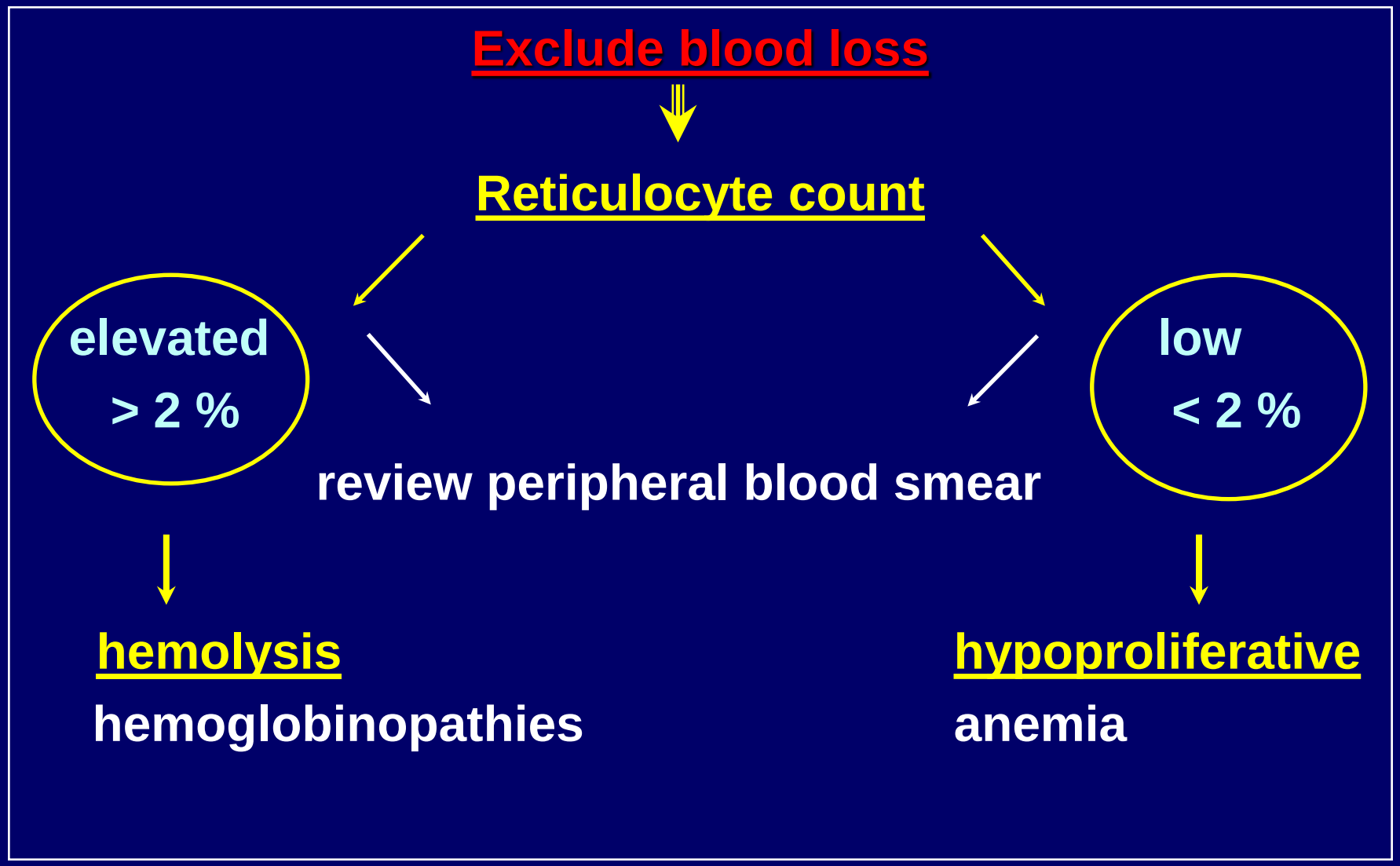
Hemolytic anemias

Jan Haber

Anemias – Diff Dg Approach



prevalence of 1 in 2000 individuals

Usefulness of the Reticulocyte (rtc)Count in the Diagnosis of Anemia

Diagnosis	Value
Hypoproliferative anemias:	Absol. rtc count <75,000/µl

	Anemia of chronic disease
	Anemia of renal disease
	Congenital dyserythropoietic anemias
	Effects of drugs or toxins
	Endocrine anemias
	Iron deficiency
	Marrow replacement

prevalence of 1 in 2000 individuals

Usefulness of the Reticulocyte (rtc)Count in the Diagnosis of Anemia

Diagnosis	Value
Hypoproliferative anemias:	Absol. rtc count <75,000/µl
Maturation abnormalities	

	Vitamin B_{12} deficiency
	Folate deficiency
	Sideroblastic anemia

prevalence of 1 in 2000 individuals

Usefulness of the Reticulocyte (rtc)Count in the Diagnosis of Anemia

Diagnosis	Value
Hemolytic anemias	Absol. rtc count >100,000/µl

	Hemoglobinopathies
	Immune hemolytic anemias
	Infectious causes of hemolysis
	Membrane abnormalities
	Metabolic abnormalities
	Mechanical hemolysis

Hemolytic anemias

A. HEREDITARY

1. RED BLOOD CELL MEMBRANE DISORDERS

Hereditary spherocytosis, elliptocytosis

Acanthocytosis, Stomatocytosis..

2. RED BLOOD CELL ENZYMES DISORDERS

non spherocytic hemolytic anemias - enzymes deficiency

- **G6PD** (glucose-6-phosphate dehydrogenase deficiency)
- **PK (**pyruvate kinase deficiency)
- other

3. DISORDERS of HEMOGLOBIN SYNTHESIS

- **hemoglobinopathy S, unstable Hb**
- **thalasemias** α, β

Hemolytic anemias

B. ACQUIRED

- **AUTOIMMUNE HEMOLYTIC ANEMIA (AIHA)**
- Paroxysmal noctural hemoglobinuria (**PNH)**
- **Drugs** a/ penicilin type
  b/ stibophen type
  c/ alfphamethyldopa type
- **Microangiopathic hemolytic anemia (MAHA sy)**
- Chemical and physical causes
- Microorganisms
- methemoglobinemia, sulfhemoglobinemia
- hypersplenism

Hemolytic anemias

Pathophysiology remarks

Extravascular and intravascular hemolysis

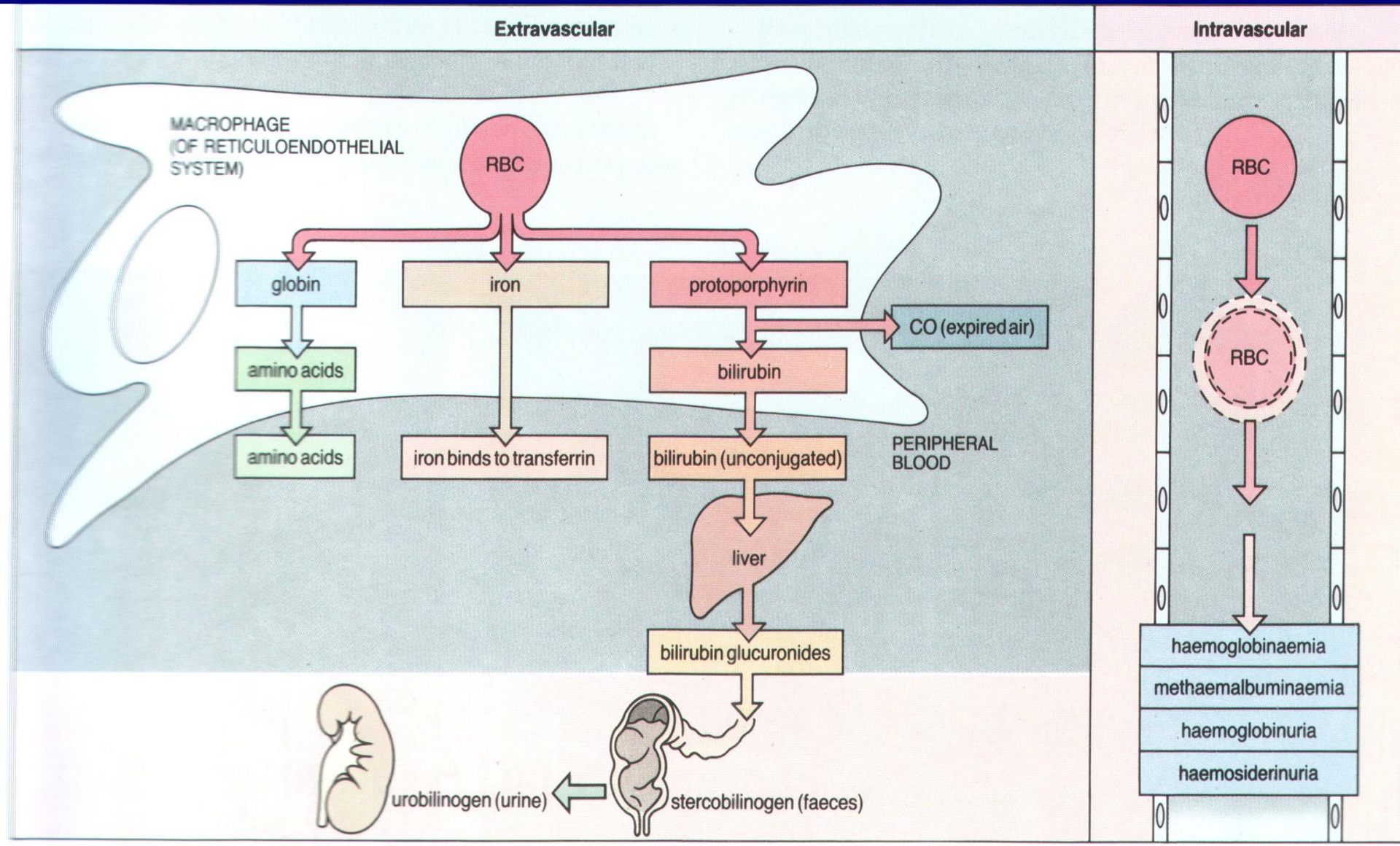


Fig. 4.1 Haemolytic anaemia: extravascular and intravascular mechanisms of red cell breakdown.

Hemolytic anemias

Definition: premature destruction of erythrocytes - congenital or acquired

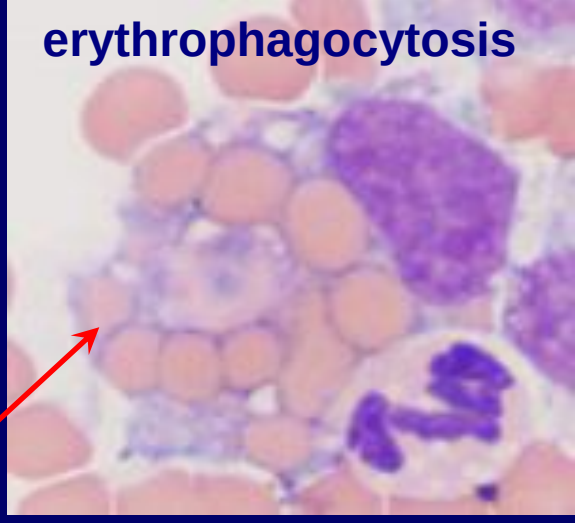
- extravascular = physiological process

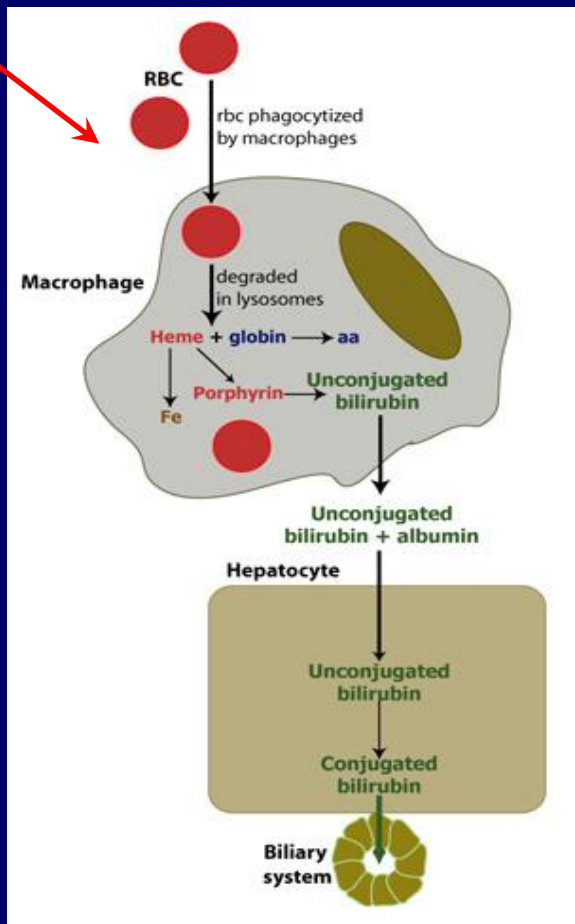
(destruction of ery in macrophages - BM, spleen, liver)

- intravascular = pathological process

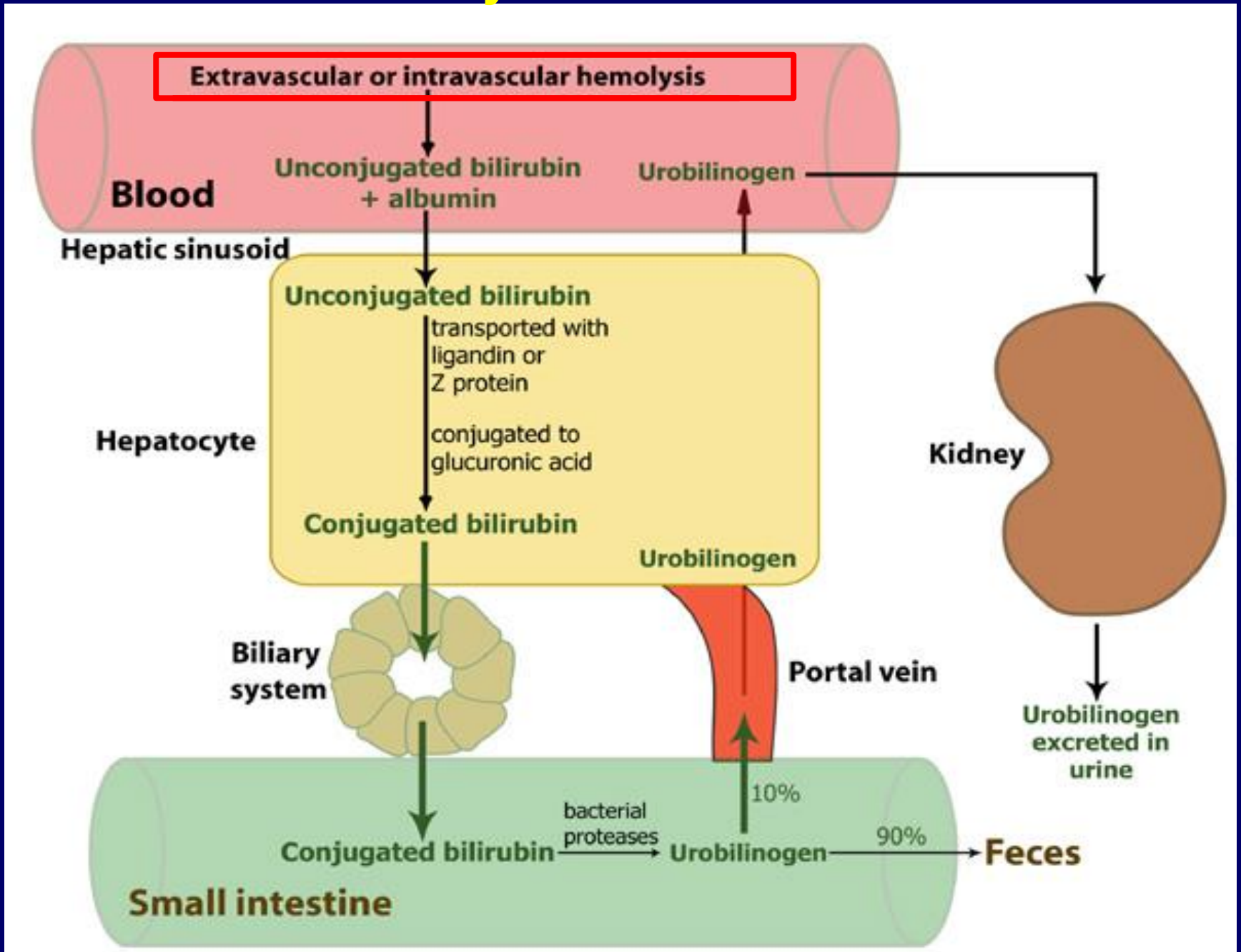
Laboratory charakteristic intravascular hemolysis

- ↘ Hb
- ↗ reticulocytosis
- ↗ indirect bilirubin
- ↗ elevated LDH
- ↘ haptoglobin



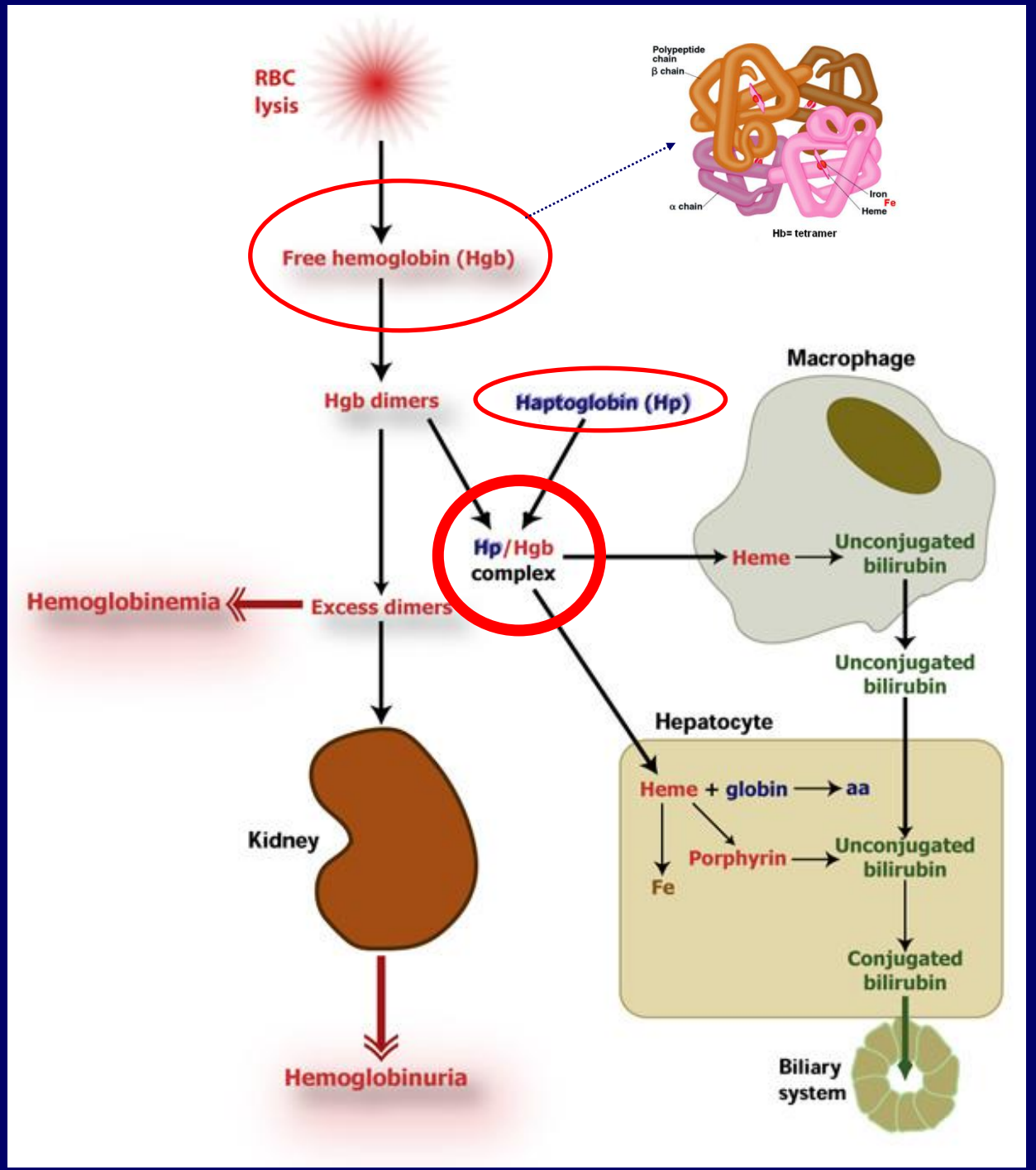


Hemolysis and bilirubin



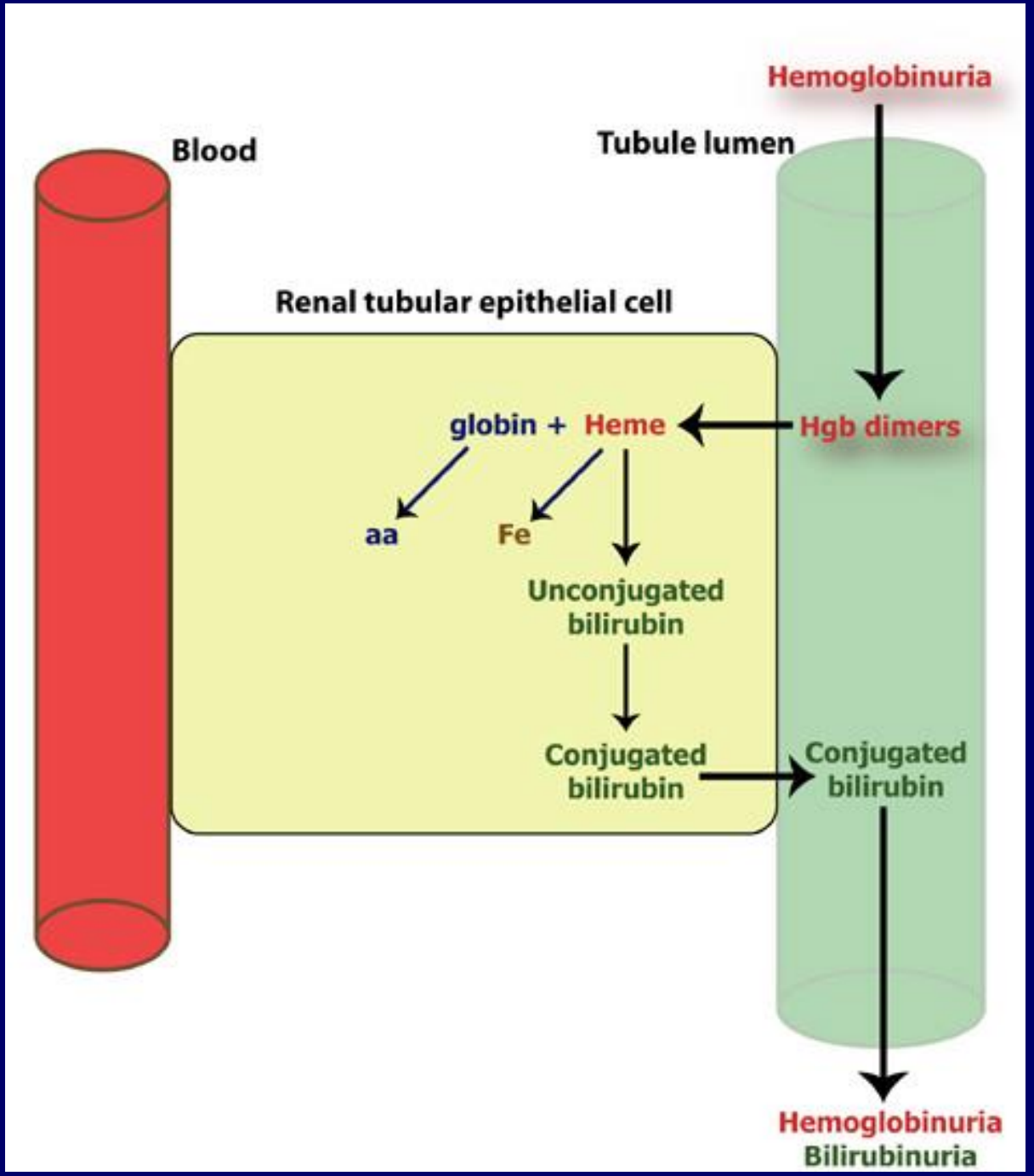
Intravascular hemolysis

↘ haptoglobin in plasma



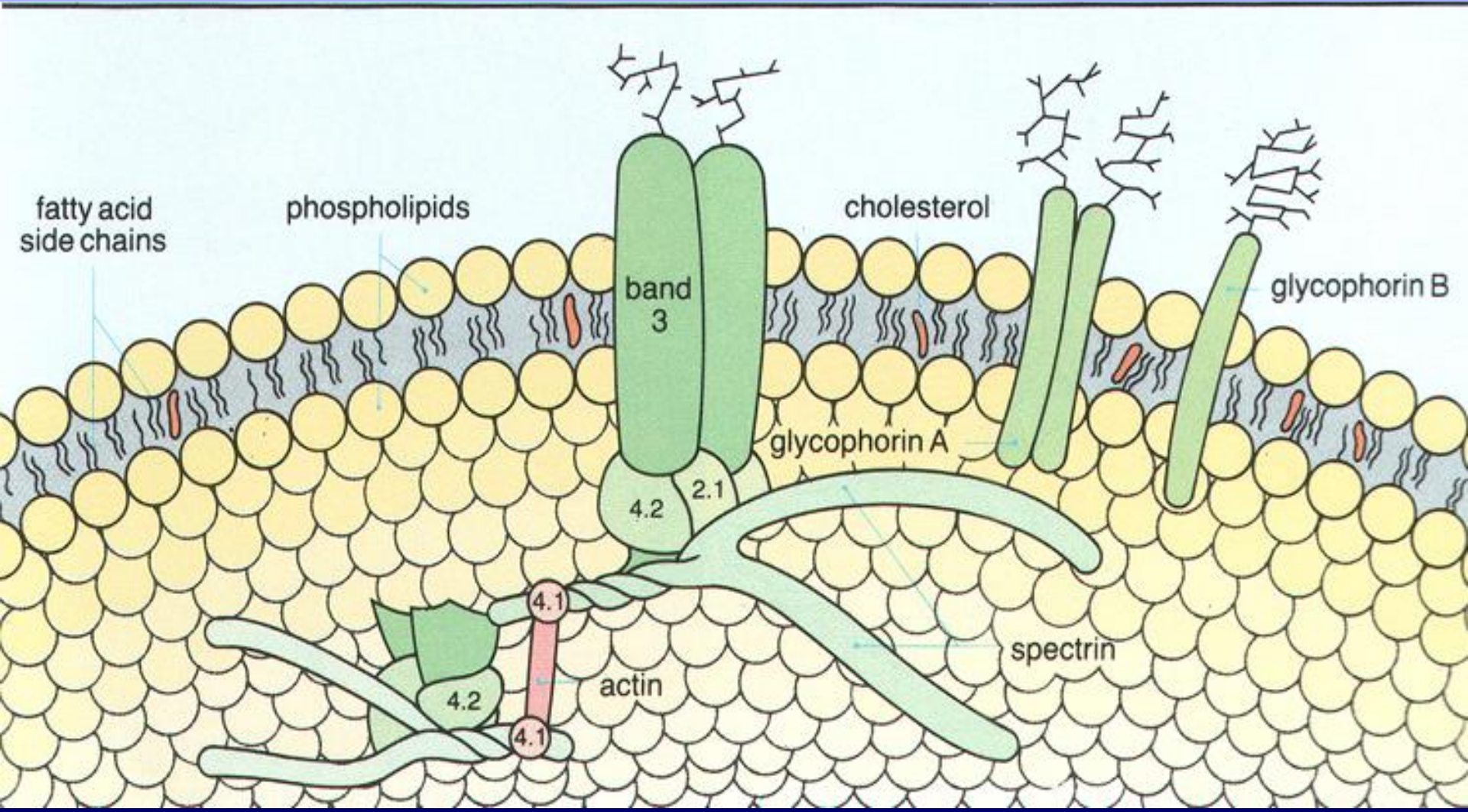
Intravascular hemolysis

hemoglobinuria



Interactions between membrane proteins of the erythrocyte forming membrane skeleton

The assembly of red cell cytoskeletal proteins form macro-complex (network) in the lipid layer



Hemolytic anemias

diagnostic approach

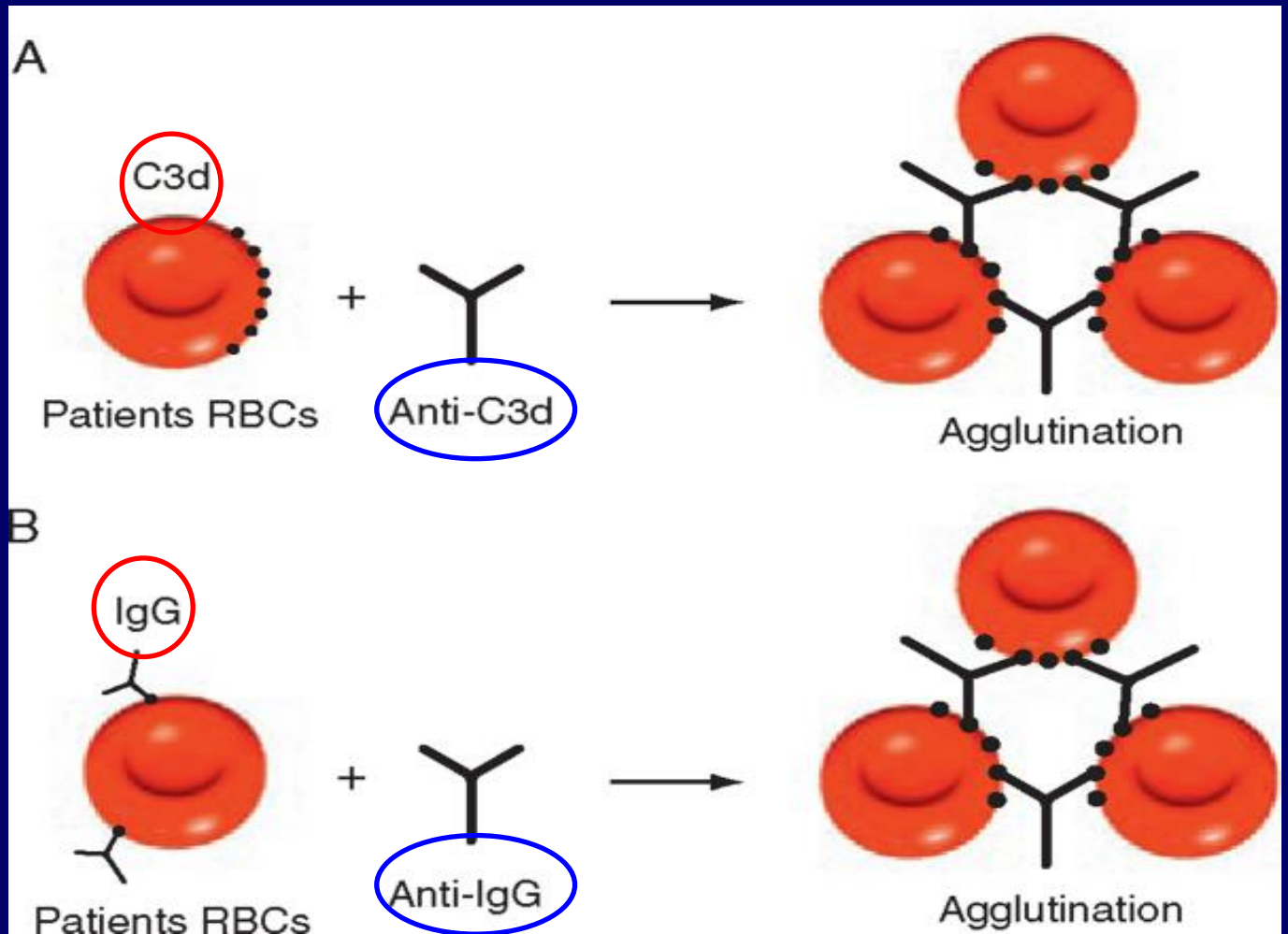
Hemolytic anemias - dg

Hb (↘)
rtc (↗)
peripheral blood smear - morphology
bilirubin, UBG v urine (↗)
free Hb in plasma **(↗)**
hemoglobinuria (↗)
haptoglobin (↘)
hemosiderin in urine (↗)
LD (LD1)
DAT , **IAT** (Coombs)

- **Special tests** (osmotic resistance, glycerin test (pink test); enzyme deficiency tests…

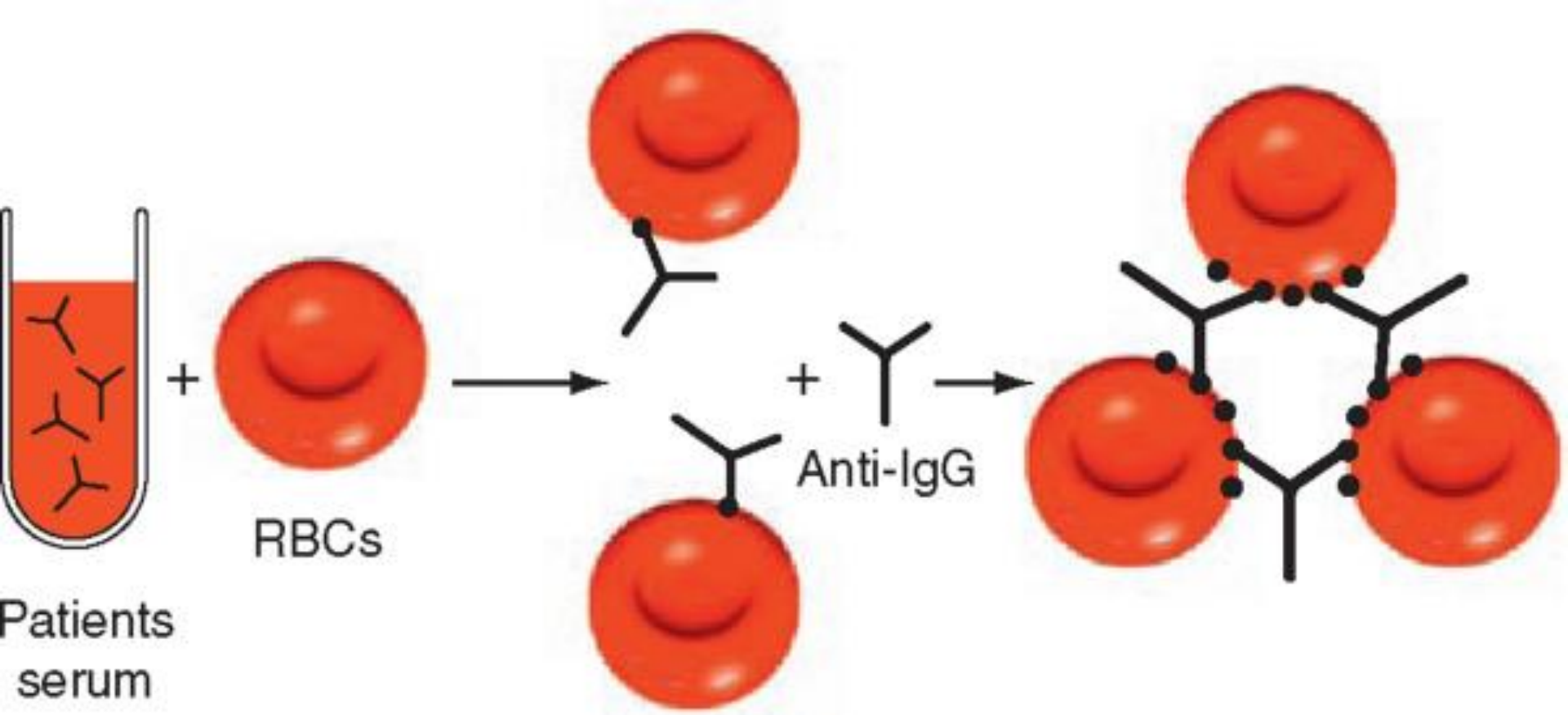
Direct antiglobulin test (DAT) for detection of (A) erythrocyte-bound C3d or (B) IgG.

Hemagglutination occurs when anti-C3d or anti-IgG can create a lattice structure by bridging sensitized red blood cells



Indirect antiglobulin test for detection of antierythrocyte antibodies (Ab) in serum.

The patient's serum (with auto Ab) is mixed with normal rbc + anti-IgG reagent is added and hemagglutination occurs.



Hemolytic anemias

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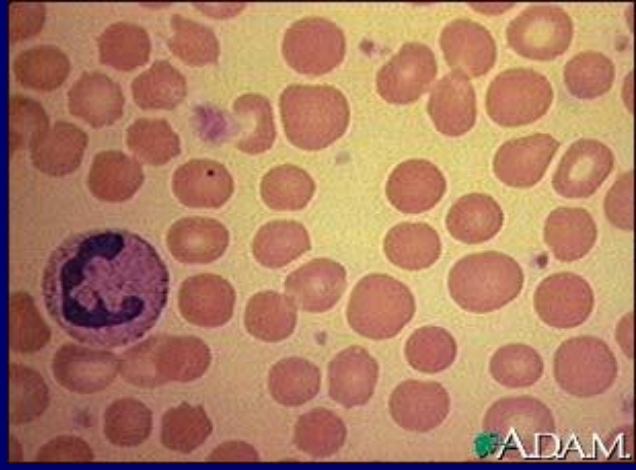
prevalence of 1 in 2000 individuals

Hereditary spherocytosis



<u>The typical features of HS</u>:

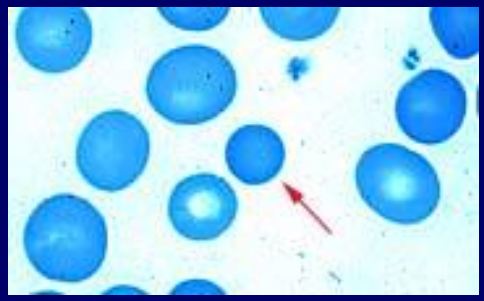
- dominantly inherited hemolytic anemia

- anemia of mild to moderate severity

- spherocytosis on the peripheral blood film

- favorable response to splenectomy

- milder forms of HS might be asymptomatic



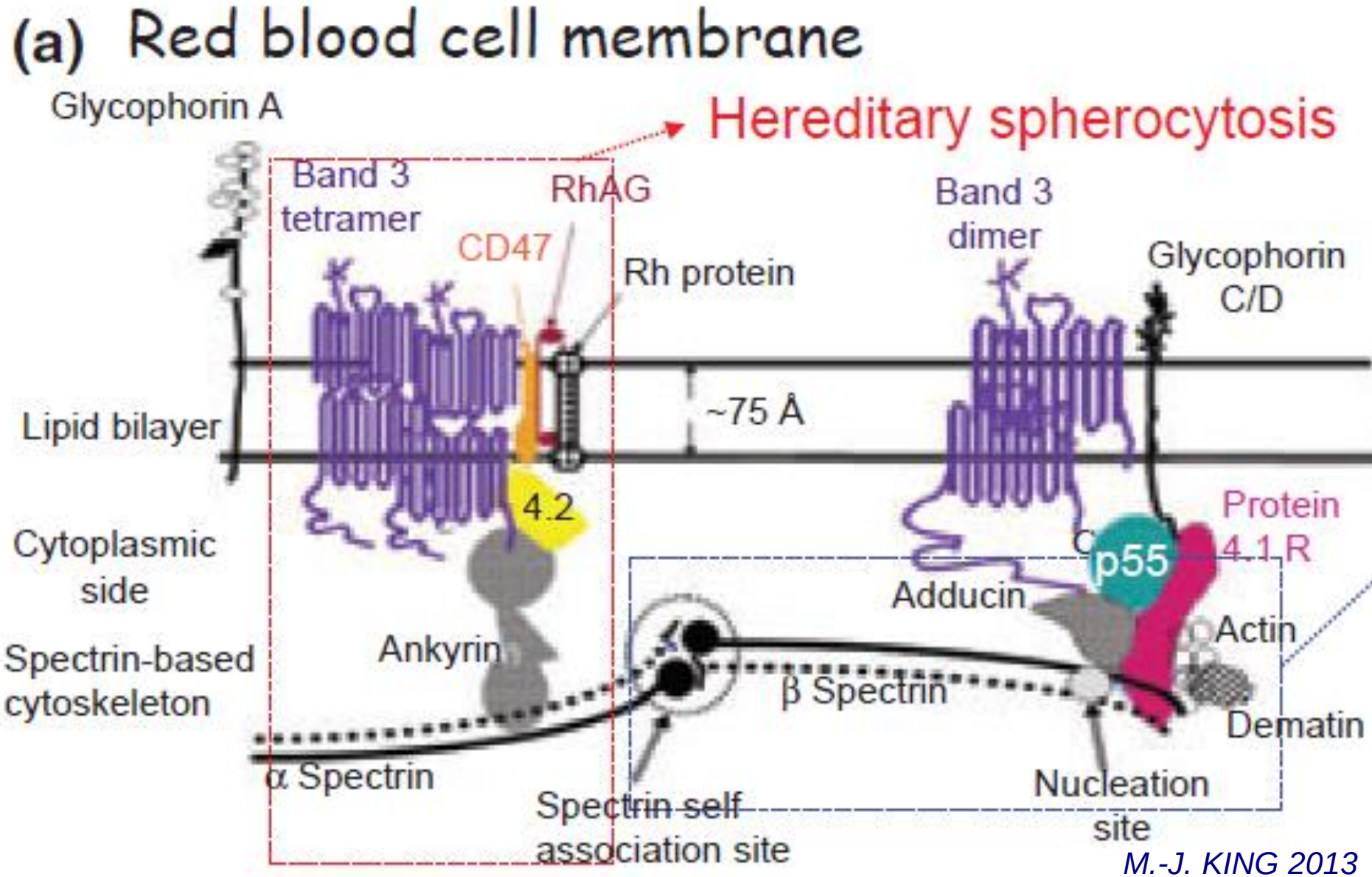
<u>Prevalence</u> of 1 in 2000 individuals

<u>Ethiology</u>

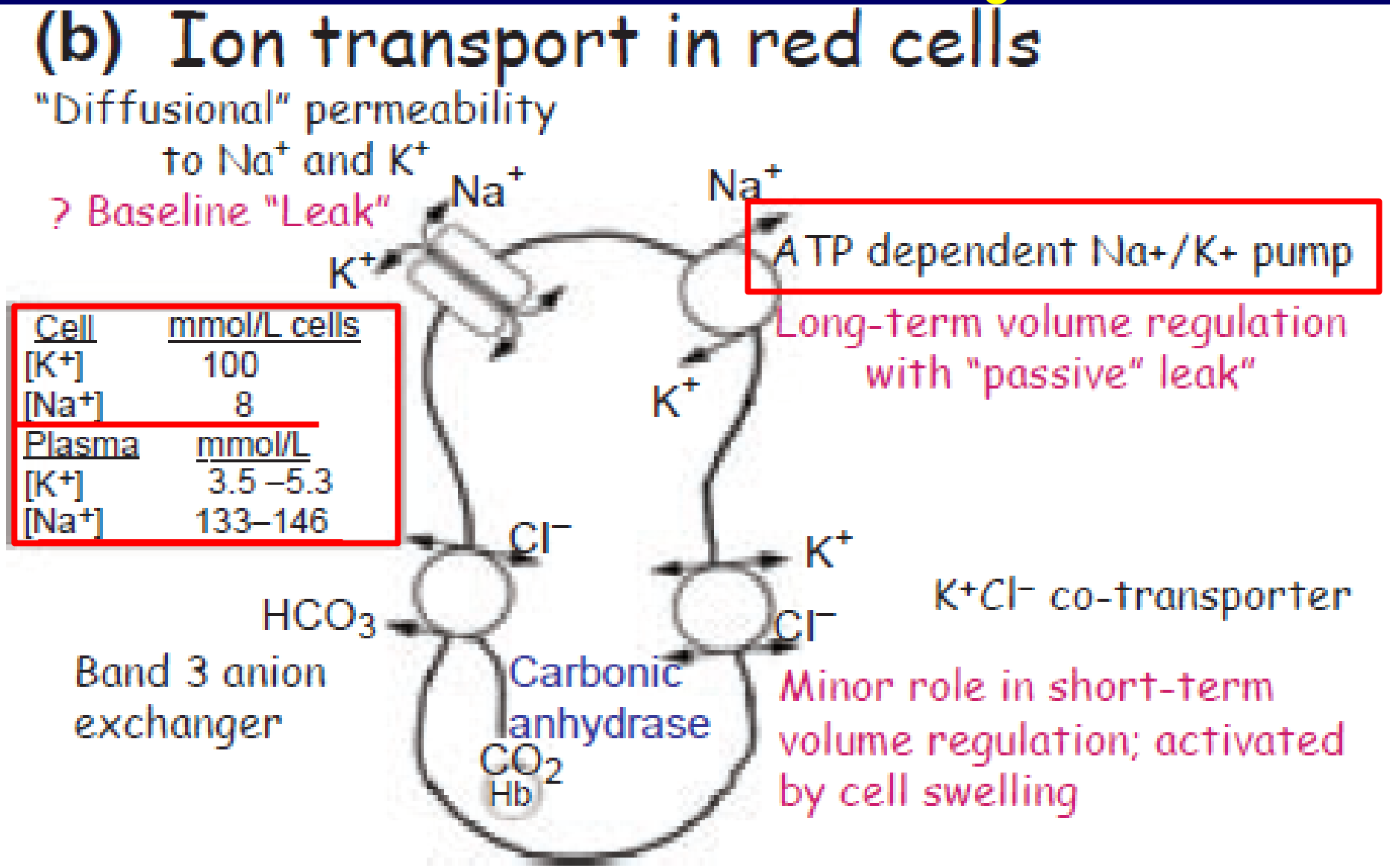
Hereditary defect of membrane protein synthesis ⟶

⟶ osmotic fragility - hemolysis

The assembly of red cell cytoskeletal proteins



Selected ion pumps and passive diffusion of cations maintain the red cell volume and intracellular Na+/K+ gradients



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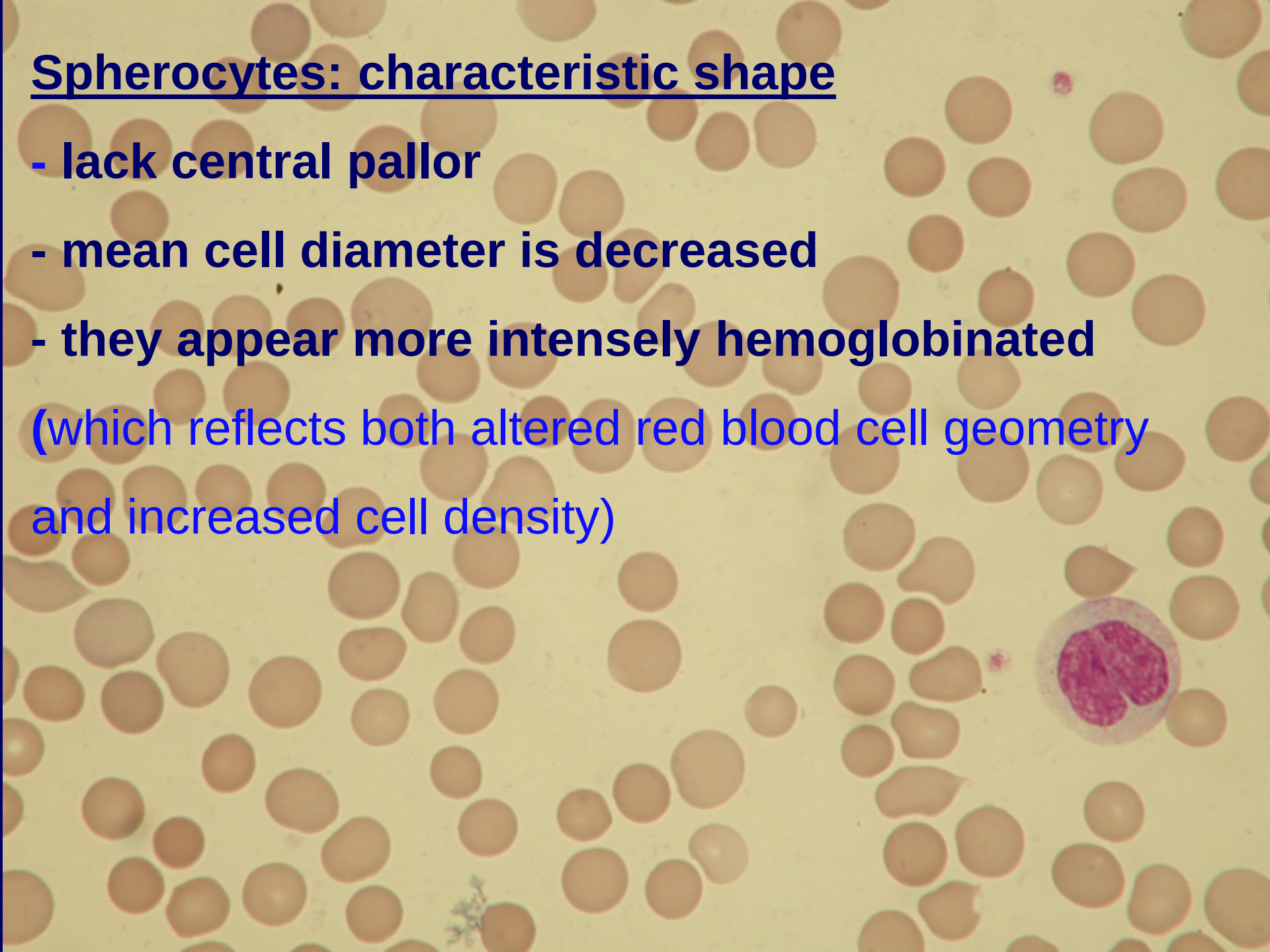
Hereditary spherocytosis

HS pathophysiology

- dominantly inherited deficiency or dysfunction of proteins of the erythrocyte membrane leads to a multistep process of accelerated HS red blood cell destruction

- poorly deformable spherocytes are selectively retained and damaged in the spleen

- splenic vasculature acts as a microcirculation filter


Spherocytes: characteristic shape
- lack central pallor
- mean cell diameter is decreased
- they appear more intensely hemoglobinated
(which reflects both altered red blood cell geometry and increased cell density)

Hereditary spherocytosis

Molecular Pathology

The molecular basis of HS is heterogeneous.

(by densitometric quantitation of membrane proteins)

1) **isolated deficiency of spectrin**
2) **combined deficiencies of spectrin and ankyrin**
3) **deficiency of band 3 protein**
4) **deficiency of protein 4.2**
5) **no abnormality identified**

Hereditary spherocytosis

Clinical Manifestations

Typical Forms - **relatively asymptomatic**

- **mild jaundice** …. „nice tan“…
- **splenomegaly gradually develops in most pts.**
- **anemia is usually mild to moderate**
- **occasionally anemia is absent, the reticulocyte count is normal or only minimally elevated, laboratory evidence of hemolysis is minimal or absent**
- ***Severe and Atypical Forms***
- **severe life-threatening hemolysis early in life**
- **Transfusion dependent during early infancy and childhood**
- ***Activation asymptomatic HS*: Parvovirus B 19, HS 6, CMV, pregnancy,** but postpartum pts conditions return to baseline level

Hereditary spherocytosis

Complications:

Bilirubin gallstones - 50% of pts with HS

Hemolytic crises – reticulocytes ⬆

= hemolysis and accelerated erythropoiesis

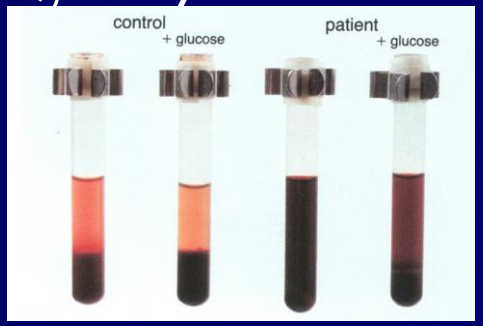
Aplastic crises - **reticulocytes - 0 !!**

= hemolysis and stop of erythropoiesis

- (Parvovirus B 019 - the virus selectively infects erythroid precursors and inhibits their growth)

Diagnosis of HS

Clinic	splenomegaly
Labor	Hb ↘, MCV ↘, MCHC ↗, RDW ↗, rtc ↗
Blood smear	morphology - spherocytes
Coombs test	negat
Hemolytic picture	indirect bilirubin, rtc
RBC haptoglobin	normal
autohemolysis	48 h incubated without glu 10-50% (N: 4%) (after glucose is added)
Bone marrow :	hypoplasia – aplasia - hyperplasia



Advanced: *Detection of the Molecular Defects*

Gel elektrophoresis (= analysis of membrane proteins)

Genetics (gen mutation for β-spectrin, protein 4.2…)

Screening:EMA test:binding of eozin-5-maleimid to protein band 3 is weakened

Hereditary spherocytosis - therapy

Splenectomy = curative

Indications for splenectomy :

- growth retardation, skeletal changes
- symptomatic hemolytic disease
- anemia-induced compromise of vital organs
- the development of leg ulcers
- appearance of extramedullary hematopoietic tumors

Hemolytic anemias

B. ACQUIRED

- **AUTOIMMUNE HEMOLYTIC ANEMIA (AIHA)**
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- Drugs a/ penicilin type
  b/ stibophen type
  c/ alfphamethyldopa type
- **Microangiopathic hemolytic anemia (MAHA sy)**
- Chemical and physical causes
- Microorganisms
- methemoglobinemia, sulfhemoglobinemia
- hypersplenism

Autoimmune Hemolytic Anemia (WARM AIHA)

Definition:

AIHA - spectrum of disorders – antibodies against self-antigens on the ery membrane – hemolysis –

- can occur as an idiopathic (primary) disorder or

- can coexist with another disease (secondary)

3 types:

1/ associated with warm-reactive IgG autoAb

***warm autoimmune hemolytic anemia* (WAIHA)**

2/ hemolytic anemias caused by cold-reactive IgM autoAbs

cold agglutinin disease

3/ sy associated with the Donath-Landsteiner antibody as

paroxysmal cold hemoglobinuria

Incidence: 1/ 80 000 / year

3 types of Autoimmune Hemolytic Anemia

Characteristic	**Warm AIHA**	**Cold Agglutinin Disease**	**Paroxysmal Cold Hemoglobinuria**
Antibody isotype	**IgG**, rare IgA, IgM	**IgM**	IgG
Direct antiglobulin test (DAT) result	**IgG and/or C3**	**C3**	C3
Antigen specificity	Multiple, primarily Rh	i/I, Pr	P
Hemolysis	Primarily **extravascular**	Primarily **extravascular**	Intravascular
Common disease associations	**B-cell neoplasia** /lymphoproliferative, collagen–vascular	Viral, neoplasia	Syphilis, viral

AUTOIMMUNE HEMOLYTIC ANEMIAS

1/ WARM AUTOIMMUNE HEMOLYTIC ANEMIA

Warm autoantibodies = IgG

Optimal binding to rbc = 37^0C

Both serious clinical picture and laboratory findings

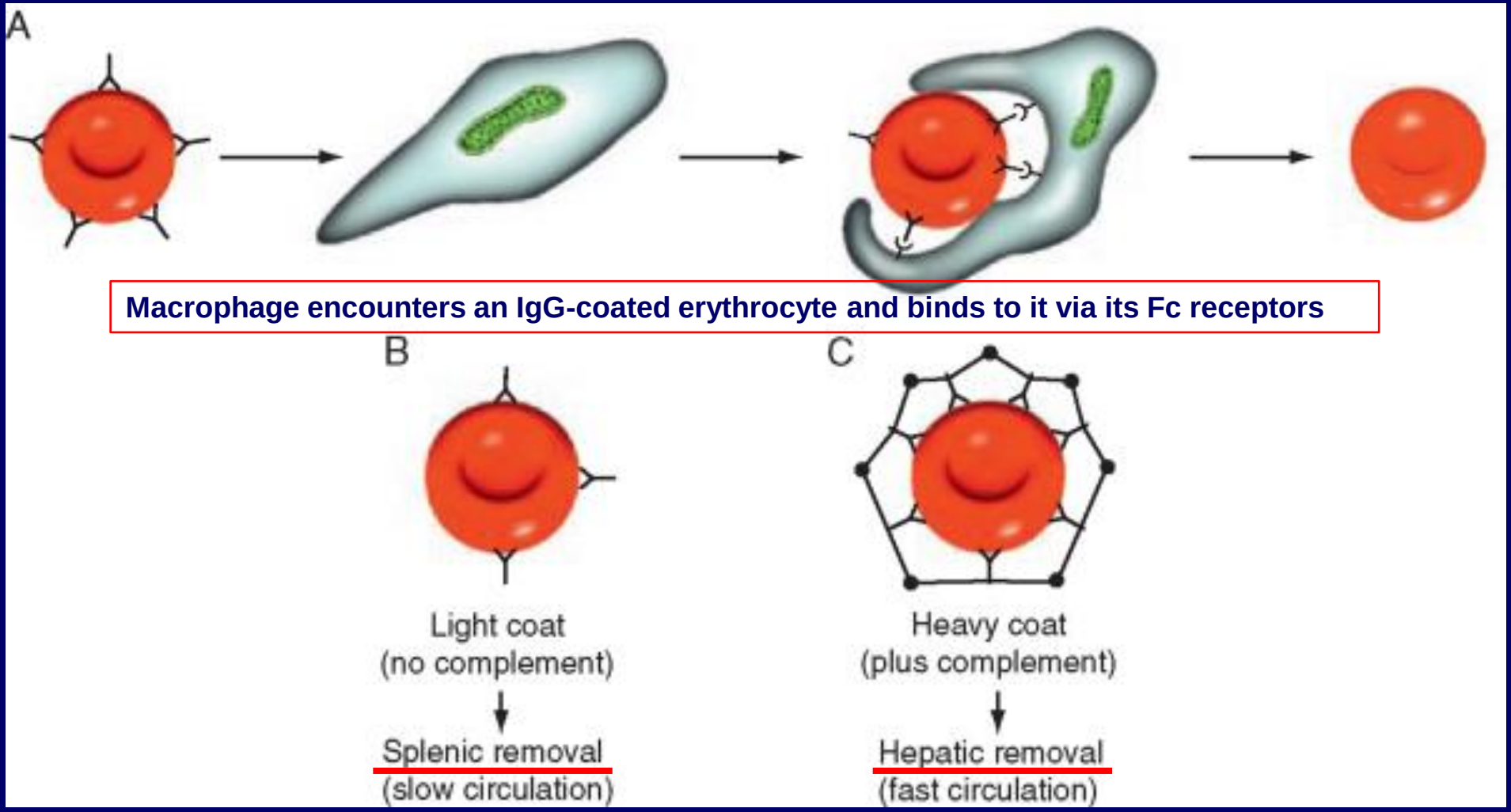
2/ Cold autoantibodies = IgM

Optimal binding to rbc = 4^0C, start 20^0C

Usually normal clinical picture and serious laboratory

3/ Mixed

Mechanism of extravascular hemolysis in autoimmune hemolytic anemia



Diseases (Rarely) Associated with Autoimmune Hemolytic Anemia (SECONDARY AIHA)

Collagen vascular disease	**Miscellaneous diseases**
Rheumatoid arthritis	Myelofibrosis with myeloid metaplasia
Scleroderma	Ulcerative colitis
Polyarteritis nodosa	Pernicious anemia
Serum sickness	Thyroid disease
Sjögren syndrome	Ovarian cysts
Lymphoreticular malignancy	Mucocutaneous lymph node sy(Kawasaki disease)
Macroglobulinemia	Evans syndrome
Hodgkin lymphoma	Congenital immunodeficiency syndromes
Multiple myeloma	Guillain-Barré syndrome
Mycosis fungoides	Primary biliary cirrhosis
Other malignancy	Multiply transfused pts with Hb-pathies
Acute leukemia, Carcinoma (colon, kidney, lung, ovary), Thymoma	

AIHA - clinical findings

Disease can occur:

acutely, with symptoms caused by rapidly developing anemia – overt anemic syndrome

gradually in a relatively asymptomatic form – mild anemia

occasionally = asymptomatic form, the blood bank provides the diagnosis through a positive antiglobulin test

When to start a search for an underlying disease –

secondary AIHA: lymphadenopathy, fever, hypertension, renal failure, rash, petechiae, or ecchymoses…

AIHA - laboratory evaluation

Blood picture: **anemia macrocytic, ↑ rtc**

Biochemistry ↑ **bili , UBG** in the urine

Immunohematology: **DAG, IAT**,

Basic characteristic of hemolysis - summary:

↓ **Hb**

- ↑ **retikulocytosis**
- ↑ **indirect bili**
- ↑ **LDH**
- ↓ **haptoglobin**

AIHA Therapy - *General Principles*

AIHA ranges from indolent to life threatening clin. course

Evaluation

AIHA is primary or secondary

Initiation of treatment

Treatment of underlying disease

Immediate transfusion

Attempts to suppress the production of autoantibodies

AIHA Therapy - *Transfusion*

Some cases of AIHA are life threatening and necessitate transfusion with RBCs

- **autoantibodies complicate the pretransfusion evaluation**
- **despite a complete blood bank workup, the patient often will receive "cross-match incompatible" blood**
- **In situations where transfusion is required, blood should not be withheld simply because it is not fully compatible.**
- **However, transfusion should be administered with particular care and close monitoring**
- **Transfusion is warranted without delay and, if necessary, before all serologic tests are completed when cardiac or cerebral function is threatened**

AIHA - therapy- summary

1/ CORTICOSTEROIDS PREDNISONE 1-2 g i.v./day x 5 days, then dose reduction to 1 mg/kg… ASAP

2/ IMMUNOMODULATORY THERAPY (Cyclof., Azathioprine …)

3/ IVIG 0,1-1,0 g /kg /day

4/ **PLASMAPHERESIS**

5/**RITUXIMAB** (anti CD20 monoklonální protilátka)

6/ **SPLENECTOMY**

Transfusion - vital indication – careful monitoring of vital signs + corticosteroids

1/ AIHA Therapy - *Corticosteroids*

- first-line - either the idiopathic or the secondary form
(to disable macrophages from clearing IgG, inactivated C3b, or C3b-coated rbc)

- begin with prednisone (there is no clear advantage to alternative forms of corticosteroids) **1 - 2 mg/kg/day** (in elderly 0.6 mg/kg/day)

- it should be continued until a response becomes evident, usually within 3 weeks

. Until - Hb level reaches ≥ 100 g/l
- transfusion should no longer be necessary

. Thereafter:
the dose can be gradually reduced
usually at a rate of 5 to 10 mg/week

2/ AIHA - *Immunomodulatory Therapy*

- **alkylating agents - 3 months or more**
  - **Cyclophosphamide 2 mg/kg/day**
  - chlorambucil
- **thiopurines**
  - **Azathioprine 1.5 mg/kg/day**
  - 6-mercaptopurine
- **Enables substantially reduced doses of prednisone**
- **Immunosuppressants:**
  - **Cyclosporine alone and in combination**
  - **2.5 mg/kg twice daily for 6 days then 3 mg/kg/day and 5 mg/day of prednisone ..**

3/ AIHA Therapy - *Intravenous IgG*

IVIG 0,1-1,0 g /kg /day

(The soluble IgG in the material may increase the lifespan of IgG-coated – RBCs: by saturating Fc receptors on macrophages prevents binding to rbc)

- for treatment of severe life-threatening AIHA

- in cases where AIHA is refractory to conventional treatment with corticosteroids

- temporizing measure prior to splenectomy

- not recommended for routine use in either acute or chronic treatment of AIHA

4/ AIHA Therapy - *Plasma Exchange*

- the therapeutic advantage - removal antibodies

- Not long-term efficacy (continuous Ab production and the large extravascular distribution of IgG in pts with IgG-mediated AIHA)

- on cessation of therapy - return to pretreatment levels

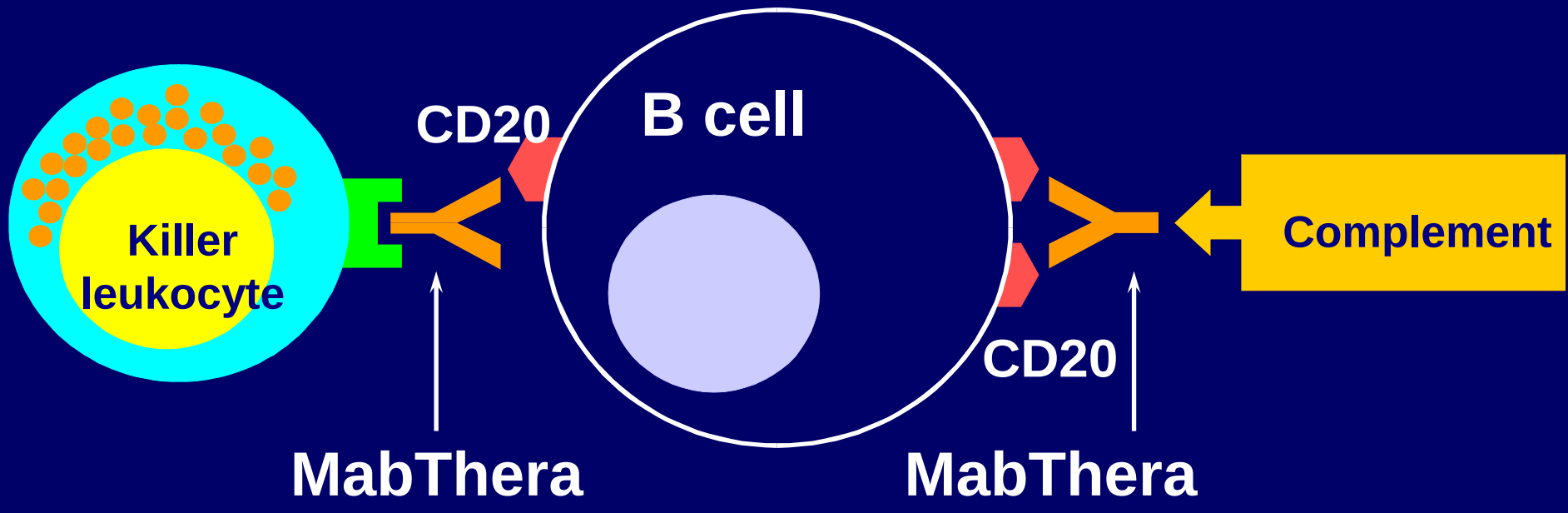
- this therapy should be reserved for:

- **patients unresponsive to trf**
- **pts in critical condition (rapid clearance of rbc)**
- **before splenectomy**

5/ AIHA Therapy - *Rituximab Therapy*

- chimeric anti-CD20 monoclonal antibody = MabThera)
  - regimen: 1xc/week x 3 weeks
- mechanism of action - multifaceted, complex
- induces cell death through complement-dependent lysis;
- antibody-dependent cellular toxicity
- antibody-dependent phagocytosis mediated by Fc, complement, and phosphatidylserine receptors
- direct antibody effects of CD20 ligation leading to inhibition of proliferation
- apoptosis and sensitization to chemotherapy
- and induction of active immunity

Proposed mechanisms of action of MabThera



6 AIHA Therapy - *splenectomy*

Indications

- failure to respond to prednisone, dosages ≥ 20 mg/day
- or intractable side effects of the corticosteroid

Highly effective

- by removing the major RES site of RBC destruction
- eliminates many phagocytosing macrophages
- and autoantibody-producing B cells

Risk

- sepsis by encapsulated organisms (Pneumococci)

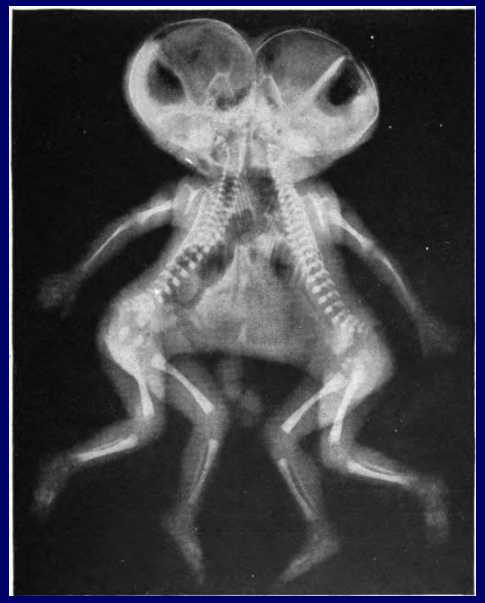
  = immunization with pneumococcal and meningococcal vaccines,

EXTRINSIC NONIMMUNE HEMOLYTIC ANEMIAS

FRAGMENTATION HEMOLYSIS: MICROANGIOPATHY

Thrombotic microangiopathy (TMA)

Thrombotic thrombocytopenic purpura–hemolytic uremic syndrome (TTP–HUS)



siamese siblings

Red Blood Cell Fragmentation Hemolysis

Damaged microvasculature
Thrombotic thrombocytopenic purpura–hemolytic uremic syndrome (TTP–HUS)
Associated with pregnancy:
Preeclampsia or eclampsia;
HELLP syndrome (hemolysis plus elevated liver enzymes plus low platelets)
Associated with malignancy, with or without mitomycin C treatment
Vasculitis: Polyarteritis, Wegener granulomatosis, acute glomerulonephritis, or *Rickettsia*-like infections
Abnormalities of renal vasculature:
Malignant hypertension, acute glomerulonephritis, scleroderma, or allograft rejection, with or without cyclosporine treatment
Disseminated intravascular coagulation
Malignant hypertension
Catastrophic antiphospholipid antibody syndrome

Atrioventricular malformations
Kasabach–Merritt syndrome
Hemangioendotheliomas
Atrioventricular shunts for congenital and acquired conditions (e.g., stents, coils, transjugular intrahepatic portosystemic shunt, Levine shunts)
Cardiac abnormalities
Replaced valve, prosthesis, graft, or patch
Aortic stenosis or regurgitant jets (e.g., in ruptured sinus of Valsalva)
Drugs: Cyclosporine, mitomycin, ticlopidine, tacrolimus, or cocaine

Clinical spectrum of thrombotic microangiopathy

- Haemolytic uremic syndrome
- Thrombotic thrombocytopenic purpura
- Malignant hypertension
- Preeclampsia – eclampsia
- Systemic lupus erythematosus
- Antiphospholipid antibody syndrome
- Systemic sclerosis
- Transplant associated
- Radiation therapy associated
- Drug induced
- Infection associated

Acquired hemolytic anemias non-immune

TTP – HUS Thrombotic thrombocytopenic purpura– hemolytic uremic syndrome

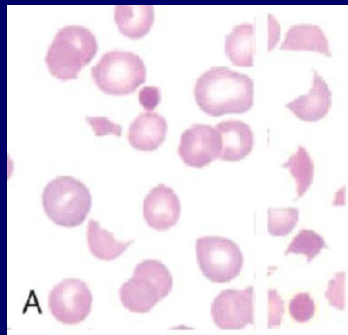
abnormally decreased ADAMTS-13 activity (A Disintegrin And **Metalloprotease** with ThromboSpondin type 1 repeats

- metalloprotease - converting the highly thrombogenic large multimers of von Willebrand factor made by platelets and endothelial cells into the smaller – attached to endothelial cell surfaces, where platelets may excessively aggregate, leading to formation of **microvascular thrombi** even in the absence of endothelial damage + **fibrin fibres** – damaged rbc = schistocytes.

ADAMTS13 deficiency: 1/ acquired (autoAb)

2/ hereditary

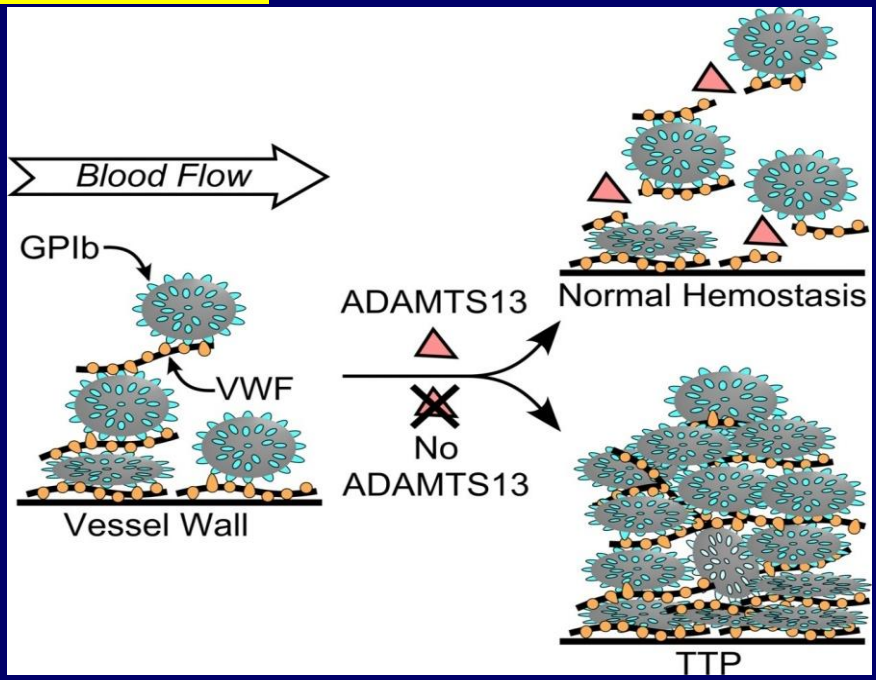
schistocytes



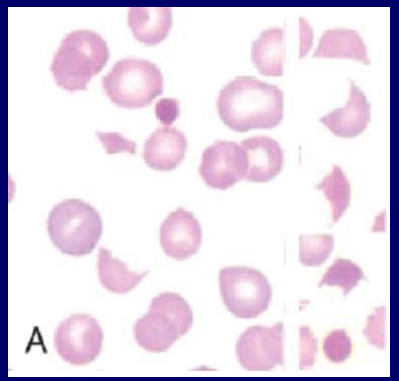
TTP – HUS Thrombotic thrombocytopenic purpura Hemolytic uremic syndrome

= ADAMTS-13 Metalloprotease (MP) defficiency:

MP cleaves the highly thrombogenic large multimers vWF, made by platelets and endothelial cells into the smaller.. attached to endothelial cell surfaces…

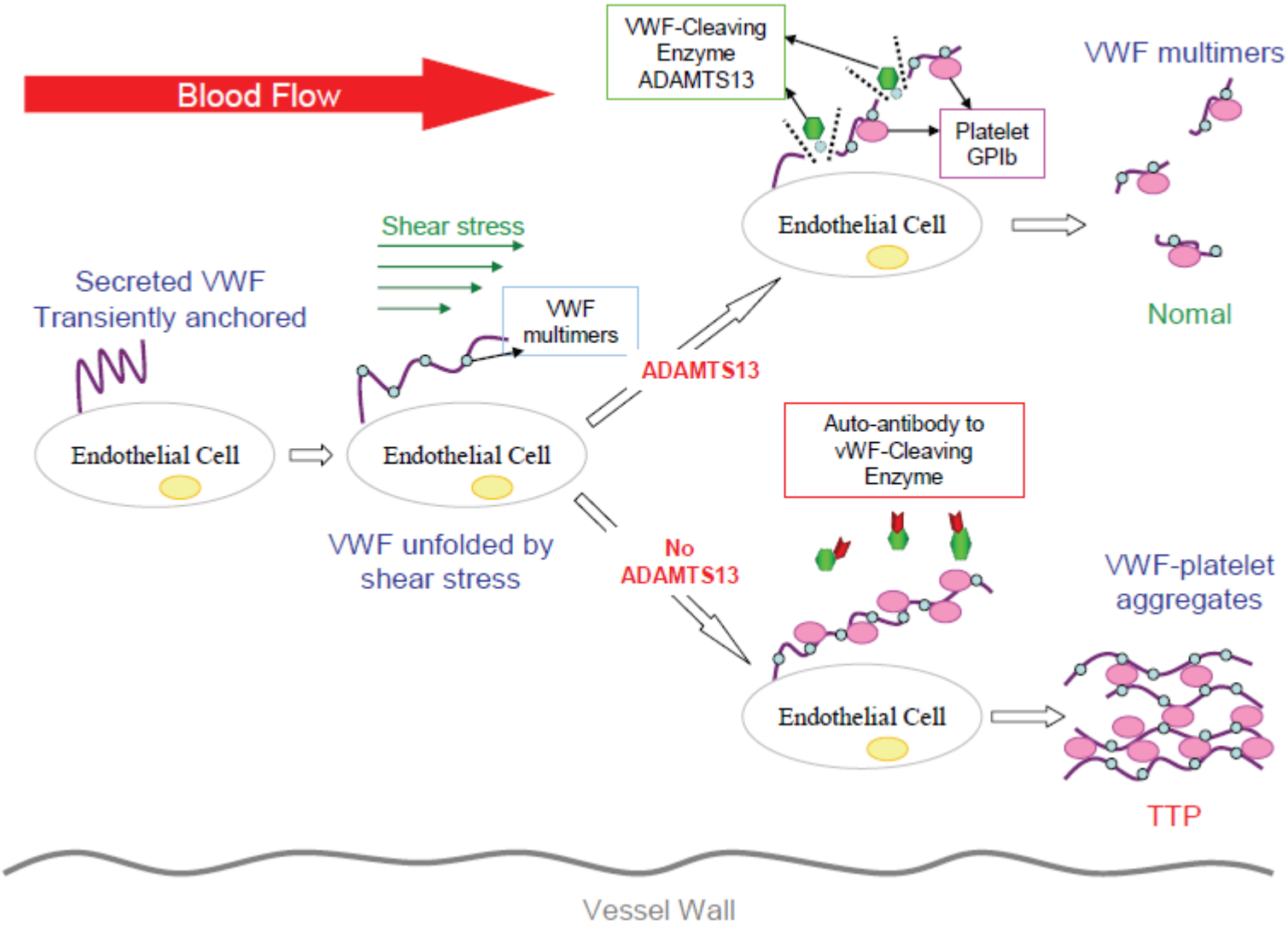


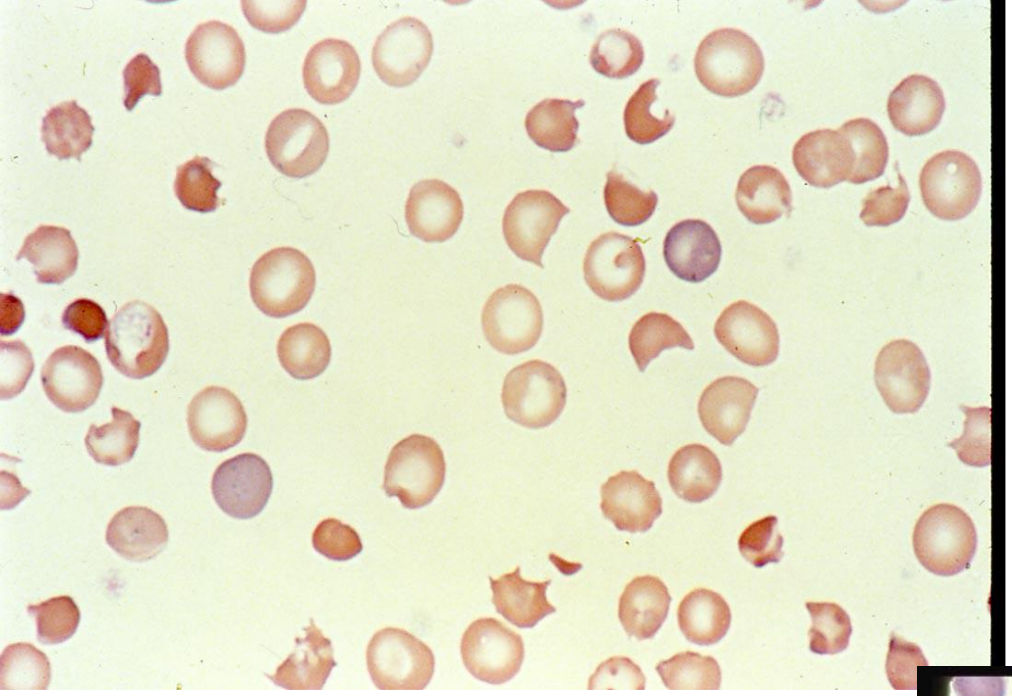
missing MP → formation of large multimers vWF → → mi**crovascular thrombi** + fibrin fibres → → damaged rbc = **schistocytes = hemolysis = manifestation of TTP**.



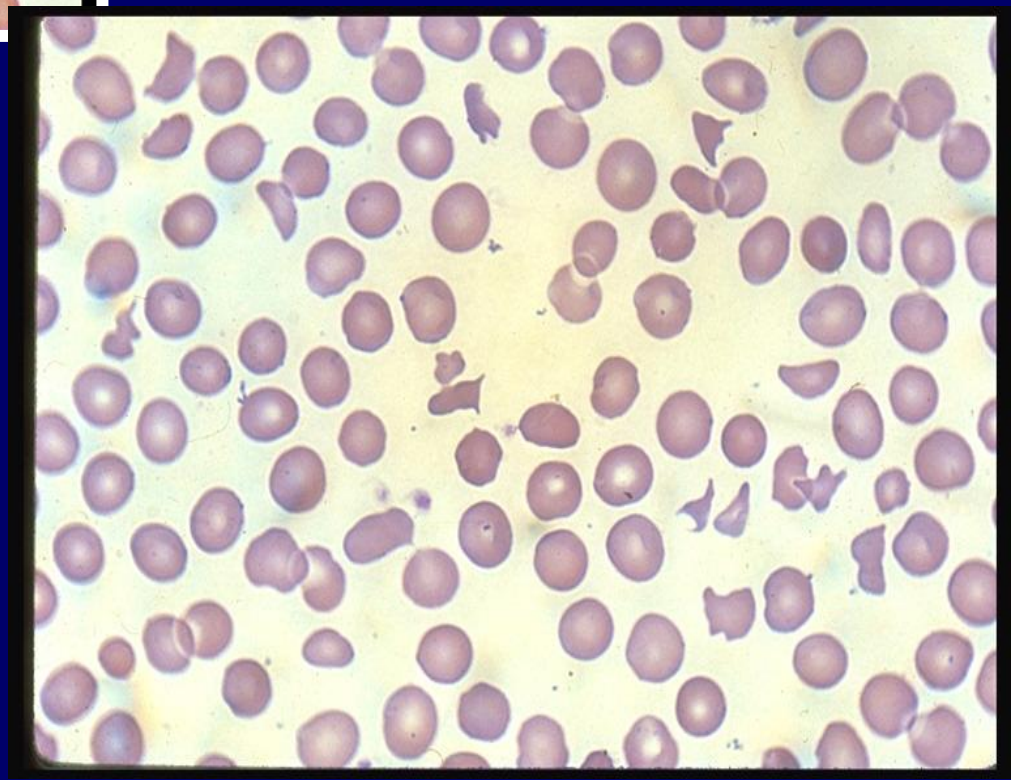
schistocytes

Pathogenesis of idiopathic thrombotic thrombocytopenic purpura: idiopathic TTP caused by ADAMTS13 deficiency due to gene mutations or autoantibodies. Multimeric VWF adheres to endothelial cells or to connective tissue exposed in the vessel wall. Platelets adhere to VWF through platelet membrane GPIb. In circulation, VWF is unfolded by shear stress and cleaved by ADAMTS13, limiting thrombus growth. If ADAMTS13 is severe deficiency, accumulation of VWF-platelet aggregation continues, eventually causing microvascular thrombosis and TTP.





schistocytes



HUS- classic triade

- Microangiopathic anemia
- Thrombocytopenia
- Acute renal failure

Symptoms	Diagnostic tools
Haemolysis	Haemoglobin ↓ red blood cells ↓
	Reticulocytes ↑
	Lactate dehydrogenase ↑
	Haptoglobin ↓ free serum haemoglobin ↑
	Direct antiglobulin test (Coombs test): negative
	Schystocytes ↑

Microangiophatic hemol anemia

Laboratory findings:

- **Anemia** + rtcytosis + **schistocytes** ↑ akanto- sphero
- **thrombocytopenia – DIC**
- elfo vWF - multimers (TTP)
- molecul. genet **ADAMTS13 Ag deficit; Coombs negat !!**
- ↑ Bili ↑ **LD**, ↓ **haptoglobin**

  ↑proteinuria, ↑ Hb-uria, ↑ free Hb, ↑ c**reatinin, urea**

Therapy TTP/HUS

FFP + exchange plasmapheresis, corticosteroids, anticoagulants, trf RBC, Plts

Acquired TTP – HUS: therapy

Untill TTP is not excluded, the TTP must be considered

1/ urgent

a/ Plasma (exchange) therapy (FFP) (30-50 ml/kg/day)

replace ADAMTS13 + remove antibodies

b/ Immunosuppression (methylprednisolone 2mg/kg/den iv.)

c/ eculizumab

d/ thrombocytes and rbc supplementation

e/ CVVH – renal failure

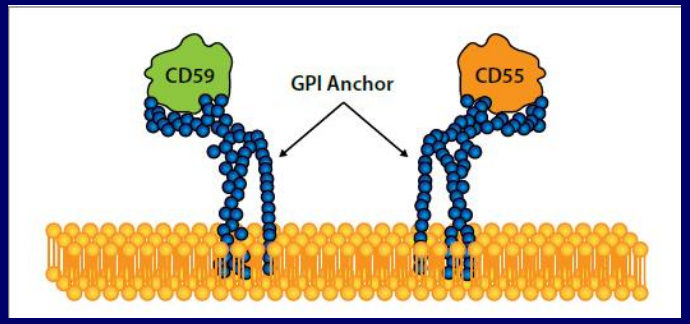
f/ anticoagulation (DIC)

Paroxysmal Nocturnal Hemoglobinuria

Marchiafava–Micheli syndrome

PNH is the only hemolytic anemia caused by an ***acquired*** intrinsic defect in the cell membrane (**deficiency** of glycophosphatidylinositol or GPI) leading to the absence of protective exterior surface proteins that normally attach via a **GPI anchor.**

GPI anchor is missing, so destruction of red blood cells by the complement system is going on

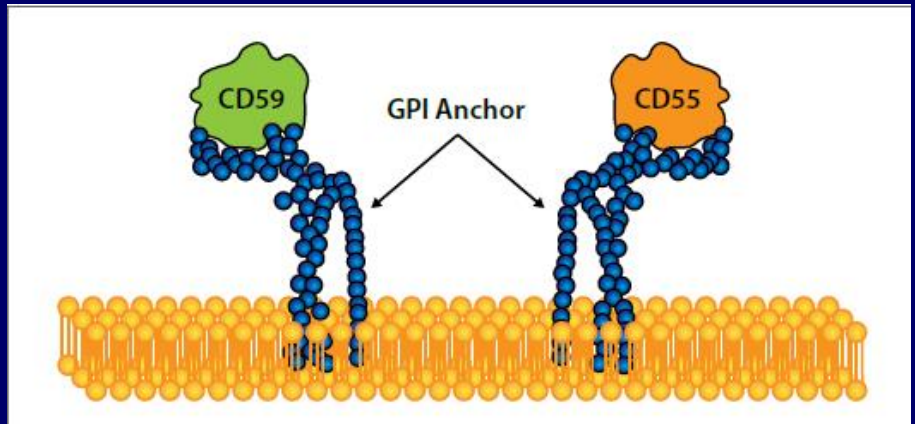


DAF / CD55 (decay-accelerating factor) is a 70 kDa membrane protein that attaches to the cell membrane via a glycophosphatidylinositol (GPI) anchor.

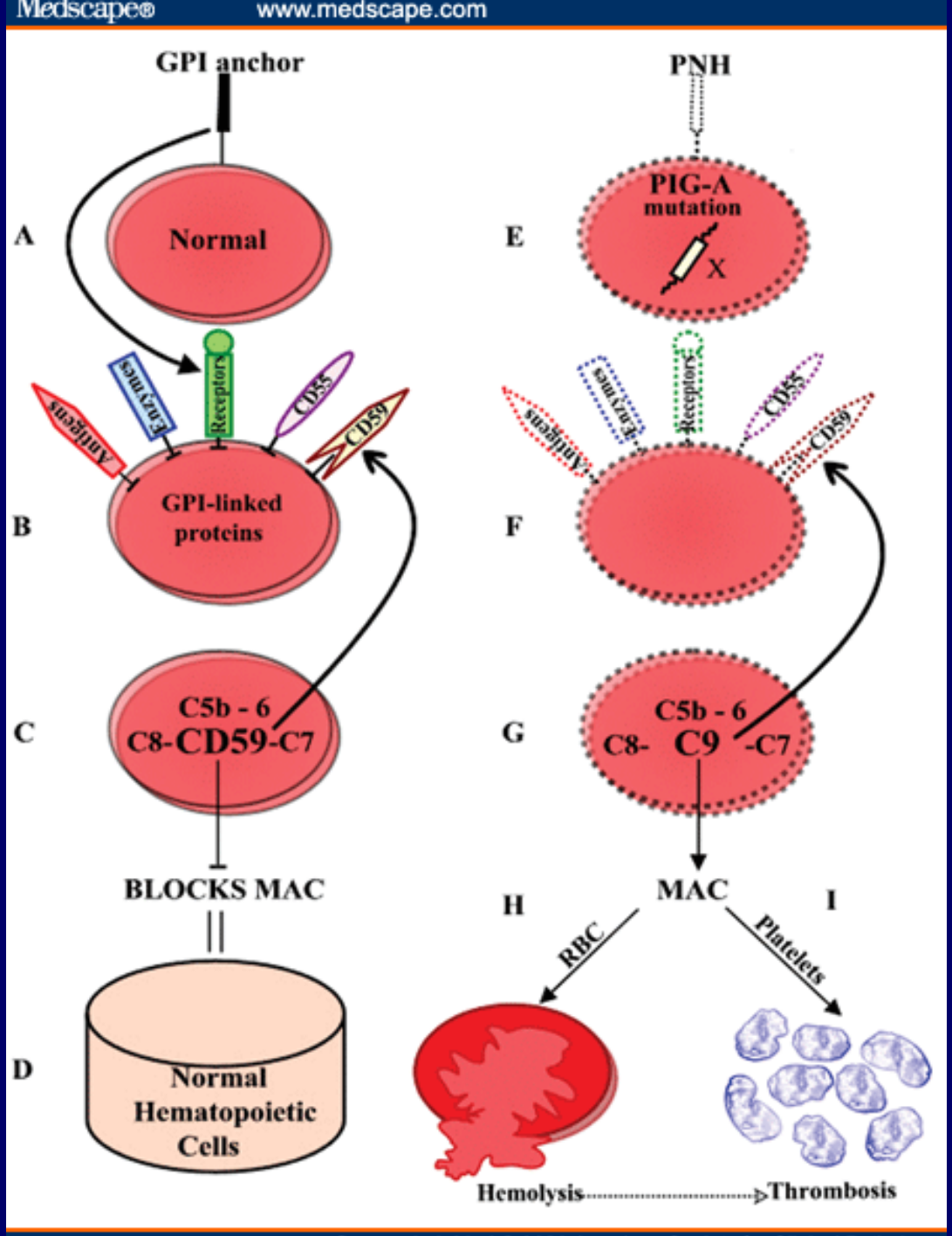
CD59 glycoprotein, **MAC-inhibitory protein** (**MAC- IP**), **membrane inhibitor of reactive lysis** (**MIRL**), or **protectin**, is a protein that in humans is encoded by the *CD59* gene

The most common defective enzyme is phosphatidylinositol glycan A (PIGA), one of several enzymes needed to make GPI.

The gene that codes for PIGA is located on the X chromosome,



Paroxysmal Nocturnal Hemoglobinuria

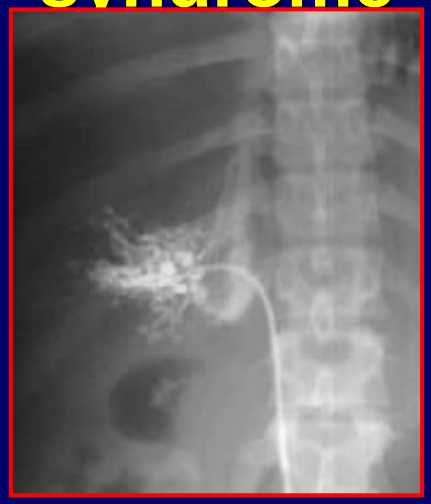


Paroxysmal Nocturnal Hemoglobinuria
Triad of Clinical Features

Haemoglobinuria

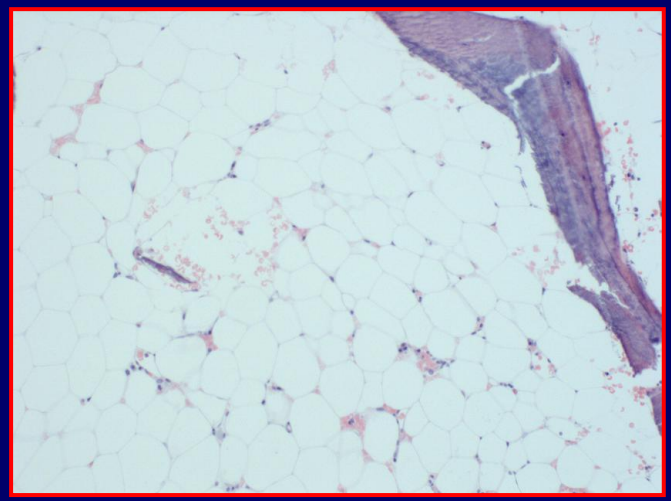
Intravascular haemolysis
→ disabling symptoms
- abdominal pain
- dysphagia
- erectile failure
- severe lethargy

Budd-Chiari syndrome



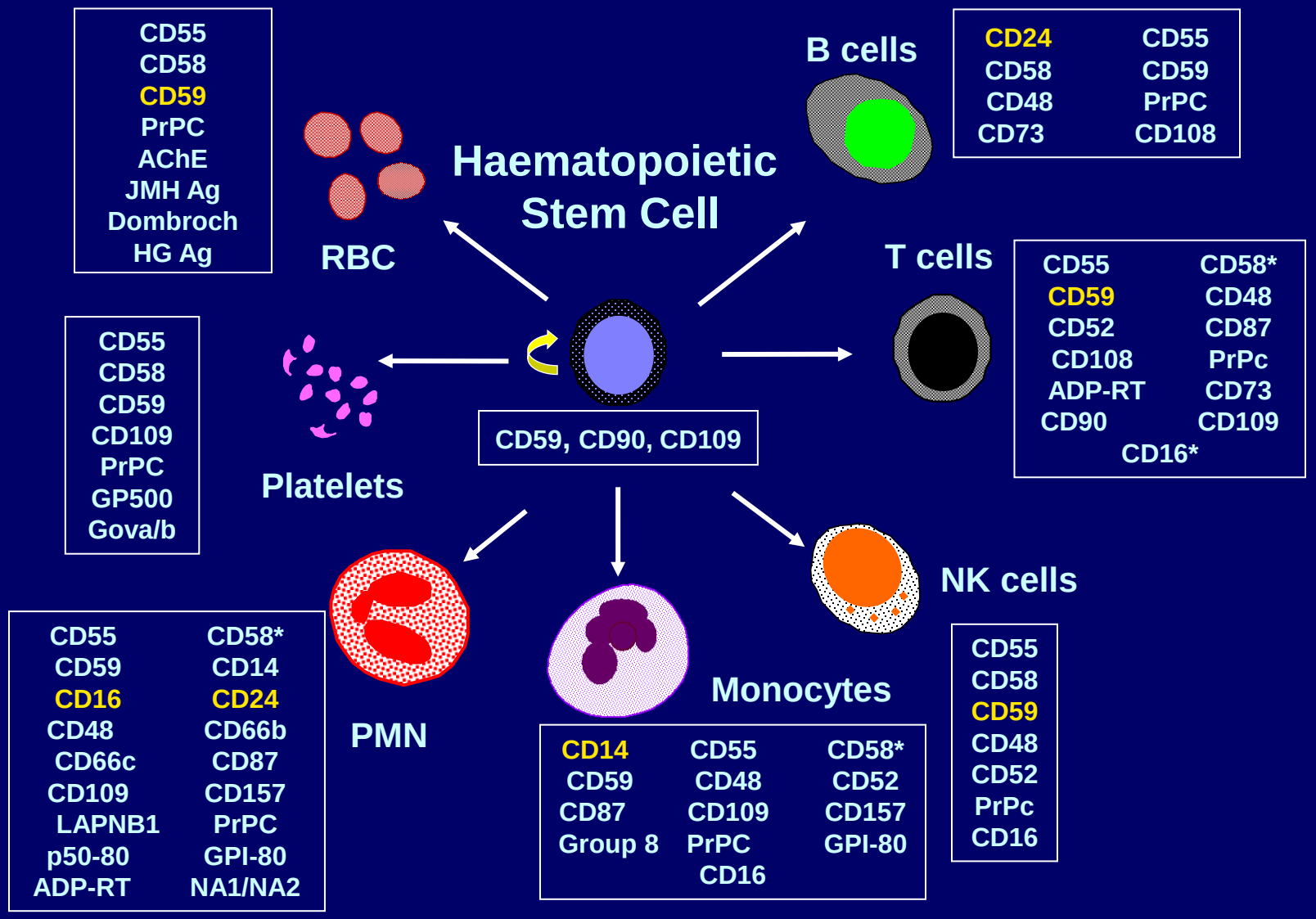
Thrombosis
- liver, cerebral
- 50% of patients
- 33% of patients is fatal

Aplastic anaemia



Bone Marrow Failure
- often precedes PNH
- selects for PNH clone

Proteins Deficient from PNH Blood Cells

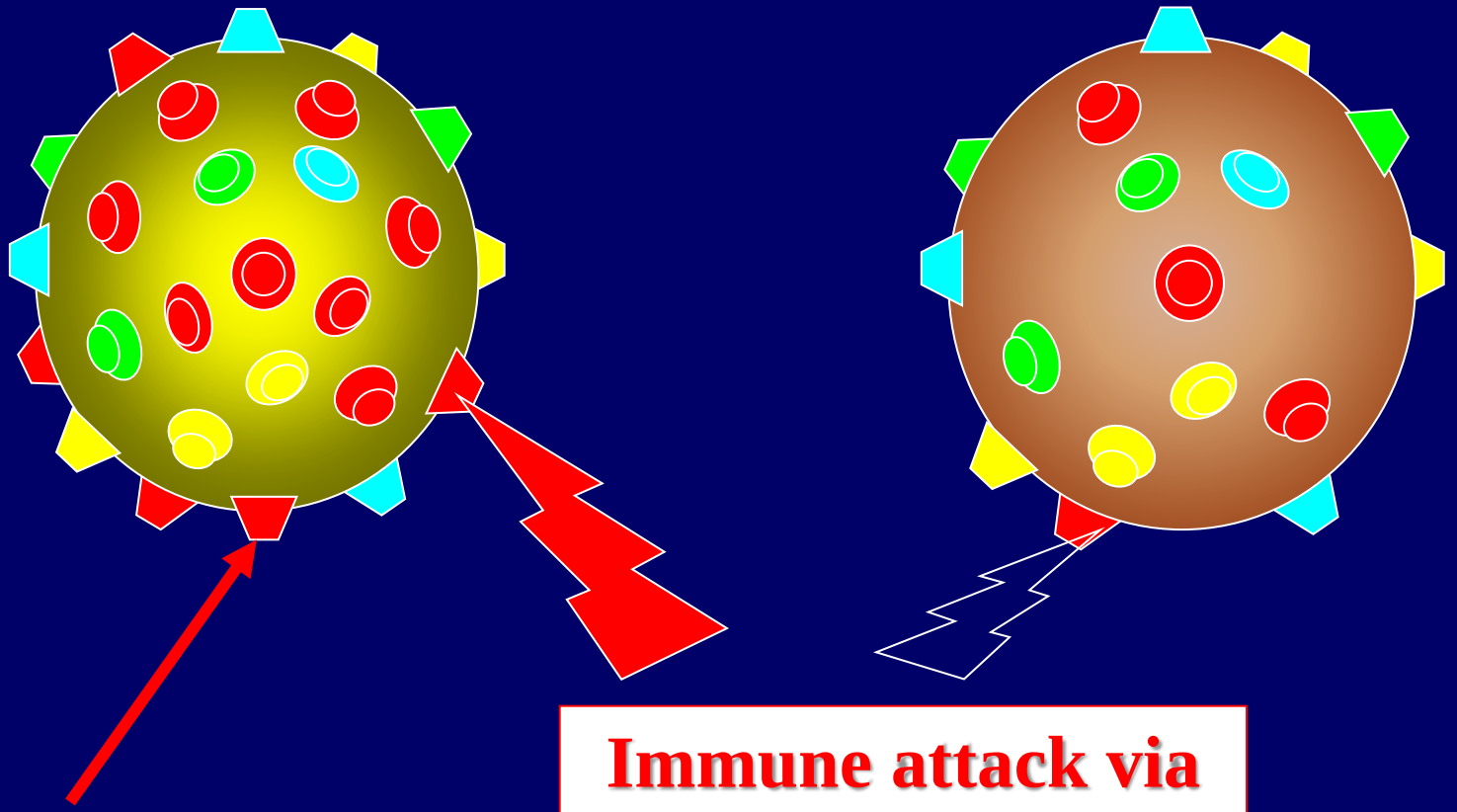


(Courtesy of Lucio Luzzatto)

Relative Growth Advantage in PNH

Normal stem cells

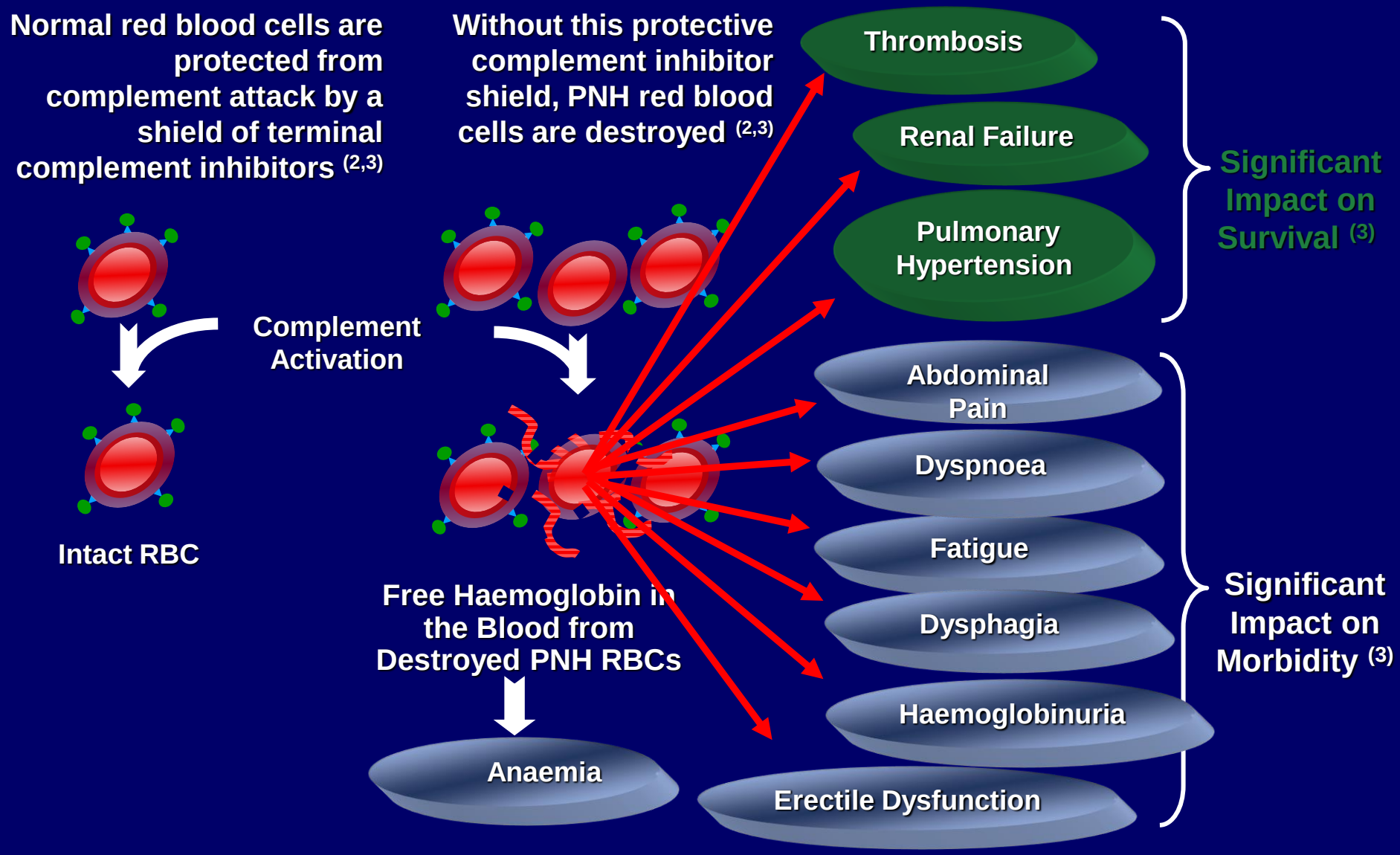
GPI-deficient (PNH) stem cells



GPI-linked antigen

Immune attack via GPI-linked antigen (aplastic anaemia)

PNH is a Progressive Disease of Chronic Haemolysis [1-4]



(1) Rother R et al. JAMA 2005;293:1653-1662; (2) Brodsky RA. Blood Rev 2008;22:65-74;
(3) Rother R et al. Nat Biotech 2007;25:1256-1264; (4) Socie G et al. Lancet 1996;348:573-577.

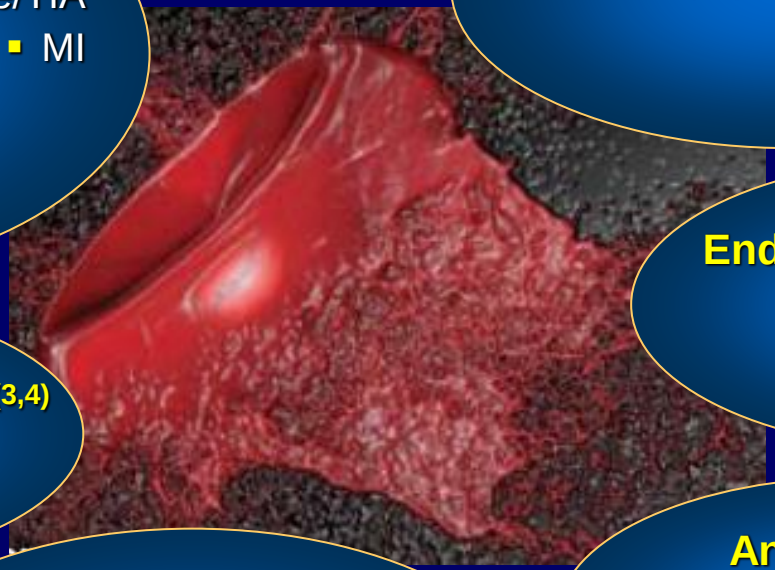
Chronic Haemolysis is the Underlying Cause of Progressive Morbidities and Mortality of PNH [1-5]

THROMBOSIS [2,4,5]

Venous
- PE/DVT
- Cerebral
- Dermal
- Hepatic/Portal
- Abdominal ischemia

Arterial
- Stroke/TIA
- MI

Chronic Kidney Disease [3,4]
- Renal insufficiency
- Dialysis
- Anaemia



End Organ Damage [2,3,4]
- Brain
- Liver
- GI

Pulmonary Hypertension [3,4]
- Dyspnoea
- Cardiac Dysfunction

Anaemia [2,4,5]
- Transfusions
- Haemosiderosis

Fatigue / Impaired Quality of Life [3,4]
- Abdominal pain
- Dysphagia
- Poor physical functioning
- Erectile dysfunction

(1) Parker C et al. Blood 2005;106:3699-709; (2) Hillmen P et al. NEJM 1995;333:1253-58; (3) Rother R et al. JAMA 2005;293:1653-62; (4) Rother R et al. Nat Biotech 2007;25:1256-1264; (5) Socie G et al. Lancet 1996;348:573-577.

Renal Damage in PNH

Chronic haemolysis and cell-free plasma haemoglobin lead to chronic kidney disease in PNH [1,2]

Renal damage in PNH may be due to repetitive exposure of tissue to cell-free haemoglobin [3,4]

64% of patients with PNH have stage 1-5 chronic kidney disease [5]

Renal failure has been identified as the cause of death in approximately 8 – 18% of PNH patients [6,7]

(1) Parker C et al. Blood 2005;106:3699-3709; (2) Rother RP et al. JAMA 2005;293:1653-1662; (3) Clark DA et al. Blood 1981;57:83-9; (4) Hillmen P et al. NEJM 1995; 333:1253-8; (5) Hillmen P et al. Blood 2007;110(11):3678: Poster at American Society of Hematology 49th Annual Meeting; (6) Nishimura JI et al. Medicine 2004;83:193-207; (7) Rosse and Nishimura. Int J Hematol 2003;77:113–20.

Routine Red Cell Analysis: Reagents

- For historical reasons, CD55 and CD59 are most commonly used
- CD59 is strongly expressed, while CD55 is weak
  - CD55 may not be necessary
  - Rare congenital CD59 deficiency cases
  - Some variation in CD59 clones
- Other GPI-anchored reagents (CD58) exist, but limited experience
- Anti-glycophorin (CD235a) may be used to identify red cells, but this may not be necessary for routine analysis
  - Can guard against failure of antibody to contact cells

Leucocyte Analysis: Routine testing

- Granulocyte PNH clone probably gives most accurate estimate of PNH clone size
- Monocyte clones can usually be determined in same tube and confirms granulocyte result, though because monocytes are less numerous, precision is lower
- Type II granulocytes can occasionally be recognized but red cells are typically better for this purpose
- Lymphocytes are not a suitable target for testing

WHAT IS FLAER?

FLuorescent AERolysin

Aerolysin is a pore-forming toxin secreted by *Aeromonas hydrophila* GPI-anchor serves as receptor

FLAER – A488-conjugated mutant aerolysin binds to GPI -anchor rather than surrogate protein and is inactive so doesn't form channels