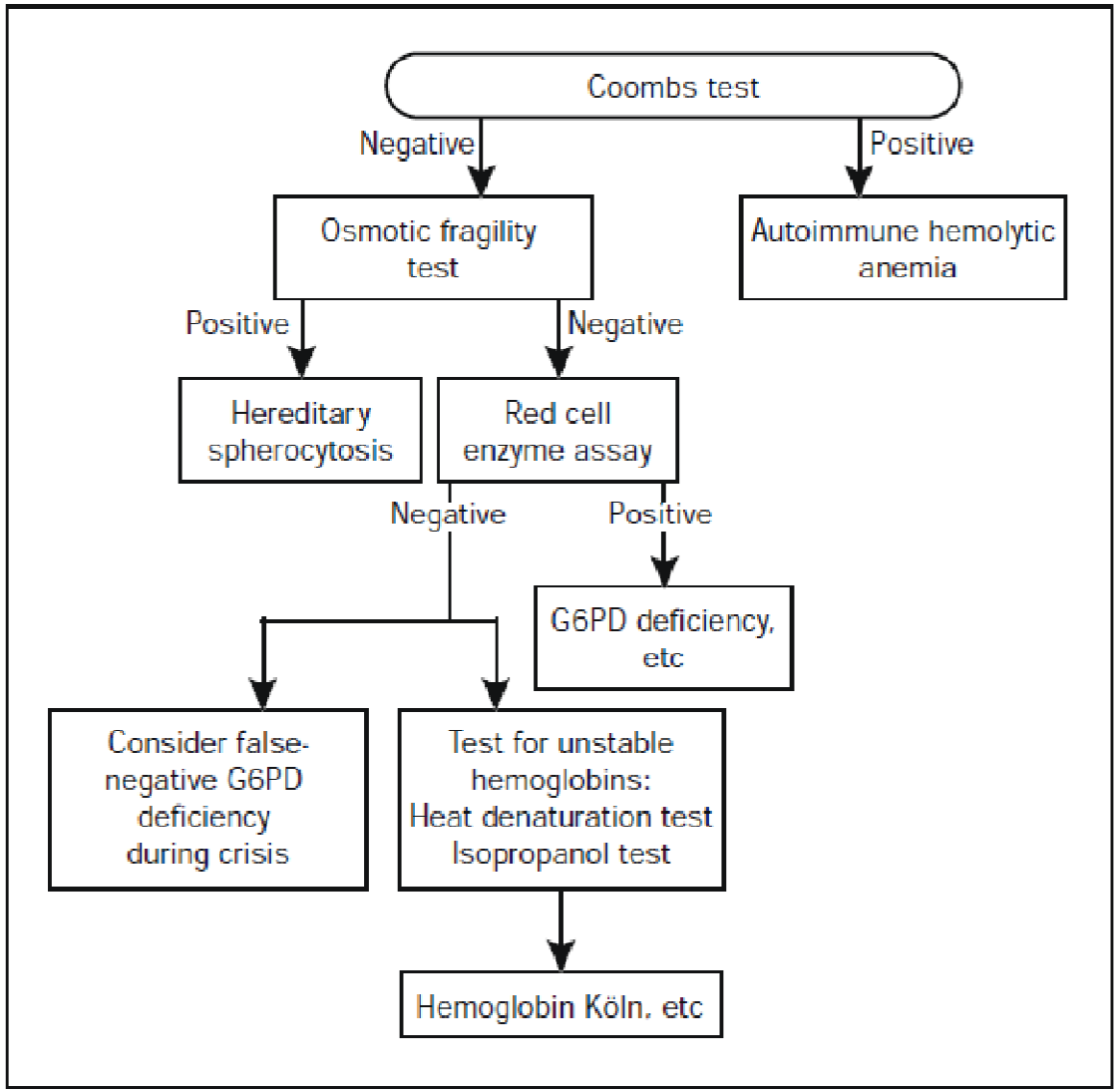
(Mayo Clin Proc, October 2003, Vol 78)



DIC = disseminated intravascular coagulation; HUS = hemolytic uremic syndrome; MAHA = microangiopathic hemolytic anemia; PCH = paroxysmal cold hemoglobinuria; PNH = paroxysmal nocturnal hemoglobinuria; TTP = thrombotic thrombocytopenic purpura.

EVALUATION OF EXTRAVASCULAR HEMOLYSIS

(Mayo Clin Proc, October 2003, Vol 78)



DIFFERENTIAL DIAGNOSIS OF INTRAVASCULAR AND EXTRAVASCULAR HEMOLYSIS

(Mayo Clin Proc, October 2003, Vol 78)

Test	Hemolytic anemias		
	All types	Intravascular	Extravascular
Reticulocyte count	Increased	Increased	Increased
Lactate dehydrogenase	Increased	Increased	Increased
Indirect bilirubin	Increased or normal	Increased	Increased or normal
Haptoglobin	Decreased	Decreased	Decreased
Urinary hemosiderin	Present or absent	Present	Absent

DIFFERENTIAL DIAGNOSIS OF HEMOLYTIC ANEMIAS AND PATHOLOGIES WHICH EVOLVES WITH ICTERIC SYNDROME

Pathology	*Hemoglo-bin*	*Reticulo-cytosis*	*Bilirubin*		*Urobilinuria*	*Functional liver tests*
			Unconjugated	*Conjugated*		
Hemolytic anemias	Decreased	Increased	Increased	Normal	Present	Normal
Parenchy-matous jaundice	Normal	Absent	Normal	Increased	Present	Altered
Mechanical jaundice	Normal	Absent	Normal	Increased	Absent	Normal
Bilirubino-pathies	Normal	Absent	Increased	Normal	Absent	Normal