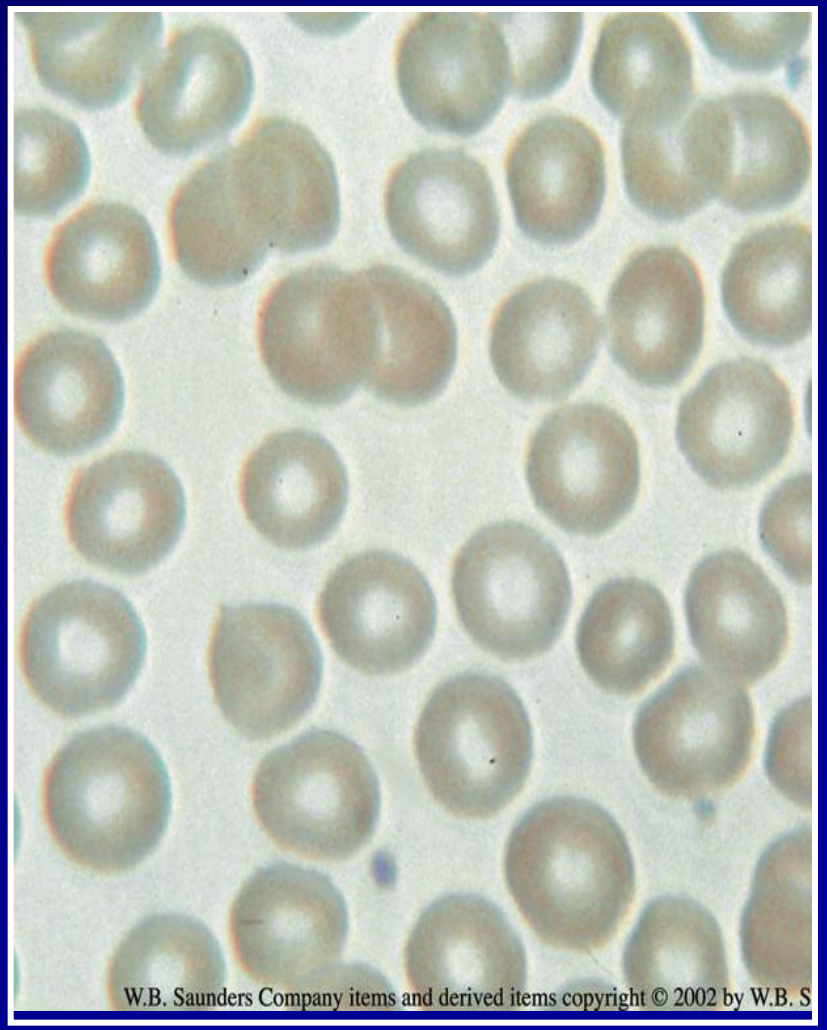
Αναιμίες

Σωτήρης Τσιόδρας
Παθολόγος-Λοιμωξιολόγος

Ερυθροκύτταρα

- Normal range 4.2-5.5 million per mm^3 in adults.
- Biconcave shape.
- Diameter 7 microns.
- Cells for transport of O_2 and CO_2.
- Life span 120 days.



Ερυθροποίηση

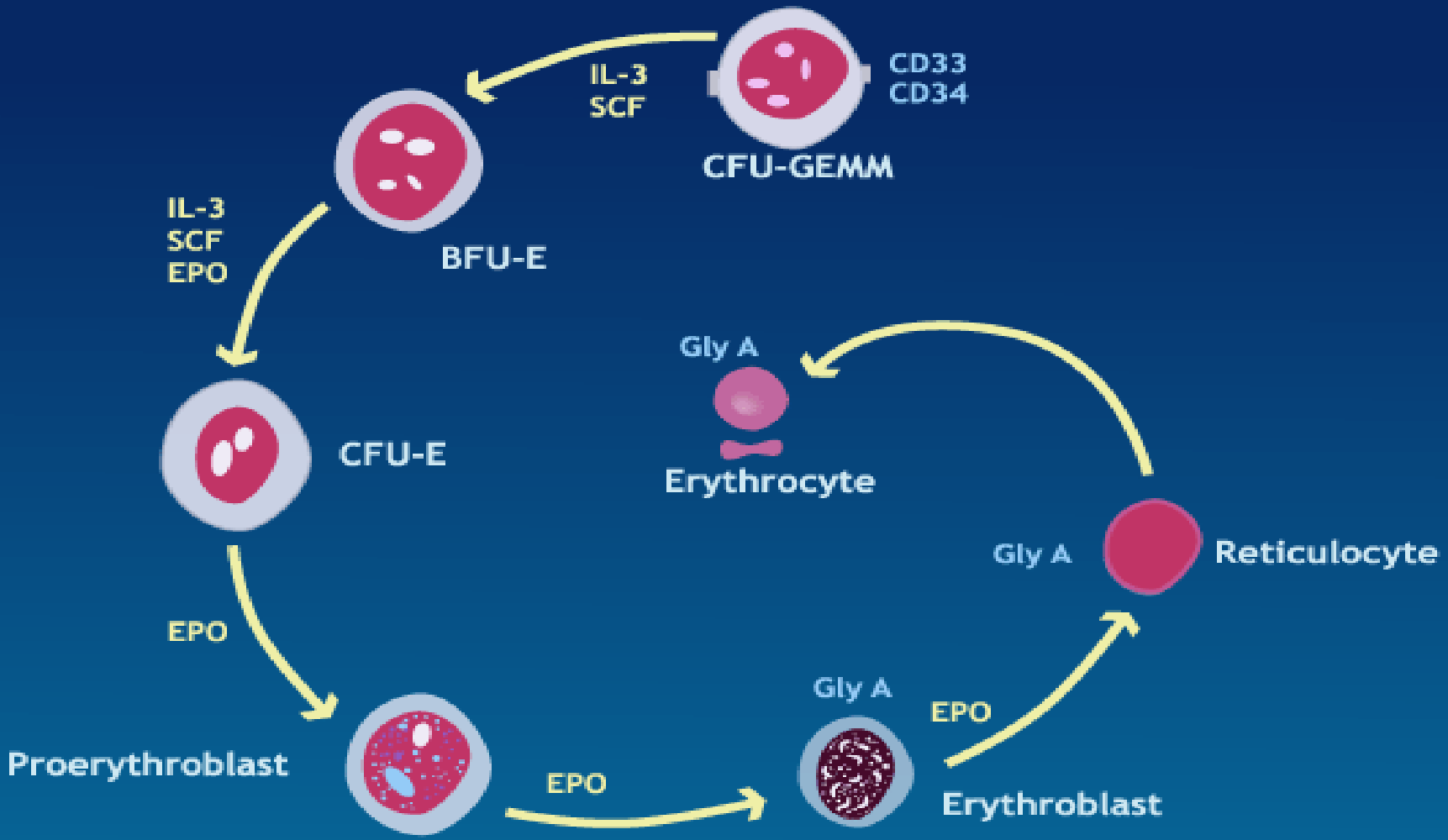
- Ο ρυθμιστής είναι η Ερυθροποιητίνη (EPO)
  - Παραγωγή στα νεφρά
    - Μικρή ποσότης στο ήπαρ
  - Κφ επίπεδα 10-25 U/L
  - T1/2 6-9 hrs
  - Σε ↓ Hb < 10-12 g/dL

Ερυθροποίηση

- Με ↑ EPO 4-5πλασιασμός παραγωγής RBC σε 1-2 εβδομάδες εξαρτώμενος από
  - Επαρκή θρεπτικά υλικά
    - Πχ Fe, B12

Erythrocytic Maturation Series

Erythrocyte Development

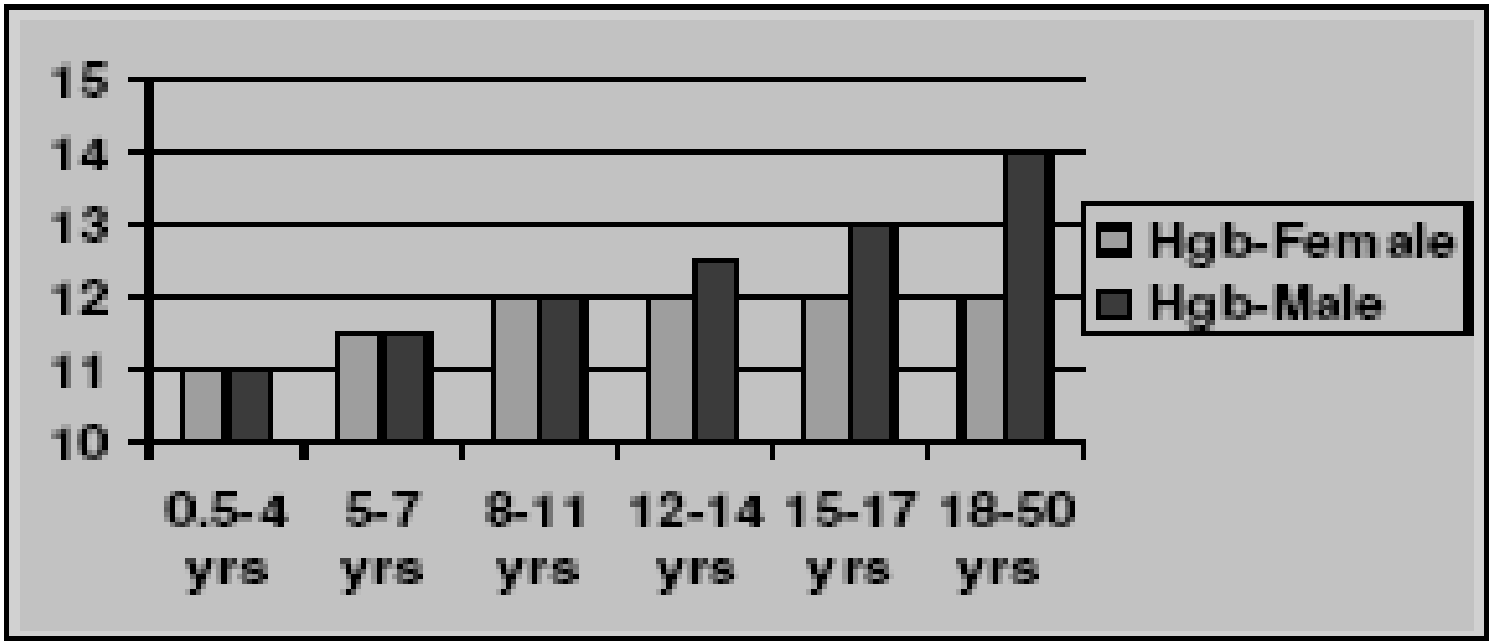


ΑΝΑΙΜΙΑ

- Άνδρες Ht 47 ± 7
- Γυναίκες Ht 42 ± 5

All Ages Anemia Definition

- Numerical -- Reduction in Hgb Level > 2 S.D. Below the Population Mean



ΑΝΑΙΜΙΑ

- Συμπτώματα και σημεία
- Οξεία
  - Αιμορραγία, Υποογκαιμία,
    - 10-15 % απώλεια
      - Αγγειοσύσπαση, ανακατανομή ροής
    - > 30 % απώλεια
      - Ορθ. υπόταση, ταχυκαρδία, δυσλειτουργία οργάνων
    - > 40 %
      - Shock, δύσπνοια, εφίδρωση, υπόταση, ταχυκαρδία

ΑΝΑΙΜΙΑ

- Συμπτώματα και σημεία
- Οξεία
  - Αιμόλυση
    - ενδαγγειακή
      - Οξύ άλγος στην οσφύ
      - Ελεύθερη Hb στο πλάσμα και στα ούρα
      - Νεφρική ανεπάρκεια

ΑΝΑΙΜΙΑ

- Συμπτώματα και σημεία
- Χρόνια
  - Εξαρτώνται από την ηλικία και αιμάτωση ζωτικών οργάνων
    - Σε νέα άτομα ασυμπτωματική παρά ↓↓↓ Ht
  - Μετρίου βαθμού
    - κακουχία, έλλειψη αντοχής, δύσπνοια, ταχυκαρδία

ΑΝΑΙΜΙΑ

- Ιστορικό
  - Μέση Ανατολή
    - G6PD deficiency
  - Έκθεση σε φάρμακα, τοξίνες
    - NSAIDS, μόλυβδος
- ΦΕ
  - Συμπτώματα και σημεία άλλων νόσων
    - Πυρετός, κακουχία, απώλεια βάρους, διαταραχές κενώσεων, αιμορραγία, οργανομεγαλία, φυσήματα, πετέχειες

ΑΝΑΙΜΙΑ

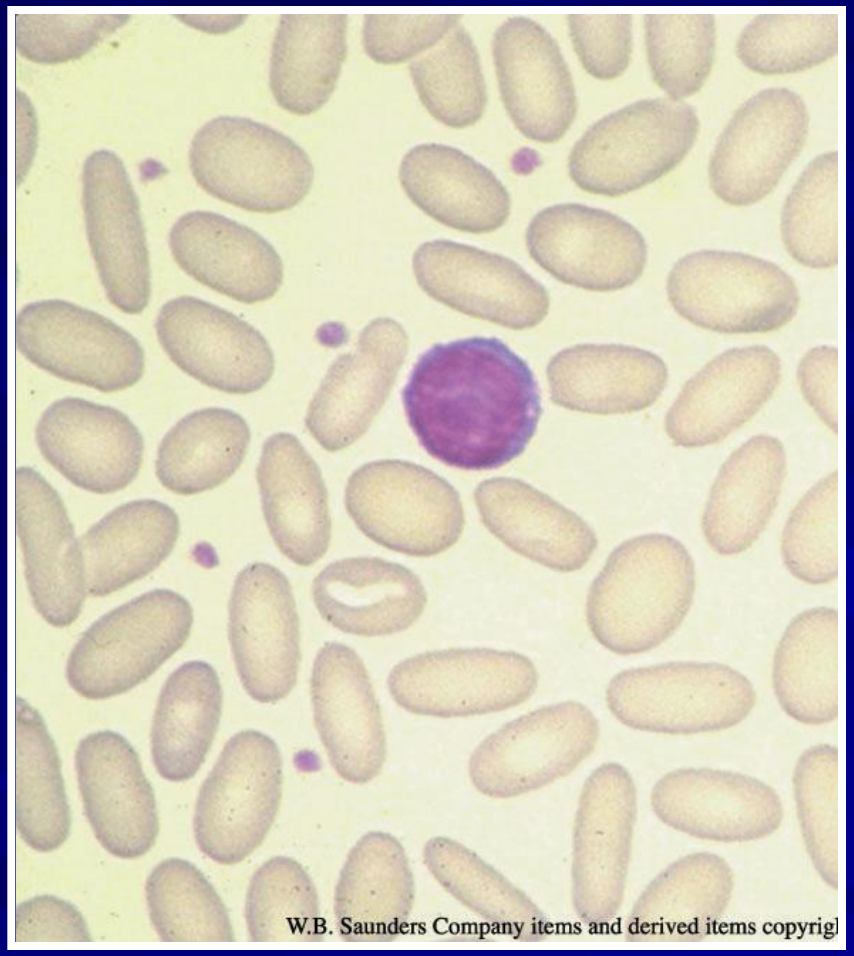
- Διαταραχές που συχνά σχετίζονται με αναιμία
  - Χρόνιες φλεγμονές
    - Π.χ. Λοιμώξεις, ΡΑ
  - Αιματολογικά νοσήματα
    - Λευχαιμίες
      - Π.χ. ΧΛΛ, Νεοπλασίες Β κυττάρων

ANAIMIA

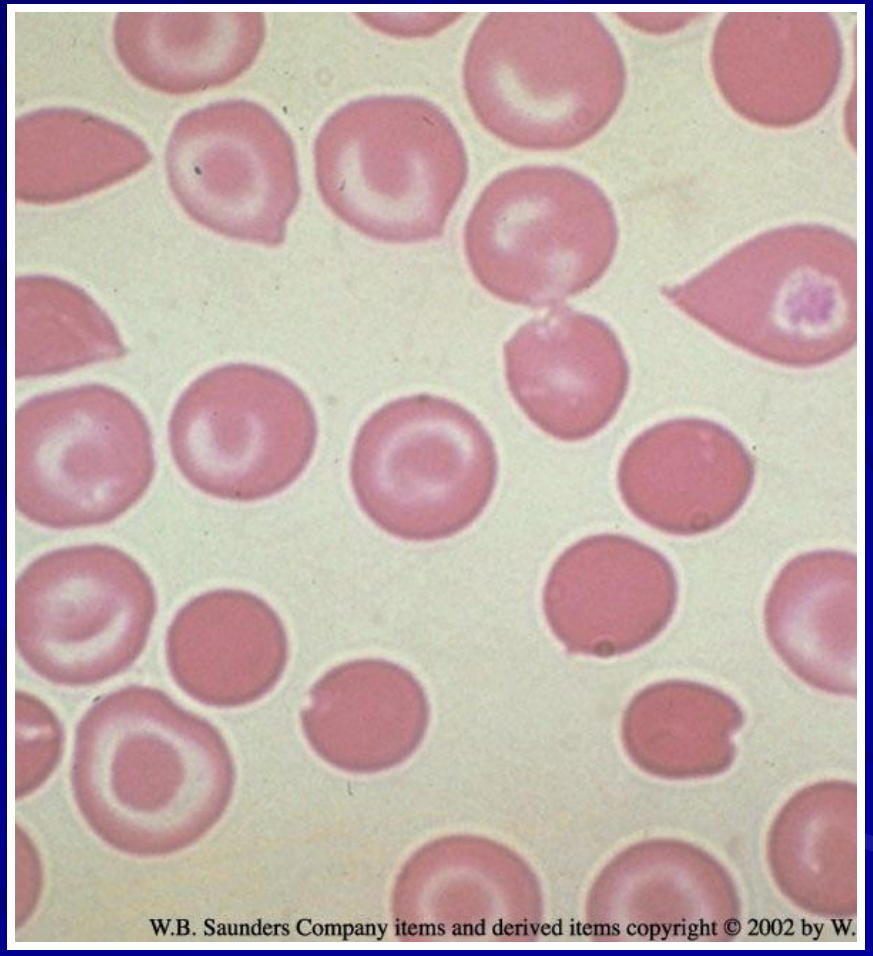
- Διαγνωστική διερεύνηση
  - CBC
    - Hb, Ht, retic count
  - RBC indices
    - MCV, MCH, MCHC, RDW
  - WBC
    - Diff, segmentation
  - PLT
  - Cell morphology
  - IRON STUDIES
    - Fe, TIBC, ferritin, marrow iron stain
  - Marrow
    - Aspirate for M/E Ratio, cell morphology, iron stain
    - Biopsy for cellularity, morphology

RBC Morphology

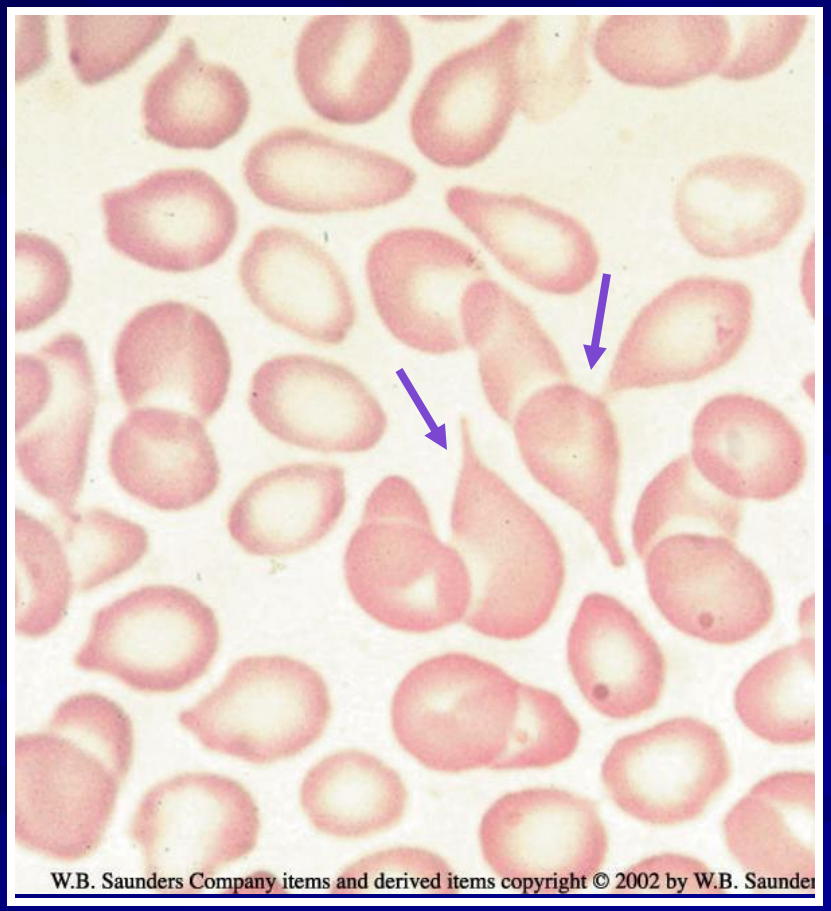
Elliptocytes



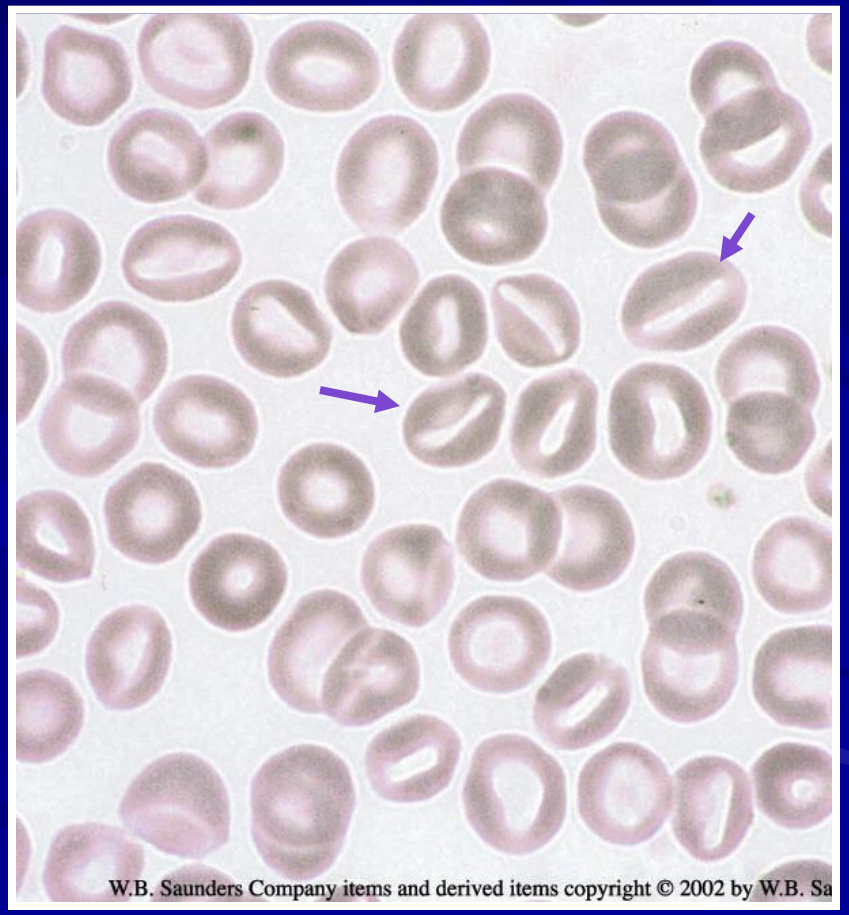
Target Cells



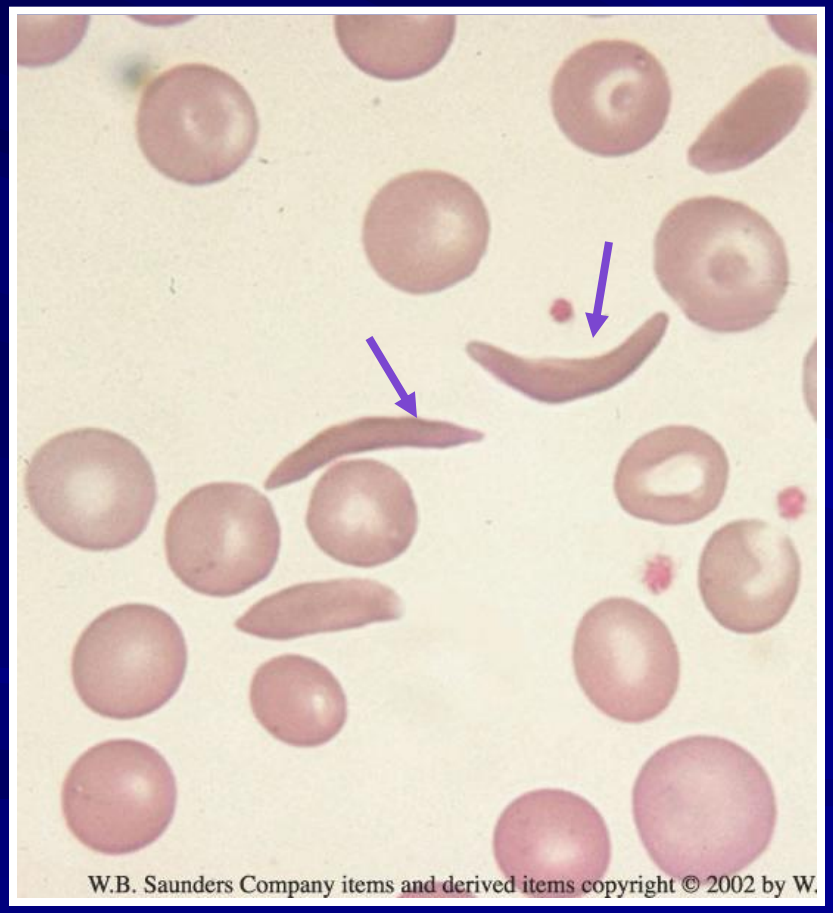
Tear Drops



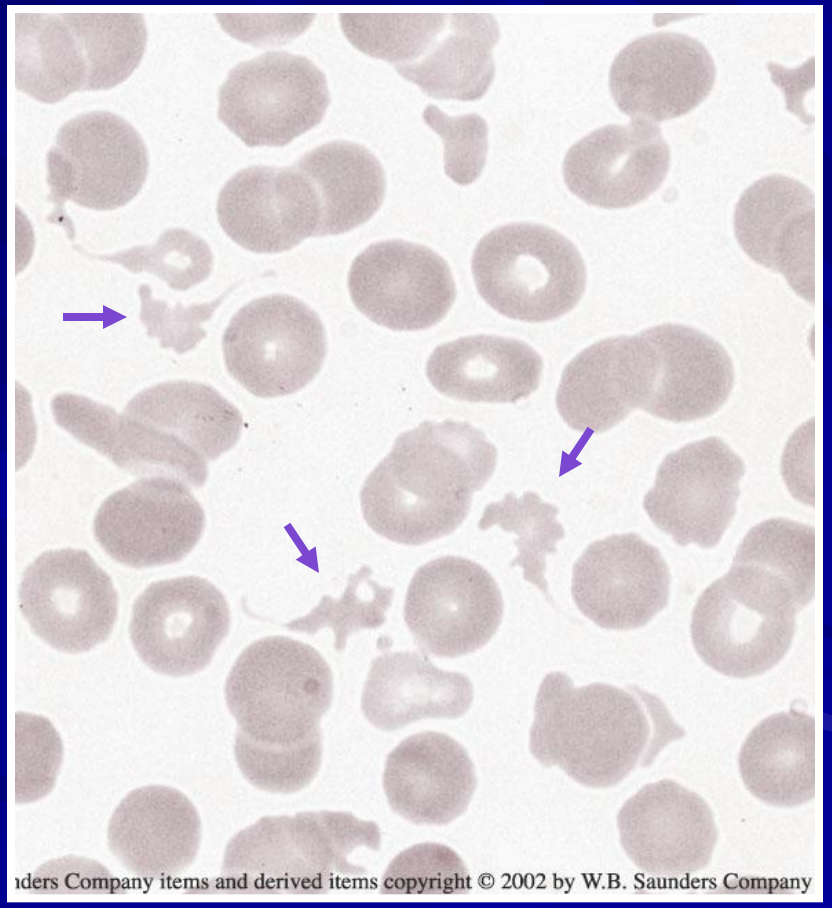
Stomatocytes



Sickle Cells



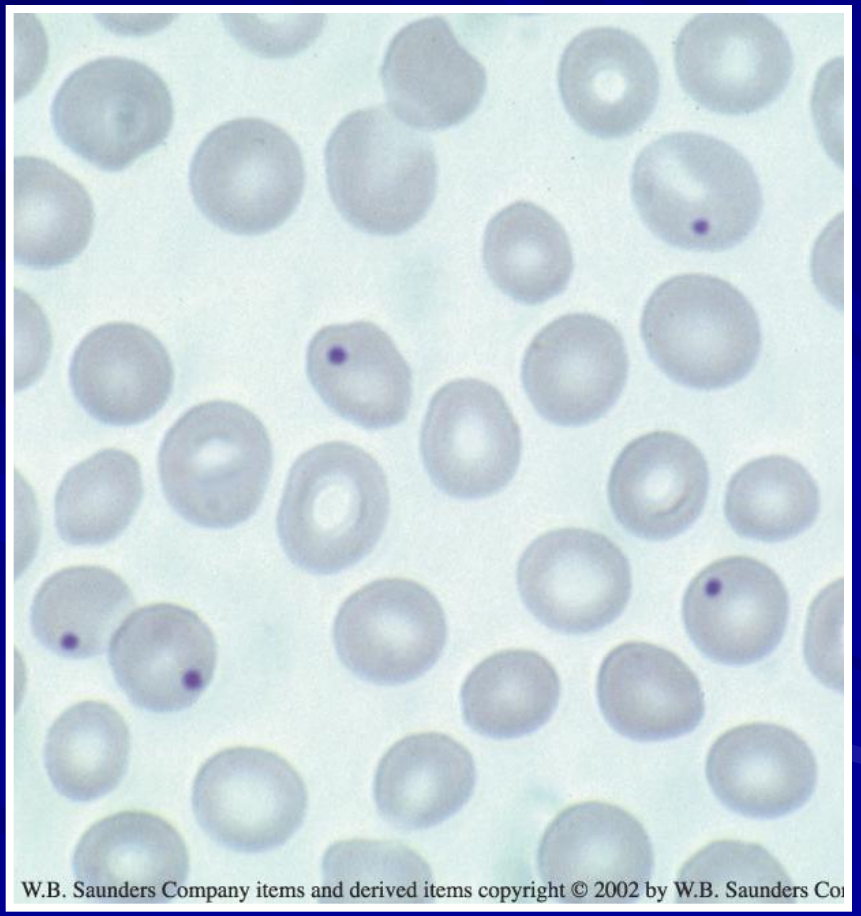
Schistocytes



RBC Inclusions

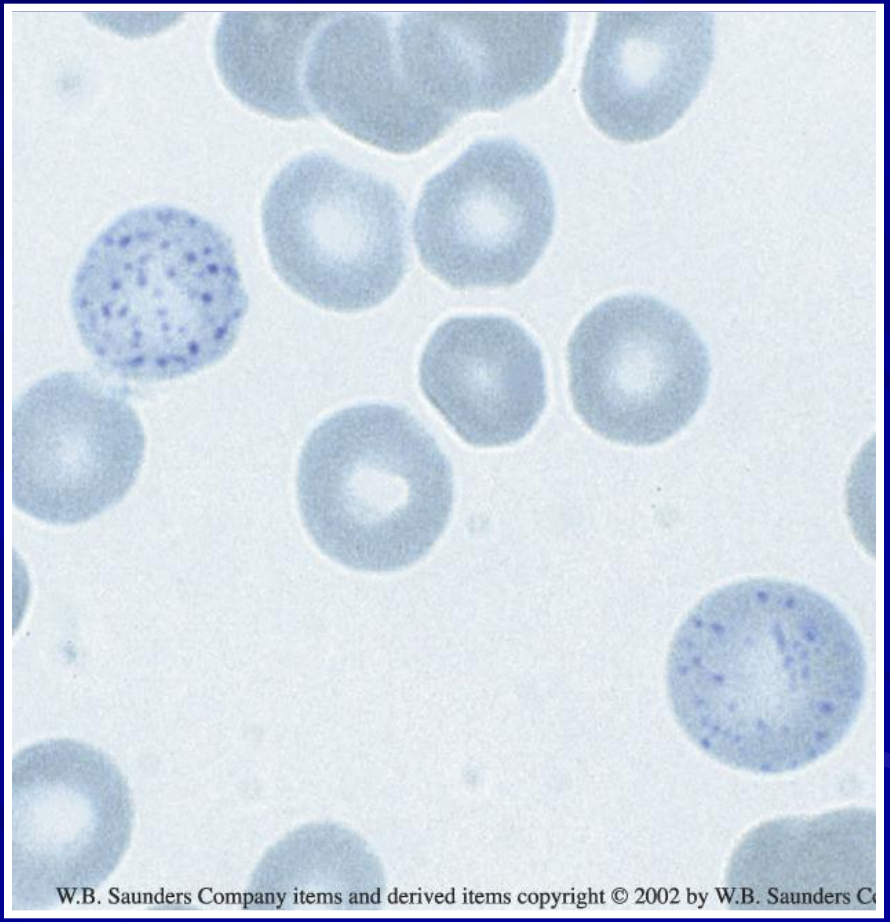
Howell-Jolly Bodies

- Round, purple inclusions in RBCs.
- Composed of DNA.
- Commonly seen in in patients with hypofunctioning spleens.
- Splenectomy.



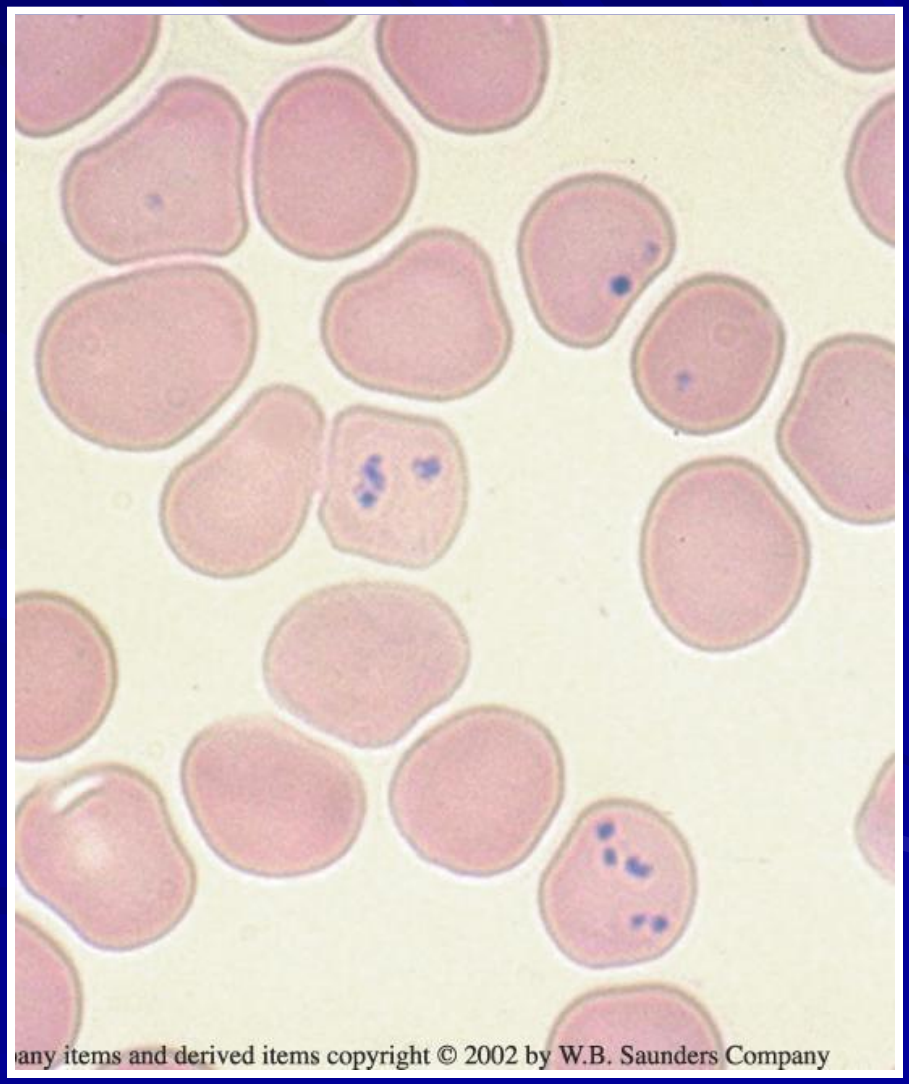
Basophilic Stippling

- Numerous, small purple inclusions in RBCs.
- Aggregates of ribosomal RNA.
- Most commonly seen in lead poisoning.

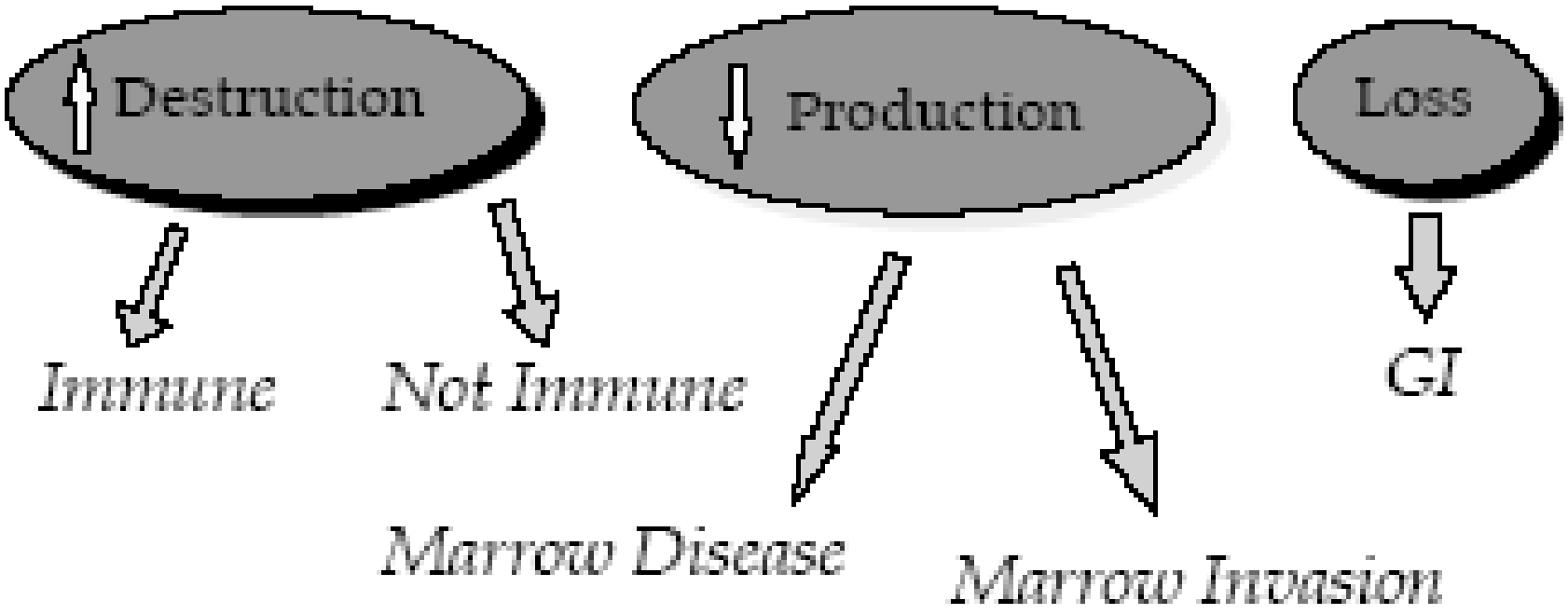


Pappenheimer Bodies

- Clusters of dark blue granules, irregular in size and shape.
- Composed of iron and ribosomal RNA.
- Seen in sideroblastic and hemolytic anemias.



Evaluating an Anemia



Decreased Production

Marrow Disease

Nutritional
- Iron; Copper; Folate

Metabolic
- Thyroid; B-12;

Failure
- Aplastic Anemia
- Myelodysplasia
- Infection

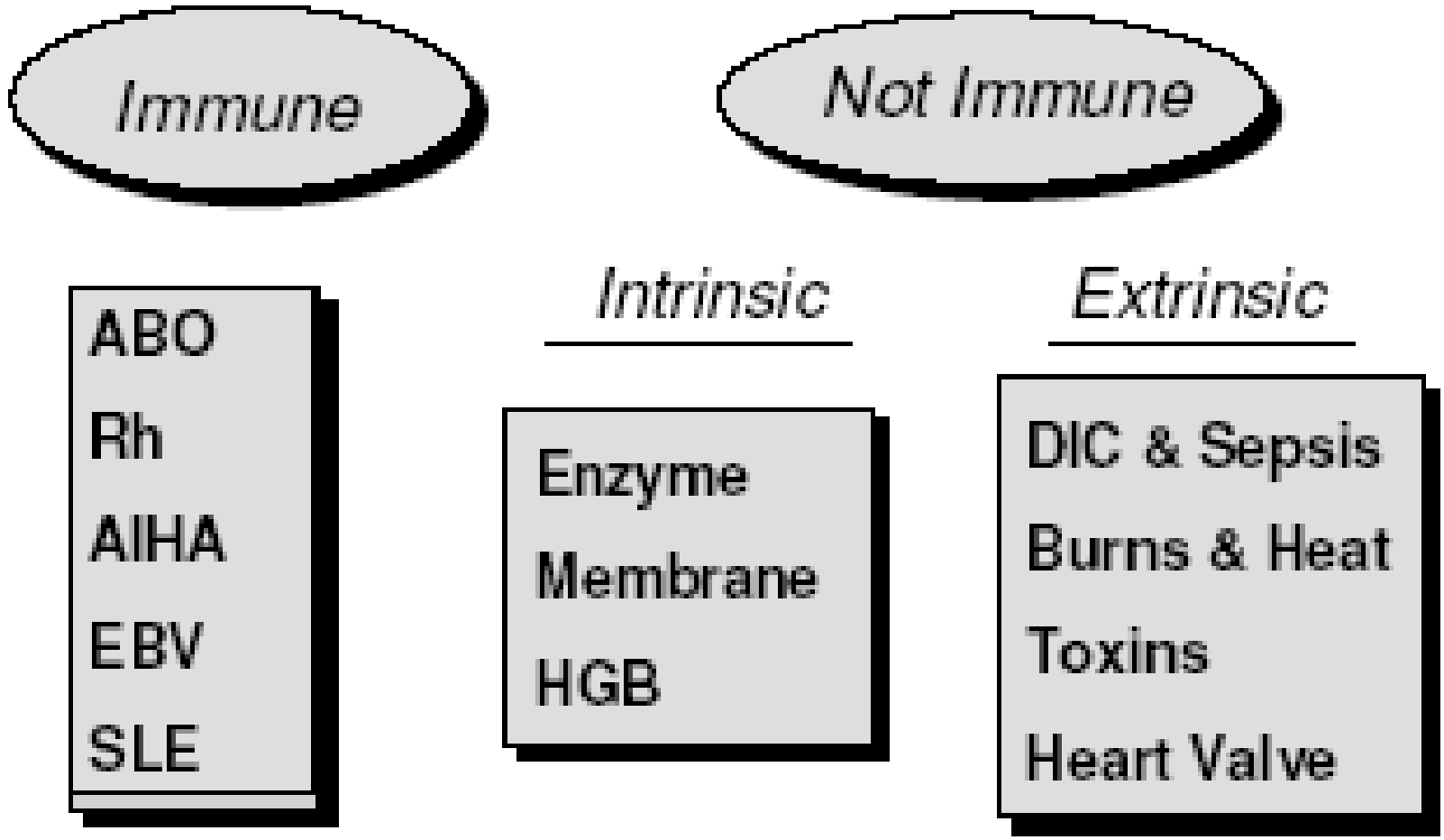
Congenital
- Fanconi; BFD

Marrow Invasion

Malignant
- Leukemia
- Neuroblastoma
- Lymphoma
- Solid Tumor

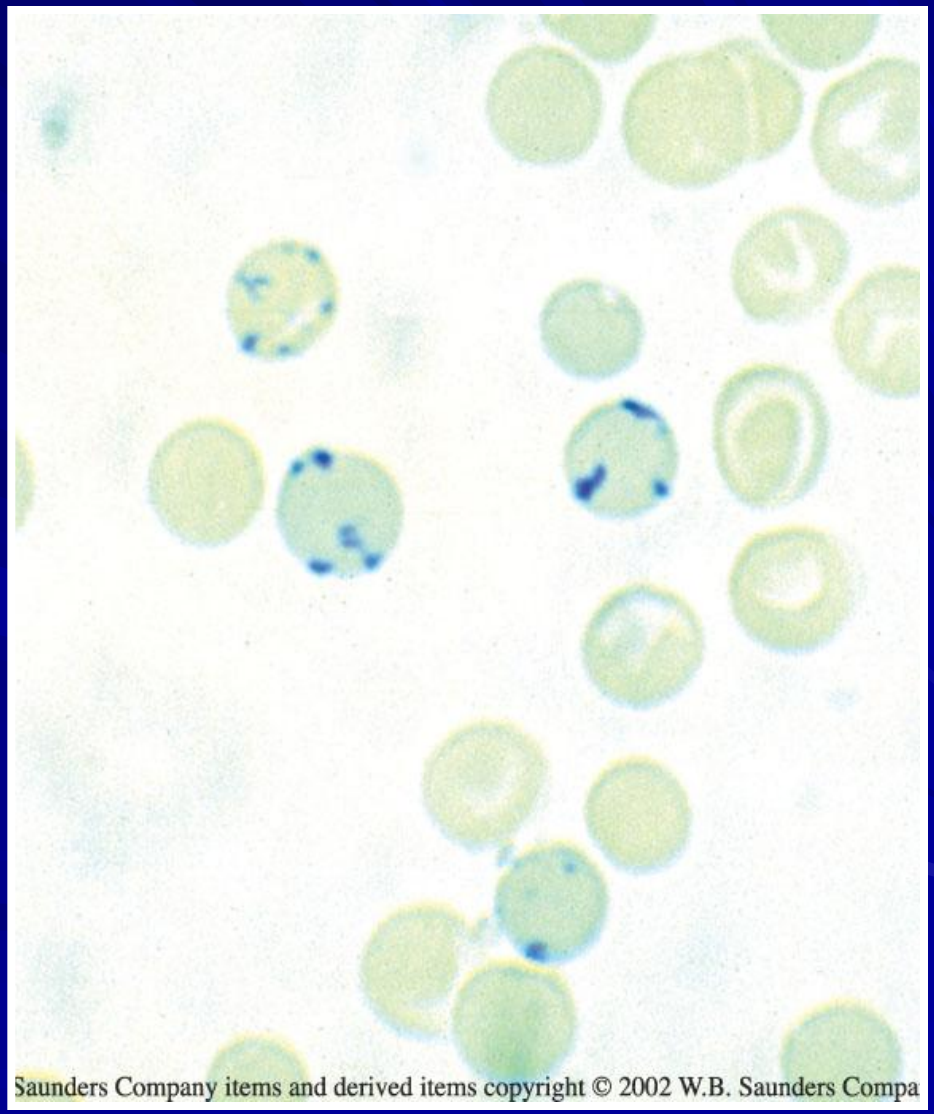
Non-Malignant
- Histiocytosis
- Storage Disease

Increased Destruction



Reticulocytes

- Immature RBCs.
- Contain residual ribosomal RNA.
- Reticulum stains blue using a supravital stain (new methylene blue).
- Counted and expressed as % of total red cells.



Reticulocyte Count

Uses supravital stain which stains cells in the living state.

Retic % = $\frac{\text{\# retics per 1000 RBCs}}{10}$

Corrected retic= $\frac{\text{\% retics x pt. HCT}}{45}$

Retic production index

DIVIDE BY FACTOR OF 2

Ταξινόμηση

<u>Σύμφωνα με το λειτουργικό έλλειμα στην παραγωγή RBCs</u>

- **Marrow production defects**
  - Hypoproliferative d/o
- **Red cell maturation defects**
  - Ineffective erythropoiesis
- **Decreased Red cell survival**
  - Blood loss / hemolysis

Ταξινόμηση

Σύμφωνα με το λειτουργικό έλλειμα στην παραγωγή RBCs

- Ret index < 2.5
  - Normocytic
    - Hypoprolif
      - Marrow damage
        - Infiltration, aplasia
      - Fe deficiency
      - ↓ stimulation
        - Inflammation, renal, metabolic

Ταξινόμηση

Σύμφωνα με το λειτουργικό έλλειμα στην παραγωγή RBCs

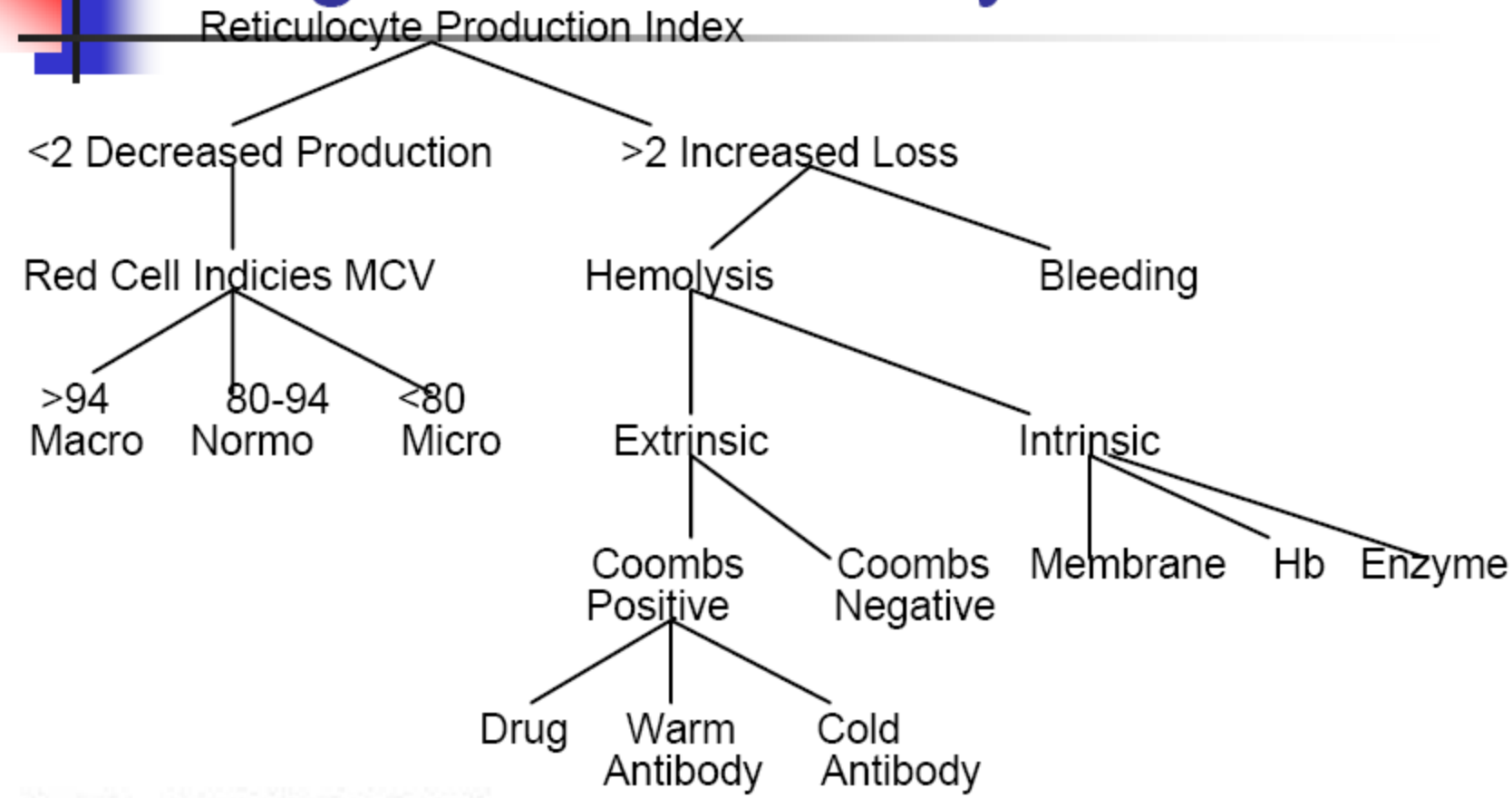
- **Ret index < 2.5**
  - **Microcytic**
    - **Maturation disorder**
      - **Cytoplasmic defect**
        - Fe deficiency
        - Thalassemia
        - Sideroblastic
      - **Nuclear defects**
        - **Folate, B12**
        - **Drug toxicity**
        - **Refractory anemia**

Ταξινόμηση

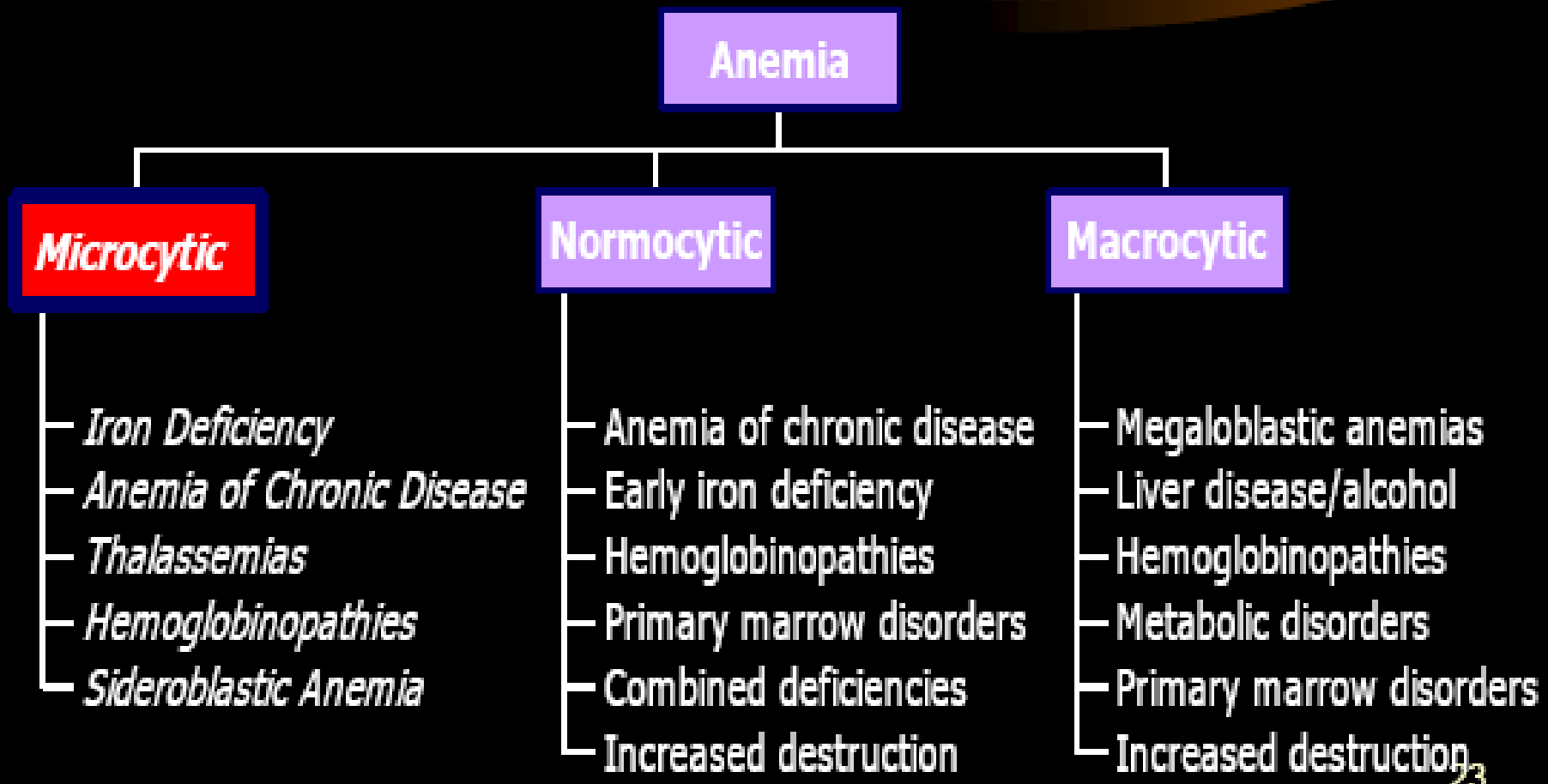
Σύμφωνα με το λειτουργικό έλλειμα στην παραγωγή RBCs

- **Ret index ≥ 2.5**
  - **Hemolysis –hemorrhage**
    - **Blood loss**
    - **Intravascular hemolysis**
    - **Metabolic defect**
    - **Membrane abnormality**
    - **Hemoglobinopathy**
    - **Autoimmune defect**
    - **Fragmentation hemolysis**

Diagnostic Pathway



Anemia Workup - MCV

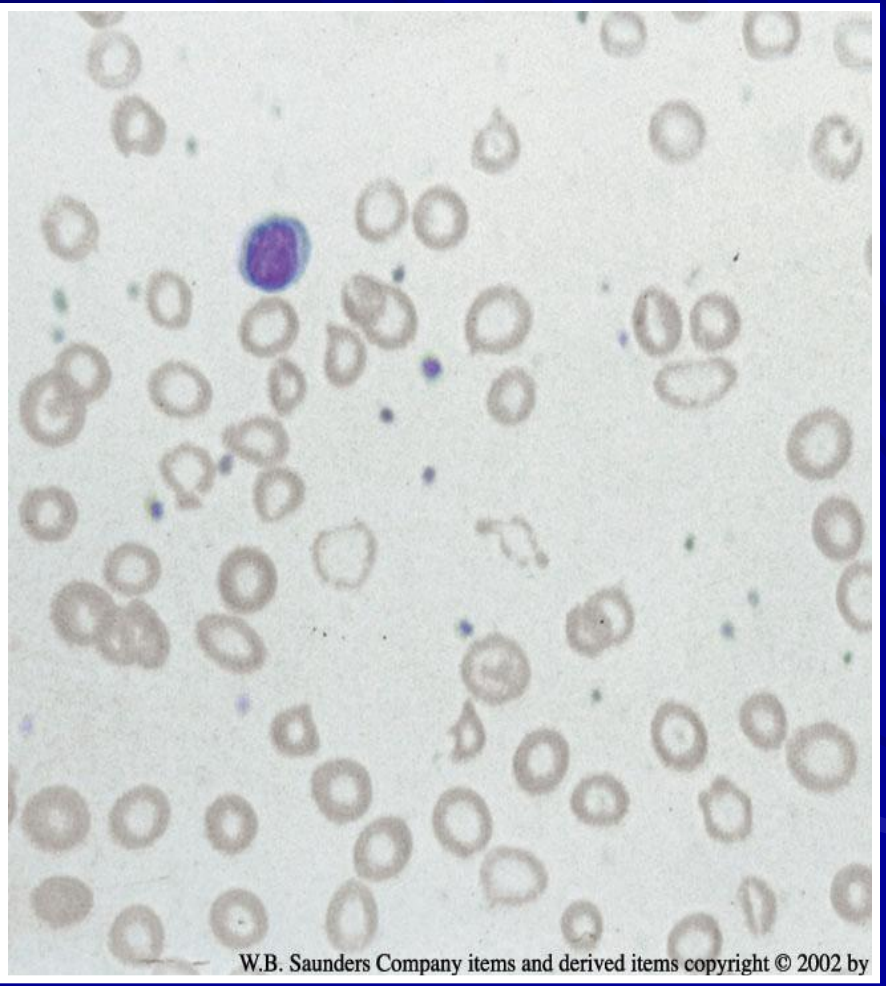


Microcytic, Hypochromic = MCV < 80

- Iron deficiency
- Sideroblastic
- Chronic disease, Inflammation
- Lead poisoning
- Thalassemia trait

Microcytic, Hypochromic

- Many RBCs smaller than nucleus of normal lymphocytes, increased central pallor.
- Iron deficiency, thalassemias, anemia of chronic disease.



Normochromic = MCV κφ

- Hereditary Spherocytosis
- Hereditary Elliptocytosis
- PNH
- G6PD deficiency
- Aplastic anemia
- Acute blood loss

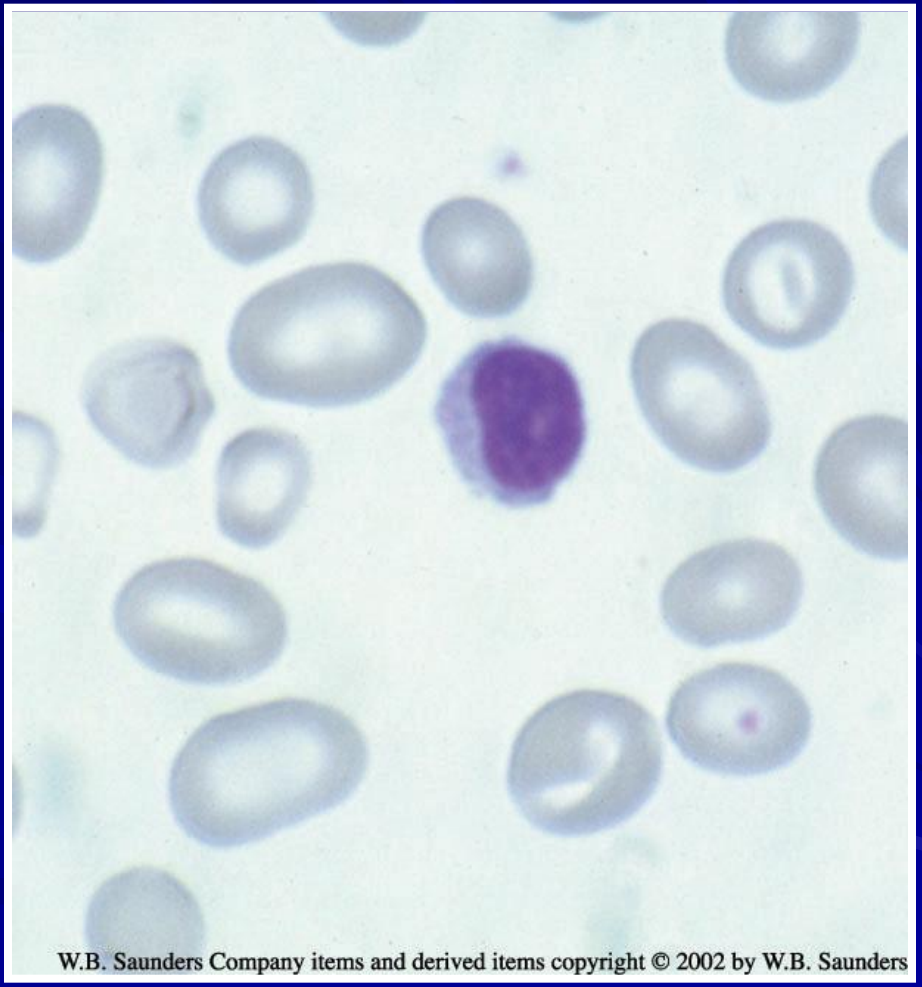
Macrocytic = MCV > 100

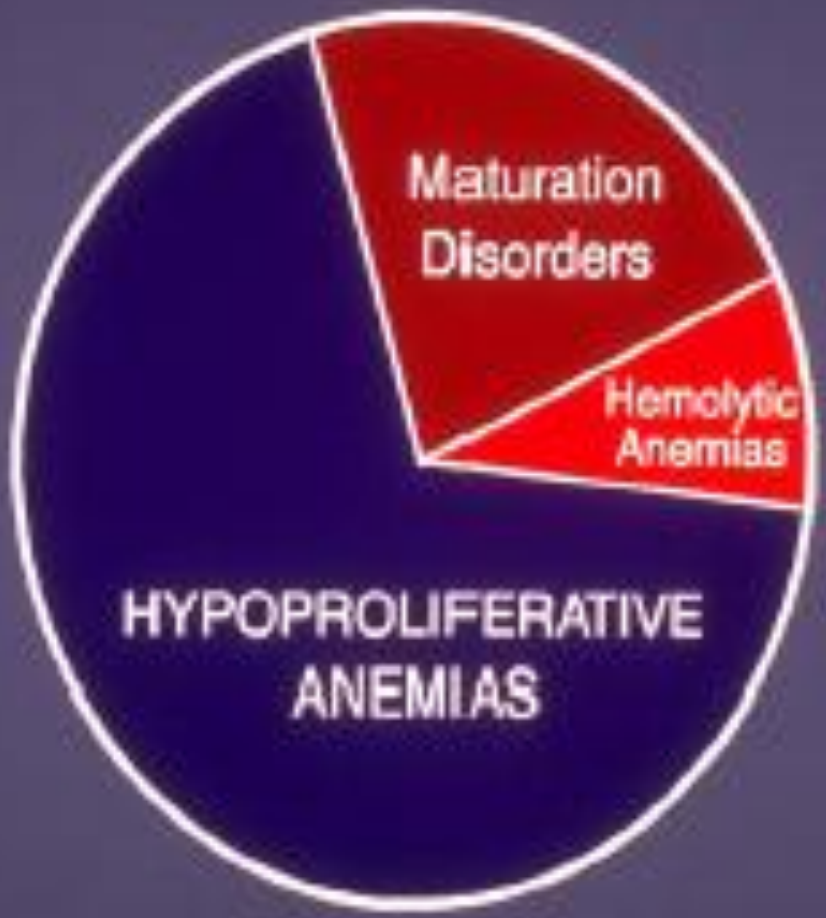
- Vitamin B12 deficiency
- Folate deficiency
- Liver disease

Macrocytic RBCs

- Most RBCs larger than nucleus of normal

  lymphocytes, increased MCV.
- Folate or Vitamin B12 deficiencies, alcoholism, and liver disease.




Maturation
Disorders
Hemolytic
Anemias
HYPOPROLIFERATIVE
ANEMIAS

HYPOPROLIFERATIVE

Σιδηροπενική αναιμία - Επιδημιολογία

- Σιδηροπενική αναιμία: 1-2% των ενηλίκων
- Σιδηροπενία χωρίς αναιμία: 11% των γυναικών (κυρίως στην αναπαραγωγική ηλικία) και 4% των ανδρών

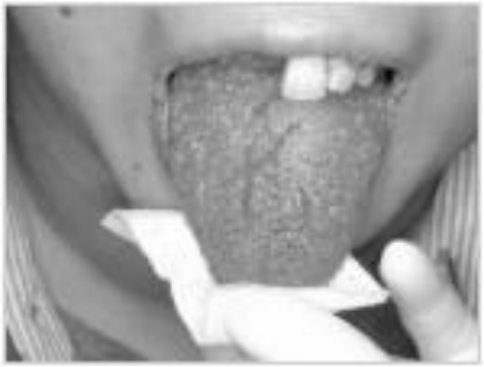
Αιτιολογία

- ↑↑ αναγκών σε Fe
  - Rapid growth, infancy, pregnancy, EPO Rx
- Απώλεια αίματος
  - menses, acute blood loss, blood donation
- Δυσαπορρόφηση
  - Crohn's, sprue
- Ενδοαγγειακή αιμόλυση
- Πνευμονική αιμοσιδήρωση

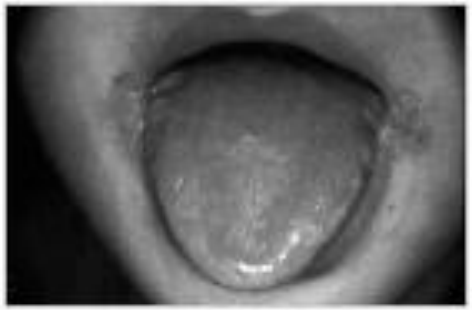
Κλινική εικόνα

- Αδυναμία, κεφαλαλγία, ευερεθιστότητα και εύκολη κόπωση
- Το σύνδρ. Plummer-Vinson (δυσφαγία με οισοφαγική στένωση) και η κοιλονυχία δεν παρατηρούνται σήμερα
- Πίκα: χαρτί, πηλός, πάγος (παγοφαγία)
- Ερυθρή χρώση ούρων μετά κατανάλωση πατζαριών

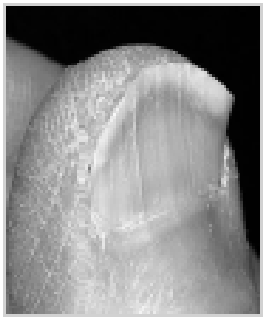
Glossitis



Angular Chelitis



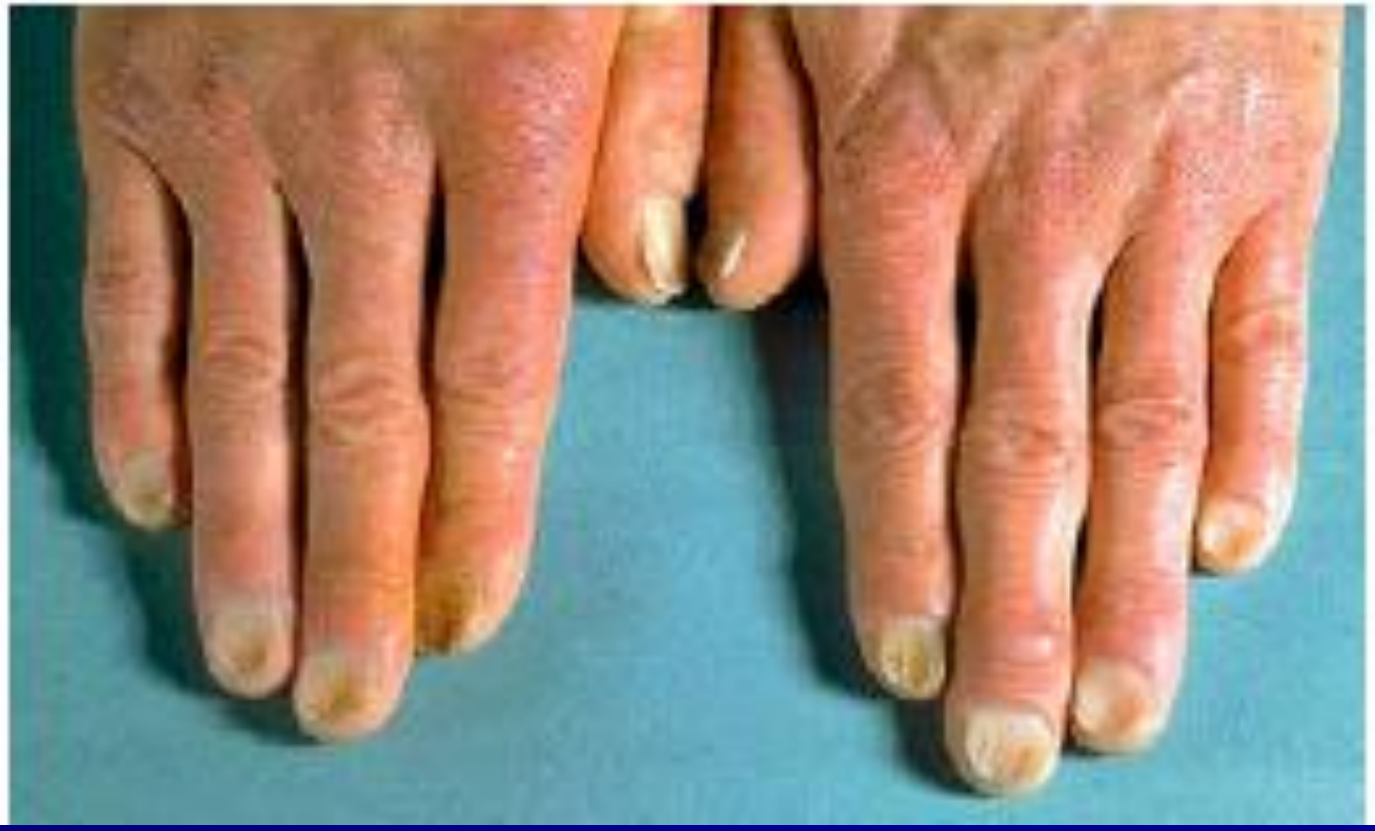
Koilonychia



Δείκτες σιδηροπενίας

- Φεριτίνη: ο καλύτερος δείκτης των αποθηκών σιδήρου
- <10-15: 99% ειδικότητα, 50% ευαισθησία
- <30: 85% ειδικότητα, 90% ευαισθησία
- > 200 τουλάχιστον κάποιες αποθήκες
- ΠΡΟΣΟΧΗ: Πρωτεΐνη οξείας φάσης (x3)
- Τρανσφερίνη / σιδηροδεσμευτική ικανότητα (TIBC) και κορεσμός τρανσφερίνης)= σίδηρος/TIBC)
- Σίδηρος ορού: μειώνεται και στην αναιμία χρονίας νόσου
- Σίδηρος μυελού: μέτρο σύγκρισης
- Υποδοχείς τρανσφερίνης: αυξάνονται

Spoon Nails – Fe Def.

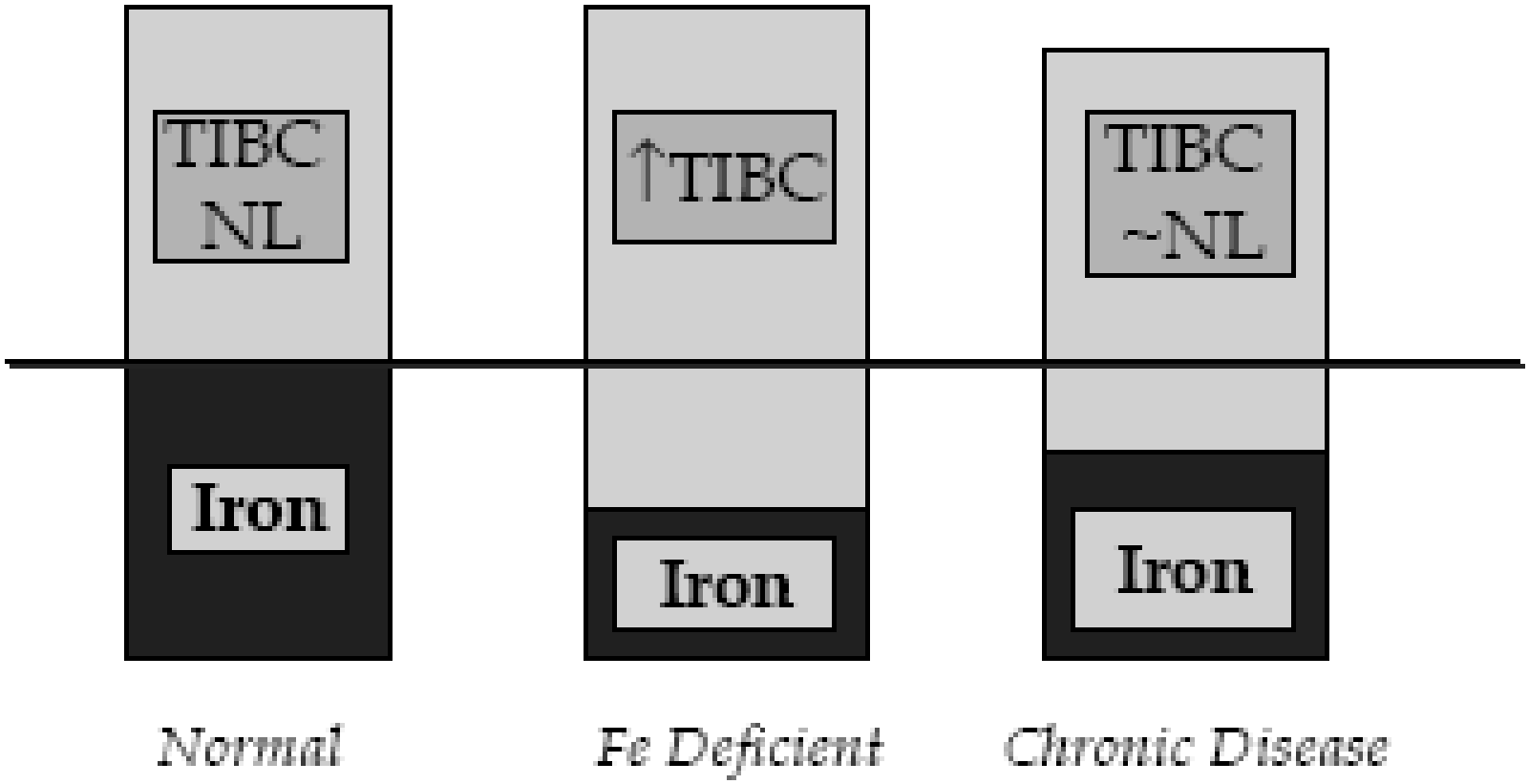


Δείκτες σιδηροπενίας

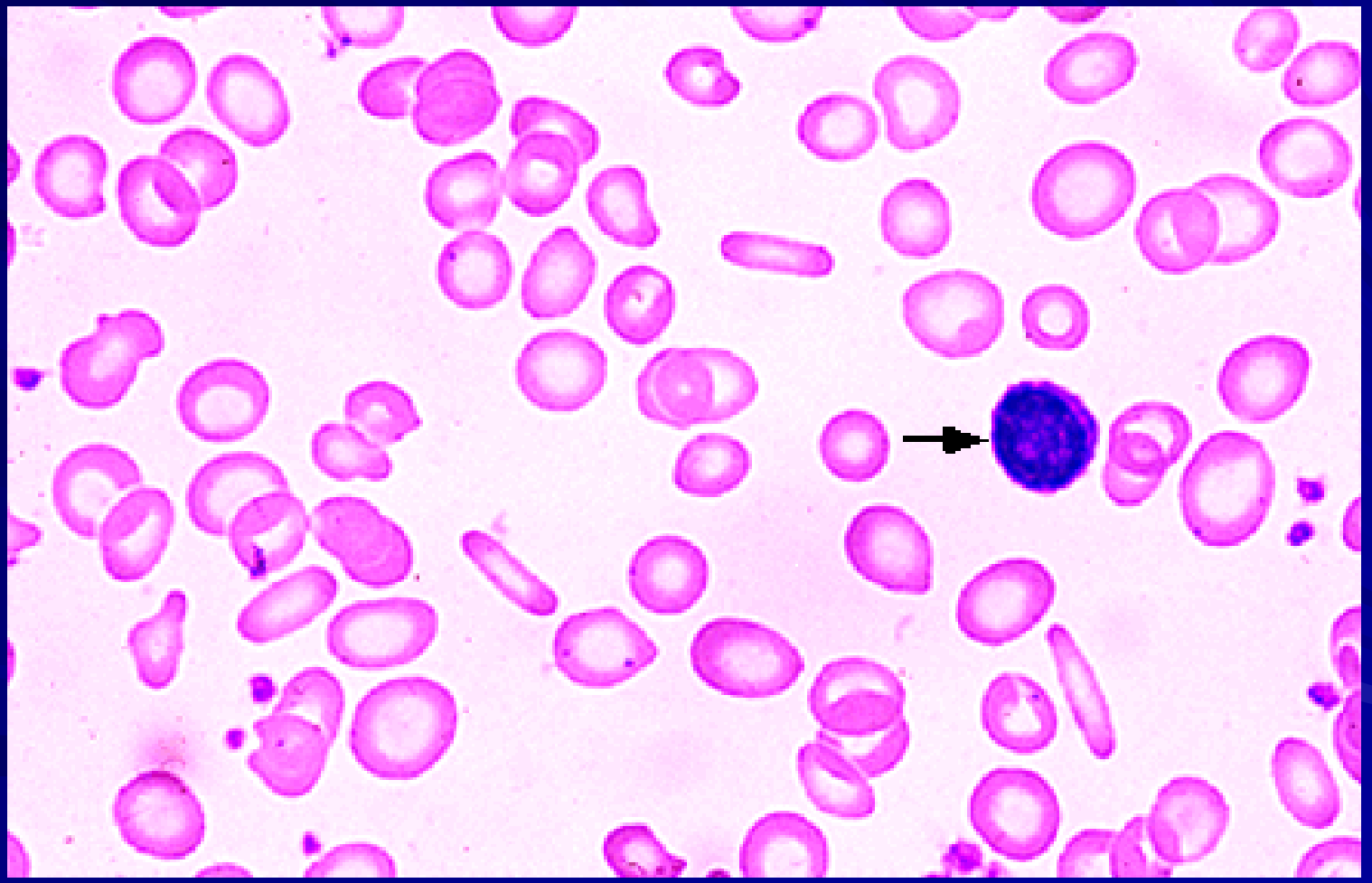
	Φυσιολογικό	Σιδηροπενία χωρίς αναιμία	Σιδηροπενία με ήπια αναιμία	Σιδηροπενία με βαριά αναιμία
Σίδηρος μυελού	2+ 3+	Καθόλου	Καθόλου	καθόλου
Σίδηρος ορού, μg/dl	60-150	60-150	<60	<40
Σιδηροδεσμευτική ικανότητα, μg/dl	300-360	300-390	350-400	>410
Κορεσμός τρανσφερίνης, %	20-50	30	<15	<10
Αιμοσφαιρίνη, g/dl	Φυσιολογική	Φυσιολογική	9-12	6-7
Μορφολογία ερυθρών	Φυσιολογική	φυσιολογική	Φυσιολογική ή ήπια υποχρωμία	Υποχρωμία και μικροκυττάρωση
Φεριτίνη, ορού ng/ml	40-200	<20	<10	0-10
Πρωτοπορφυρίνη ερυθρών, ng/ml	30-70	30-70	>100	100-200
Αλλοιώσεις βλεννογόνων	ΟΧΙ	ΟΧΙ	ΟΧΙ	ΝΑΙ

- *Iron*
- *Ferritin*
- *TIBC (transferrin)*

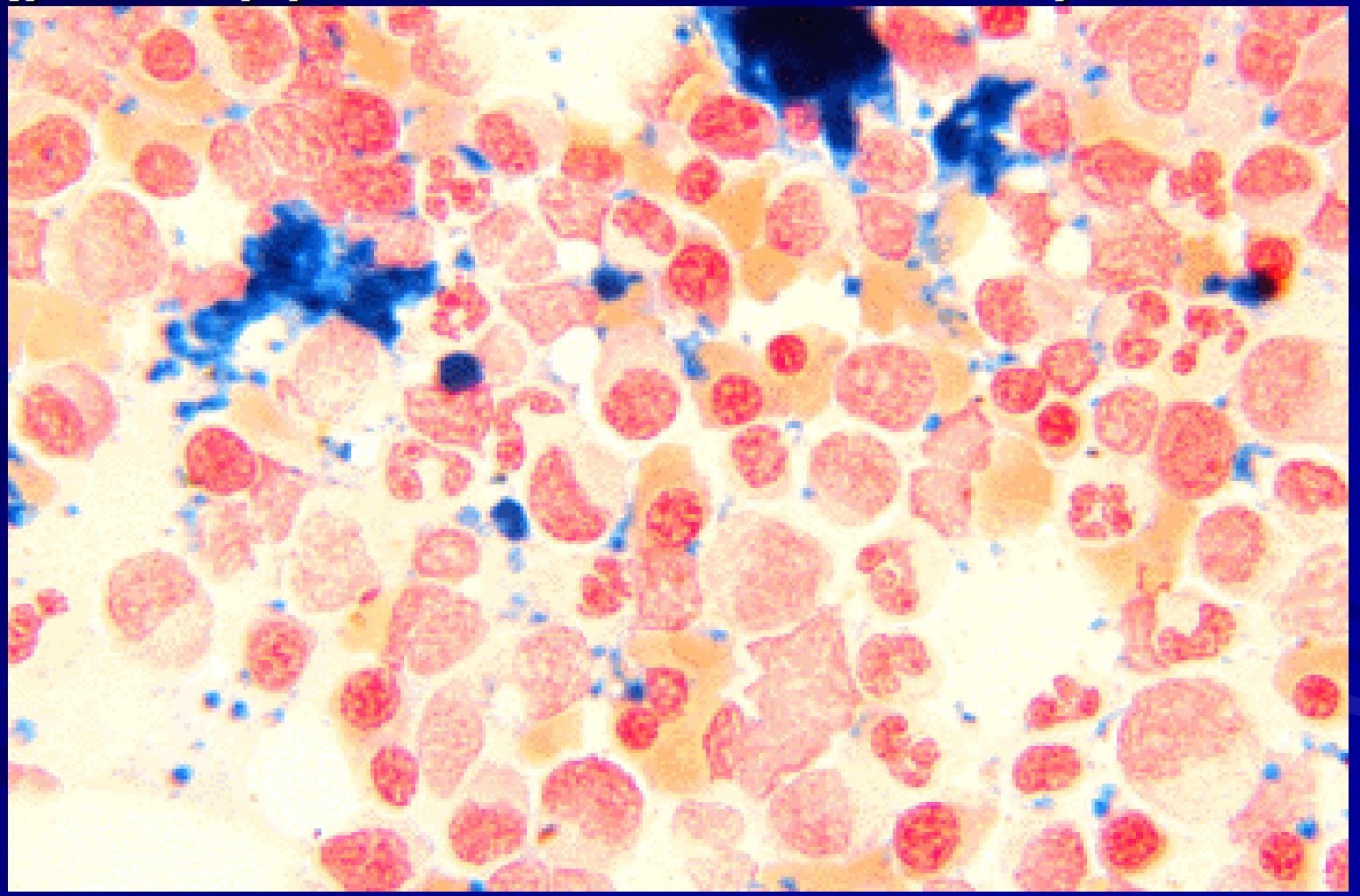
Iron Studies



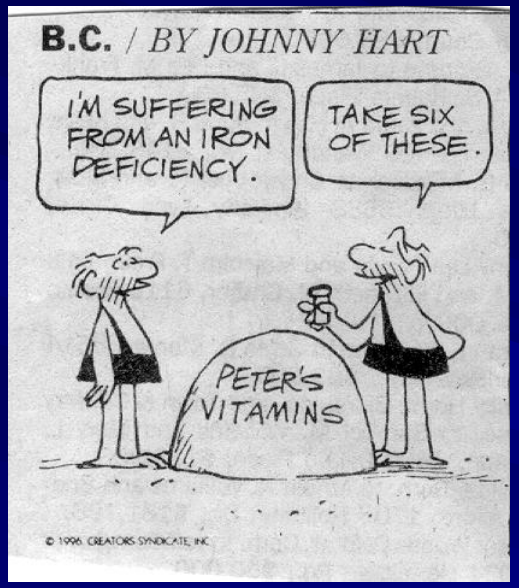
Υποχρωμία - μικροκυττάρωση

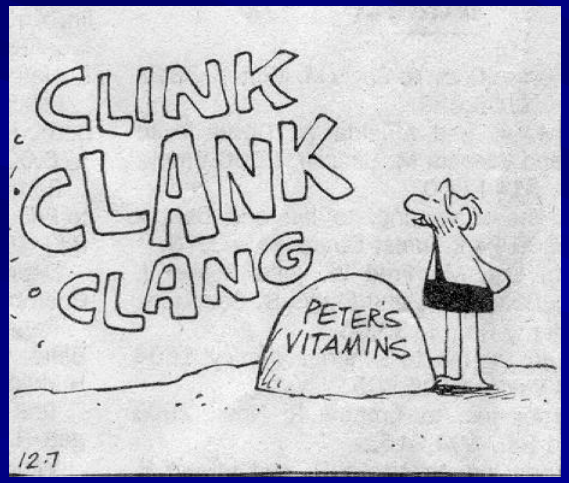


Σίδηρος μυελού
χρώση με κυανούν του Βερολίνου



Therapy of Iron Deficiency





Θεραπεία

- Μετάγγιση
- Από του στόματος Fe
  - 200-300 mg/d = απορρόφηση 50 mg
  - 6-12 μήνες
  - ΑΕ
    - Κοιλ άλγος, ναυτία, εμετός, δυσκοιλιότητα
- Parenteral iron
  - Body weight x 2.3 x(15-Pt Hb) + 500 or 1000 mg (for stores)

HYPOPROLIFERATIVE

Άλλες

Άλλες hypoproliferative

- Αναιμία χρονίας νόσου
  - Χρόνιες φλεγμονές, νεοπλάσματα
    - Ρόλος TNF-a, interferon b σε νεοπλάσματα
    - Ρόλος IL-1, interferon γ σε PA
- Νεφρικής νόσου
- Ενδοκρινικής νόσου, θρεπτικών διαταραχών
- Ηπατοπάθειας

Εργαστηριακά ευρήματα

- Συνήθως ήπια αναιμία, σε 20% Hb<8 g/dl
- Ορθόχρωμη, ορθοκυτταρική και σπανιότερα υπόχρωμη μικροκυτταρική
- Σίδηρος και TIBC μειωμένα, κορεσμός τρανσφερίνης φυσιολογικός
- Φεριτίνη ↑

Iron studies in IDA and ACD

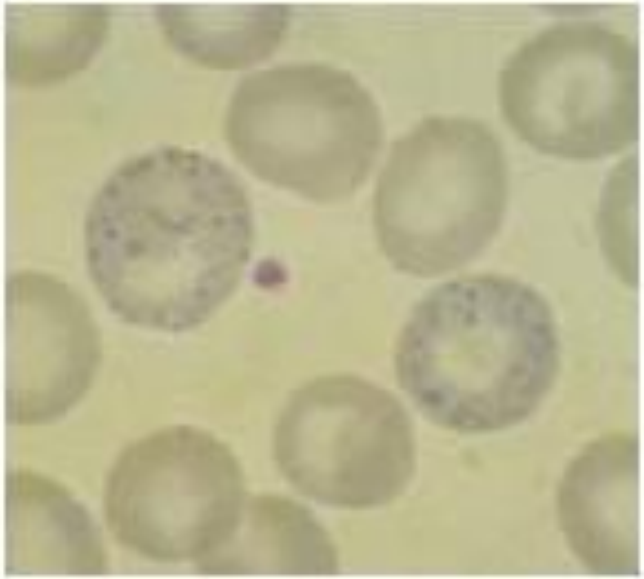
Test	**IDA**	**ACD**
serum iron	low	low
TIBC	high	normal or low
transf. sat.	low	low
serum ferritin	low	normal or increased
marrow iron	absent	normal or increased

Anemia of chronic renal failure

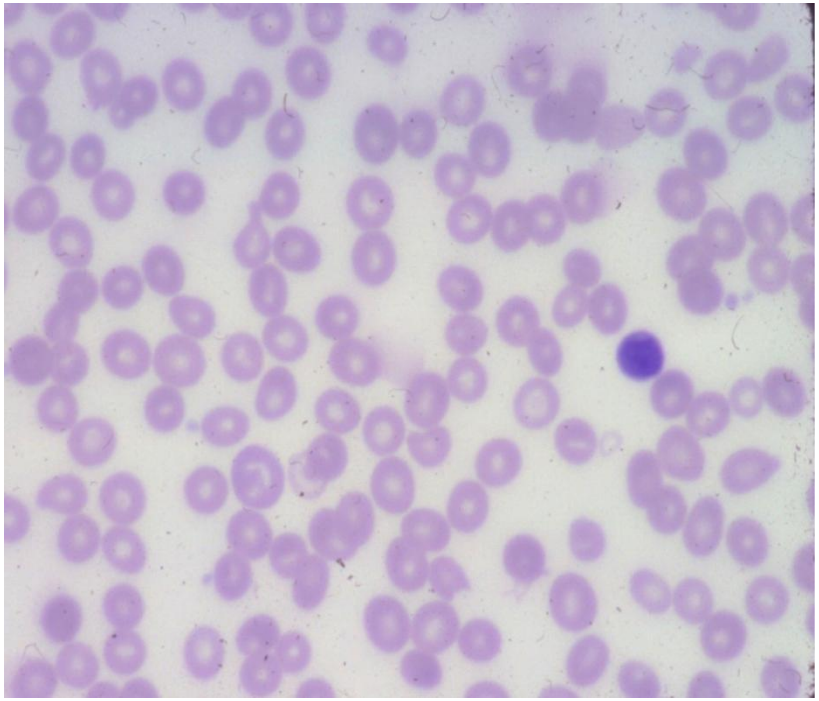
- Mechanism:
  - mainly due to reduced production of erythropoietin by diseased kidneys
  - also iron or folate deficiency, chronic inflammation, shortened red cell survival
- Treatment
  - erythropoietin thrice weekly
  - dialysis

Sideroblastic

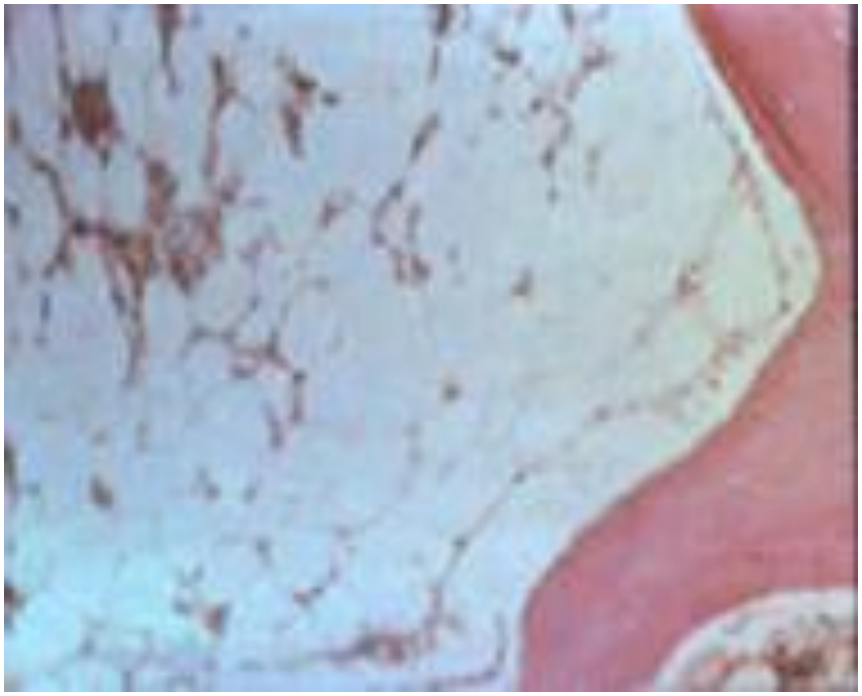
- Ring sideroblasts in bone marrow
- Serum iron is increased and TIBC normal resulting in a high saturation. Serum ferritin is increased
- Basophillic stippling
- Lead toxicity is suspect



Aplastic Anemia



Blood

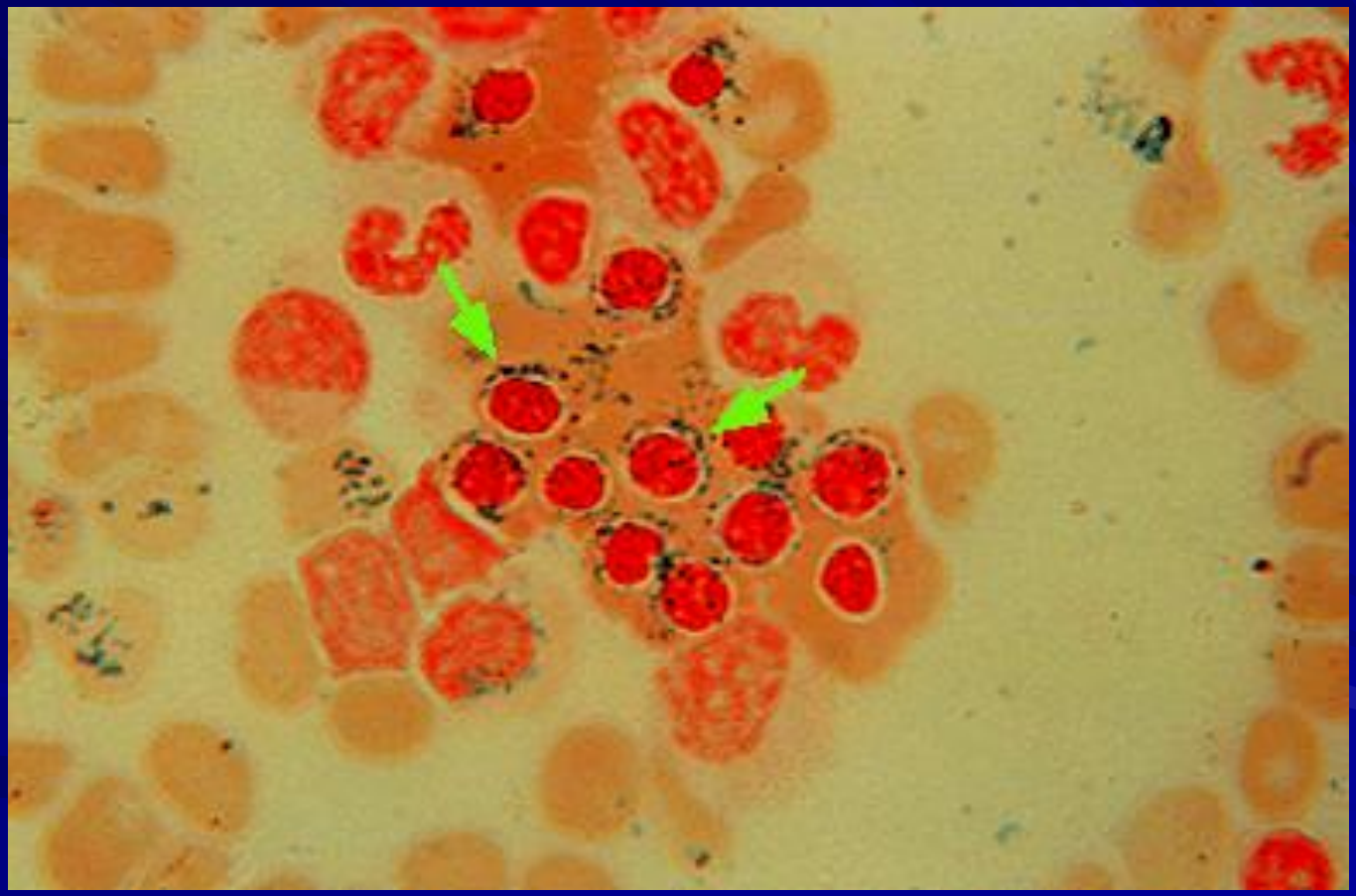


Bone Marrow Biopsy

Μυελοδυσπλαστικό σύνδρομο

- Μέση ηλικία εμφάνισης 65 έτη
- Σπάνια <50 ετών
- Κλωνική διαταραχή των πρόδρομων αιμοποιητικών κυττάρων (stem cells)
- Αιτιολογία: περιβαλλοντικοί παράγοντες, χημειοθεραπεία (δευτεροπαθές)
- Αναιμία (μακροκυττάρωση, δυσπλαστικός μυελός)
- Λοιμώξεις (δυσλειτουργία ουδετεροφίλων, ουδετεροπενία 50%)
- Θρομβοπενία 25%

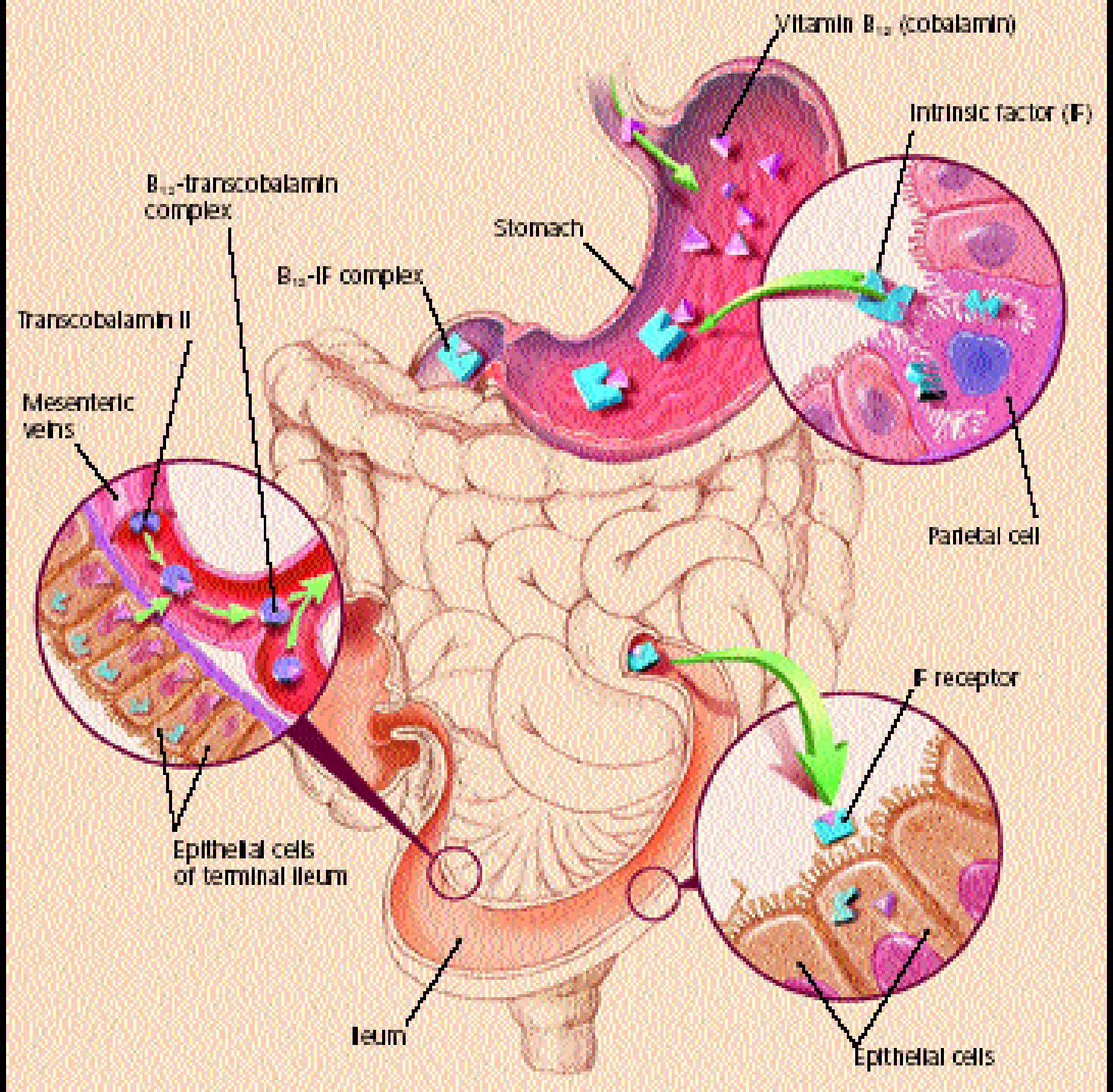
Δακτυλιοειδείς σιδηροβλάστες



Ταξινόμηση WHO

	Περιφερικό αίμα	**Μυελός οστών**
Ανθεκτική αναιμία (RA)	Αναιμία χωρίς βλάστες	Δυσπλασία ερυθράς σειράς
Ανθεκτική αναιμία με δακτυλιοειδείς σιδηροβλάστες (RARS)	Αναιμία χωρίς βλάστες	Δυσπλασία ερυθράς σειράς Δακτ. σιδηροβλάστες
Ανθεκτική κυτταροπενία πολλαπλών σειρών (RCMD)	Κυτταροπενία 2 ή 3 σειρών	Δυσπλασία 2 ή 3 σειρών
Ανθεκτική κυτταροπενία πολλαπλών σειρών με δακτυλιοειδείς σιδηροβλάστες (RCMD-RS)	Κυτταροπενία 2 ή 3 σειρών	Δυσπλασία 2 ή 3 σειρών Δακτ σιδηροβλάστες
Ανθεκτική αναιμία με περίσσεια βλαστών 1 & 2 (RAEB 1&2)	Κυτταροπενία βλάστες<5–19%	Βλάστες 5-20%
Αταξινόμητο ΜΔΣ (MDS-U)	Κυτταροπενία	Δυσπλασία μυελικής ή μεγακ. σειράς
ΜΔΣ με del5q (5q- syndrome)	Αναιμία Θρομβοκυττάρωση	del5q
Χρόνια μυελομονοκυτταρική λευχαιμία (CMML)	Μονοκύτταρα >1000/μl Βλάστες <5-19%	Βλάστες <10-19%

MATURATION DISORDER


Vitamin B_{12} (cobalamin)
Intrinsic factor (IF)
B_{12}-transcobalamin complex
Stomach
B_{12}-IF complex
Transcobalamin II
Mesenteric veins
Parietal cell
IF receptor
Epithelial cells of terminal ileum
Ileum
Epithelial cells

Έλλειψη Β12

- Ανεπάρκεια κοβαλαμίνης (βιτ. Β12)
  - Κακοήθης αναιμία (Biermer): αντισώματα έναντι:
    - ενδογενούς παράγοντα
    - τοιχωματικών κυττάρων
  - Γαστρεκτομή
  - *H. pylori*
  - Δυσαπορρόφηση: ν. Crohn, σ. τυφλής έλικας
  - Φυτοφαγία
- Ανεπάρκεια φυλικού
  - Ελλειπής δίαιτα
  - Αυξημένες ανάγκες: κύηση, αιμολυτικές αναιμίες
  - Φάρμακα

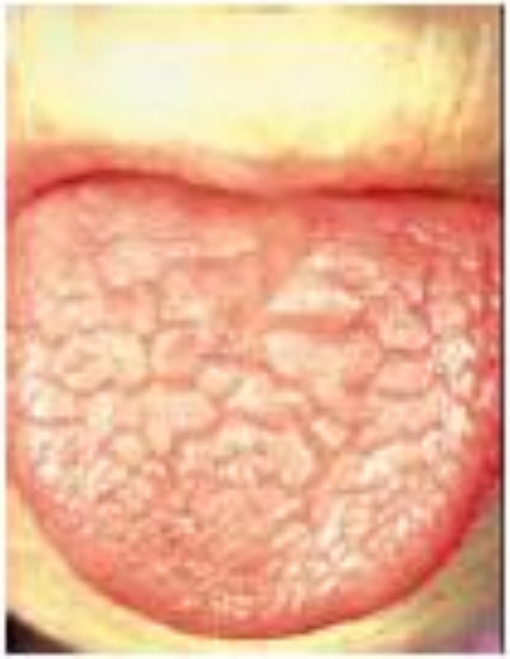
Μεγαλοβλαστική αναιμία

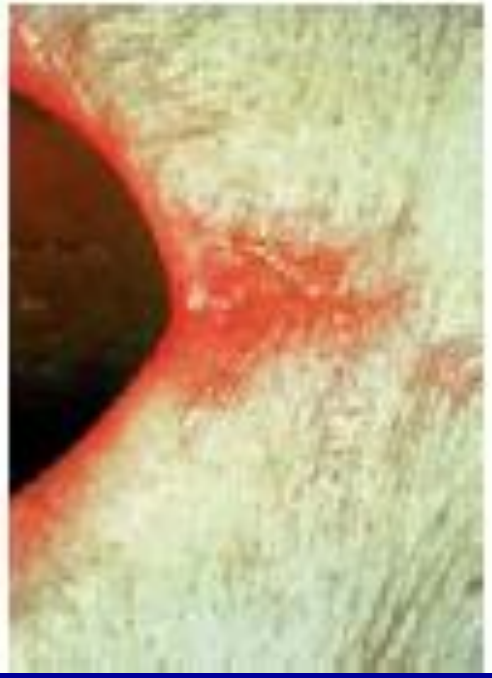
- Ανεπάρκεια κοβαλαμίνης (βιτ. B12)
  - Κακοήθης αναιμία (Biermer): αντισώματα έναντι:
    - ενδογενούς παράγοντα
    - τοιχωματικών κυττάρων
  - Γαστρεκτομή
  - *H. pylori*
  - Δυσαπορρόφηση: ν. Crohn, σ. τυφλής έλικας
  - Φυτοφαγία
- Ανεπάρκεια φυλικού
  - Ελειπής δίαιτα
  - Αυξημένες ανάγκες: κύηση, αιμολυτικές αναιμίες
  - Φάρμακα

Κλινικές εκδηλώσεις

- Αναιμία (λεμονοειδής χροιά δέρματος)
- Νευροψυχιατρικές εκδηλώσεις (ΜΟΝΟ σε ανεπάρκεια Β12)
  - διαταραχή μνήμης – συγκέντρωσης, άνοια
  - Περιφερική νευροπάθεια (διαταραχή εν τω βάθει αισθητικότητας, παραισθησίες, αδυναμία, σπαστικότητα, αστάθεια βάδισης) – υποξεία συνδυασμένη εκφύλιση (οπίσθιων και πλάγιων δεματίων)
- Γλωσσίτιδα

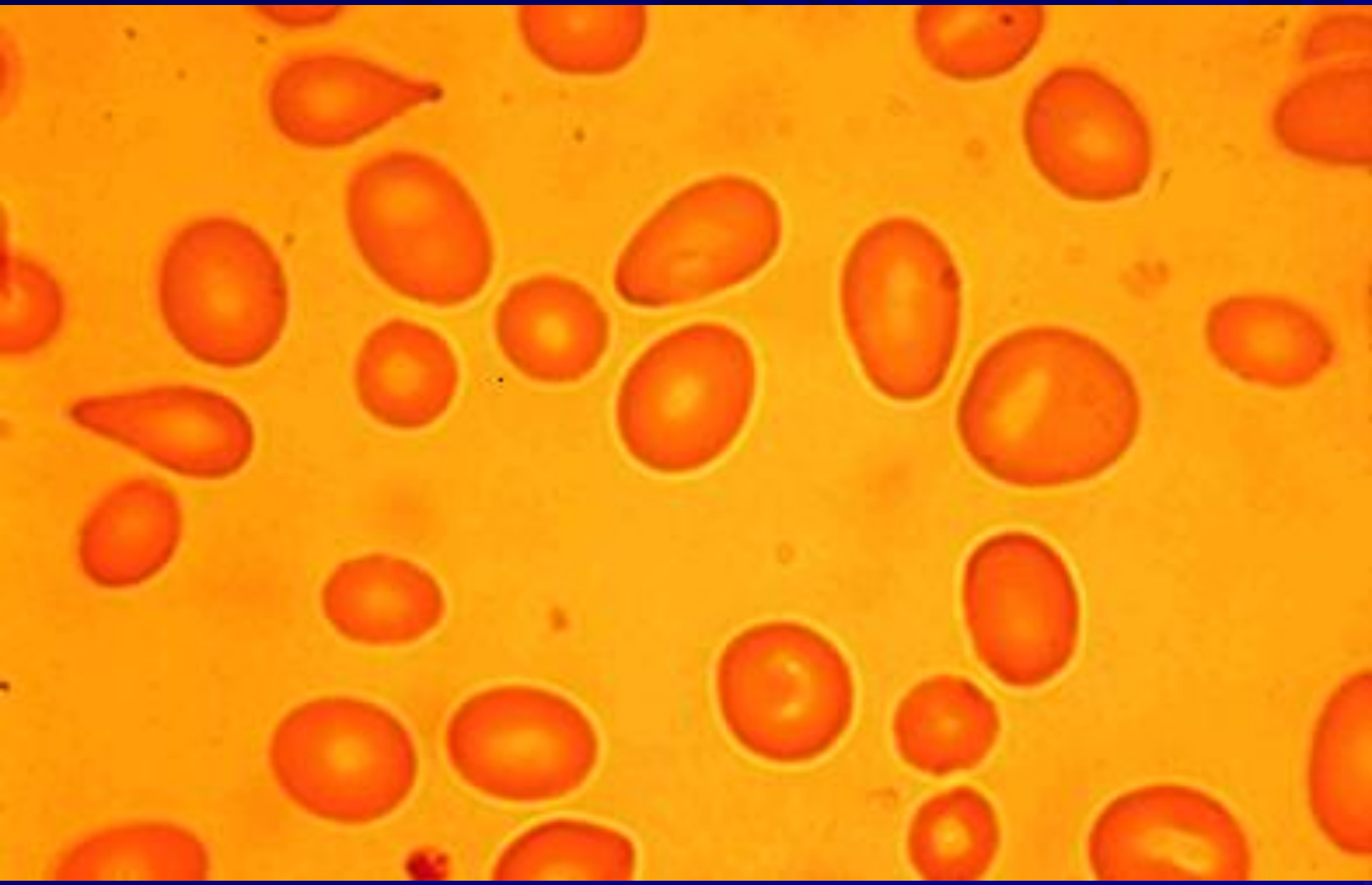
Glossitis and Chelosis – Fe and B12



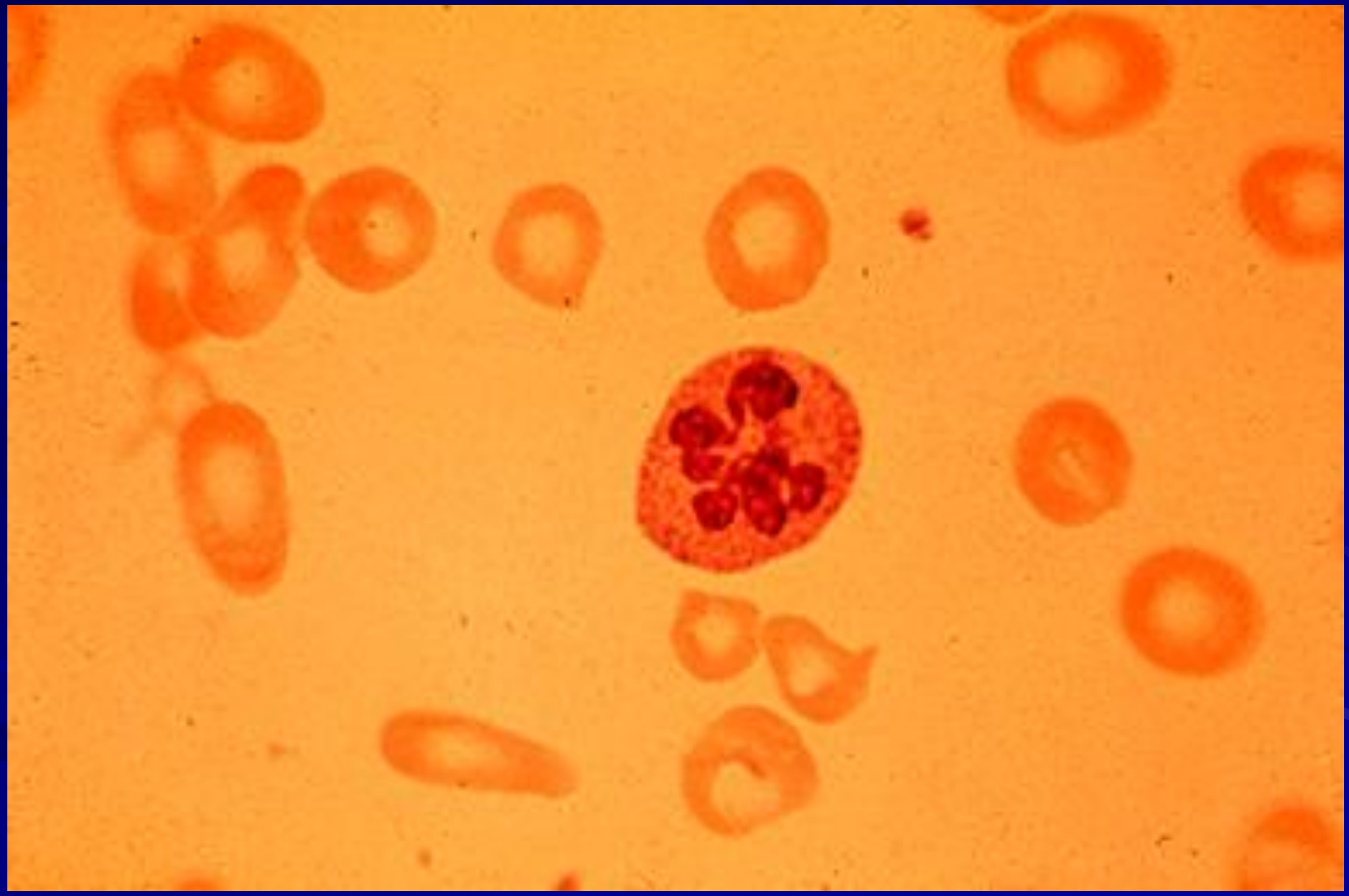


Εργαστηριακά ευρήματα

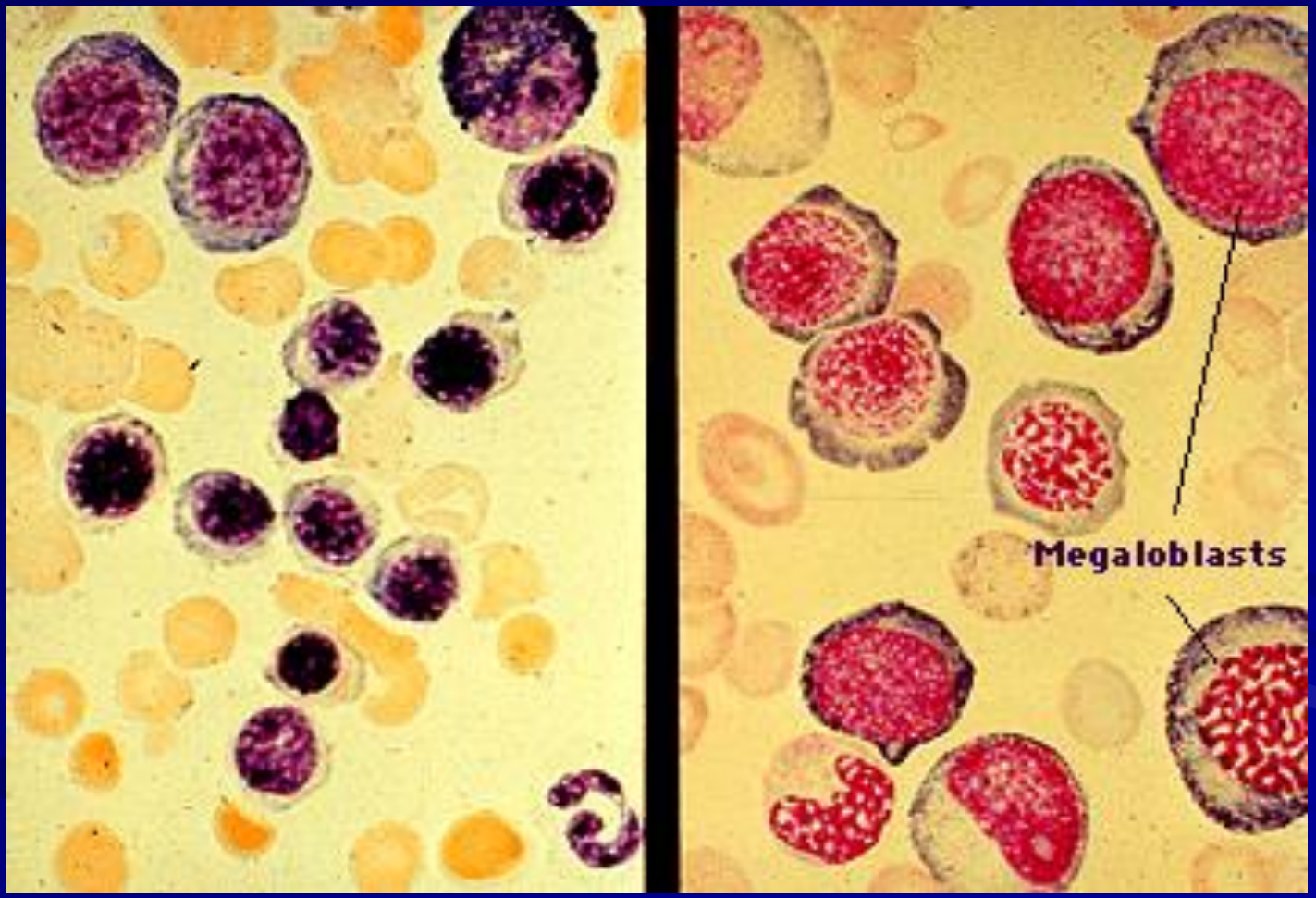
- Μακροκυττάρωση – MCV>100
- Πολυκατάτμητα ουδετερόφιλα
- Μεγαλοβλάστωση μυελού
- Αύξηση LDH – έμμεση υπερχολερυθριναιμία
- Λευκοπενία – θρομβοπενία
- Αύξηση ομοκυστεΐνης και μεθειονίνης



Πολυκατάτμητα πολυμορφοπύρηνα



Μεγαλοβλάστες



ΘΕΡΑΠΕΙΑ

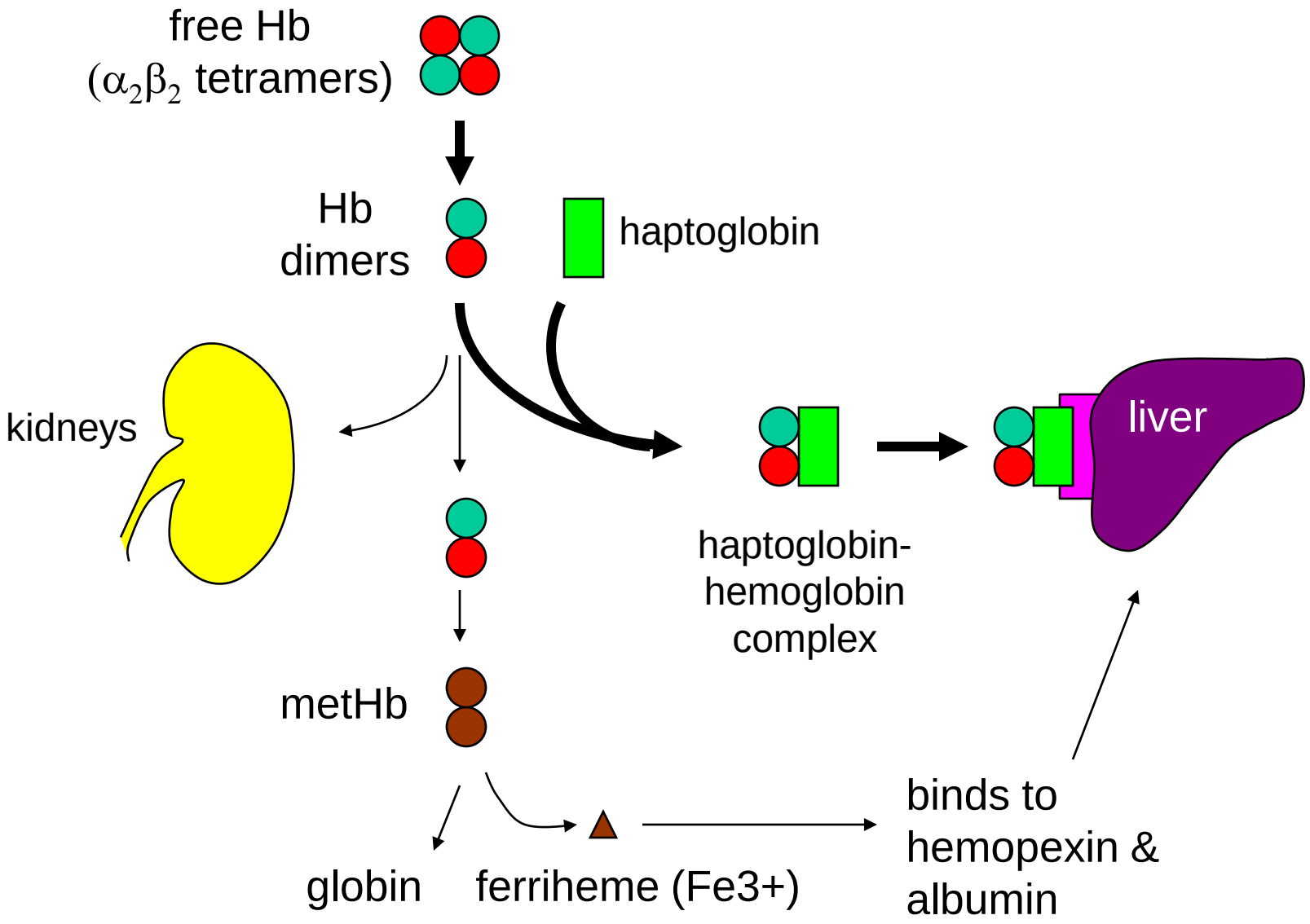
- Αντικατάσταση
  - 1000 μg / wk X 8 wks im
  - 1000 μg / month im / for life
  - *or*
  - 2 mg crystalline B12 pos / daily
  - 1 mg folate /daily
  - Folinic acid if due to drugs
    - 100 – 200 mg/d
    - rescuvolin

ΑΙΜΟΛΥΣΗ/ΑΙΜΟΡΡΑΓΙΑ

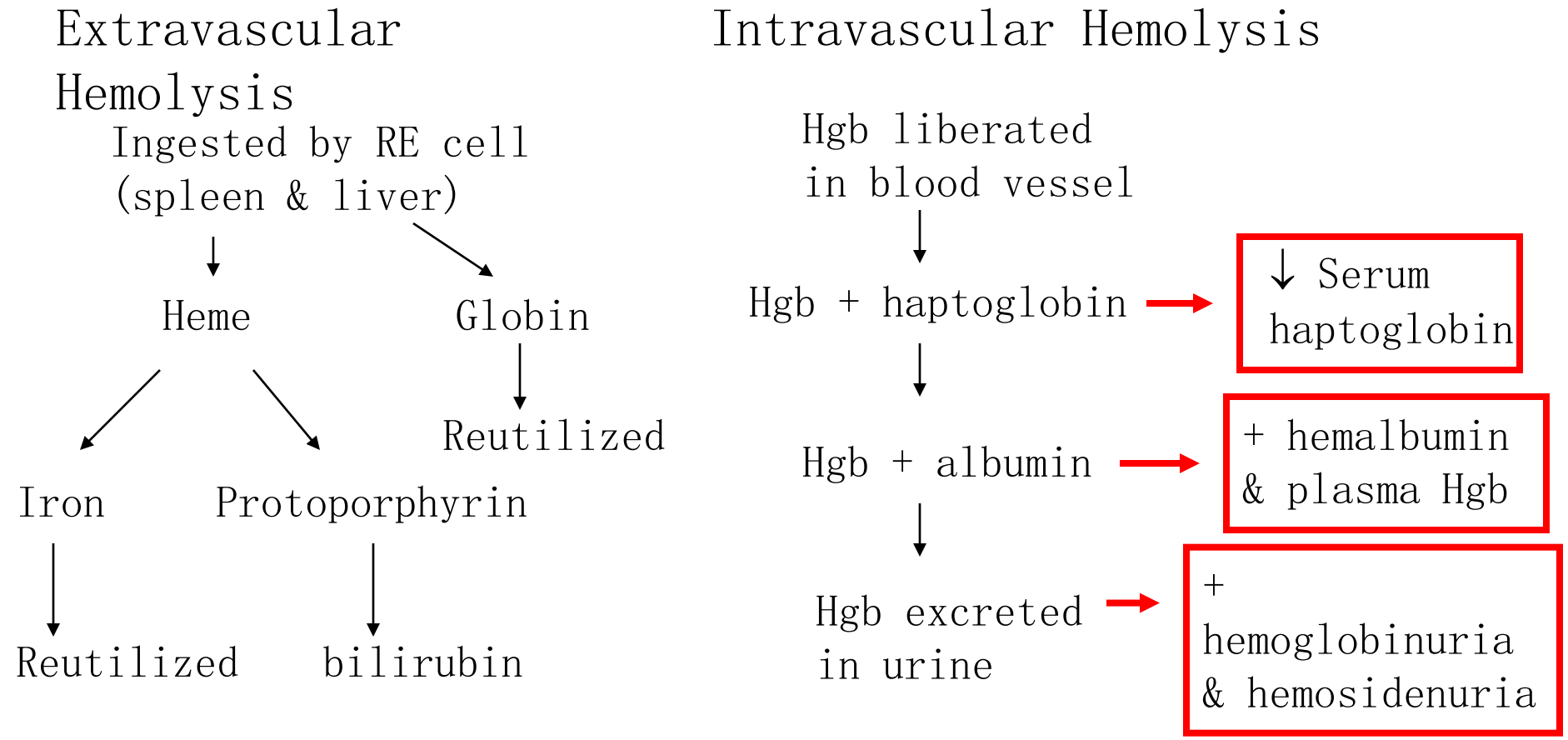
Normal red cell turnover

- normal RBC survival of ~ 120 days
- macrophages of the reticuloendothelial (RE) system removes RBC's
  - unclear what marks a red cell for removal
  - spleen is major site of RBC clearance
- RE system is extravascular
  - 90% of normal RBC destruction occurs without release of hemoglobin into circulation.

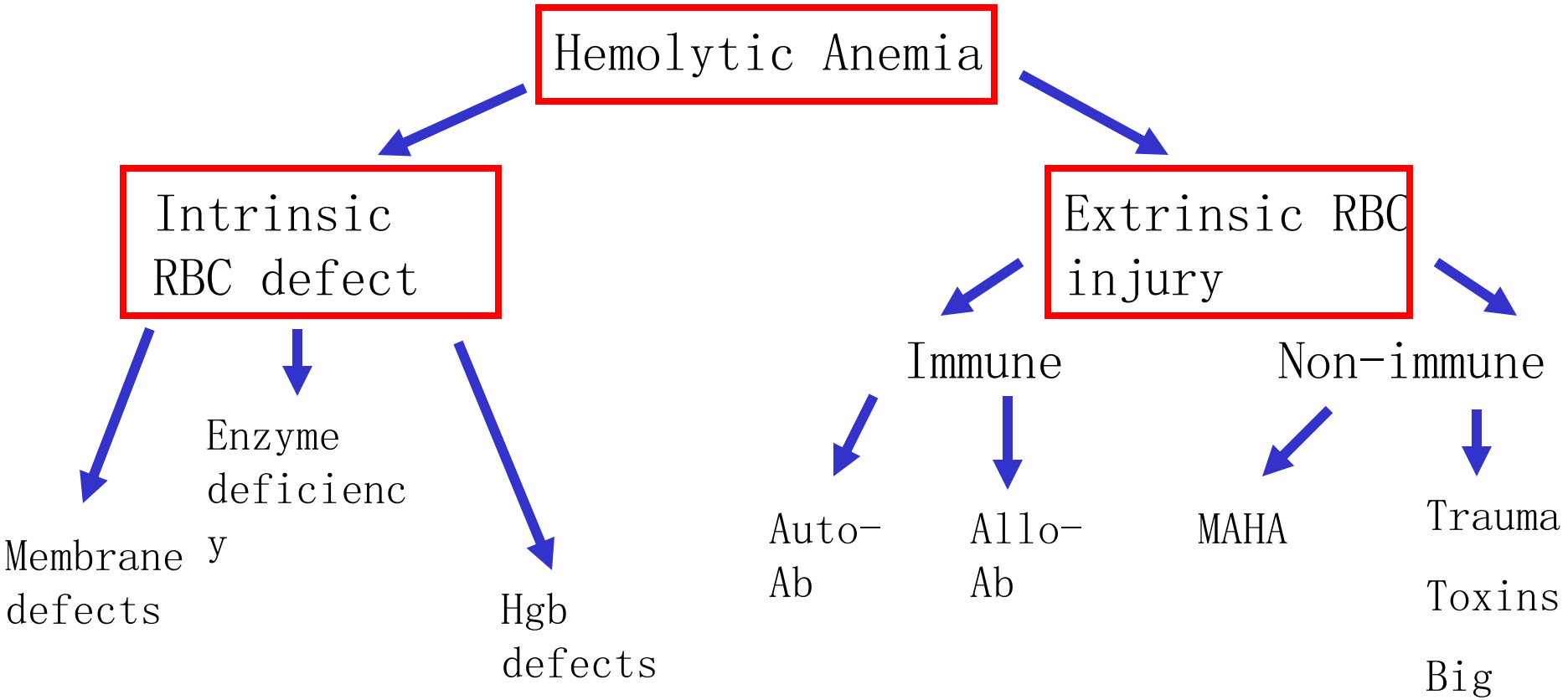
The fate of intravascular hemoglobin



Αιμόλυση καταστροφή RBCs



Διαφορική διάγνωση

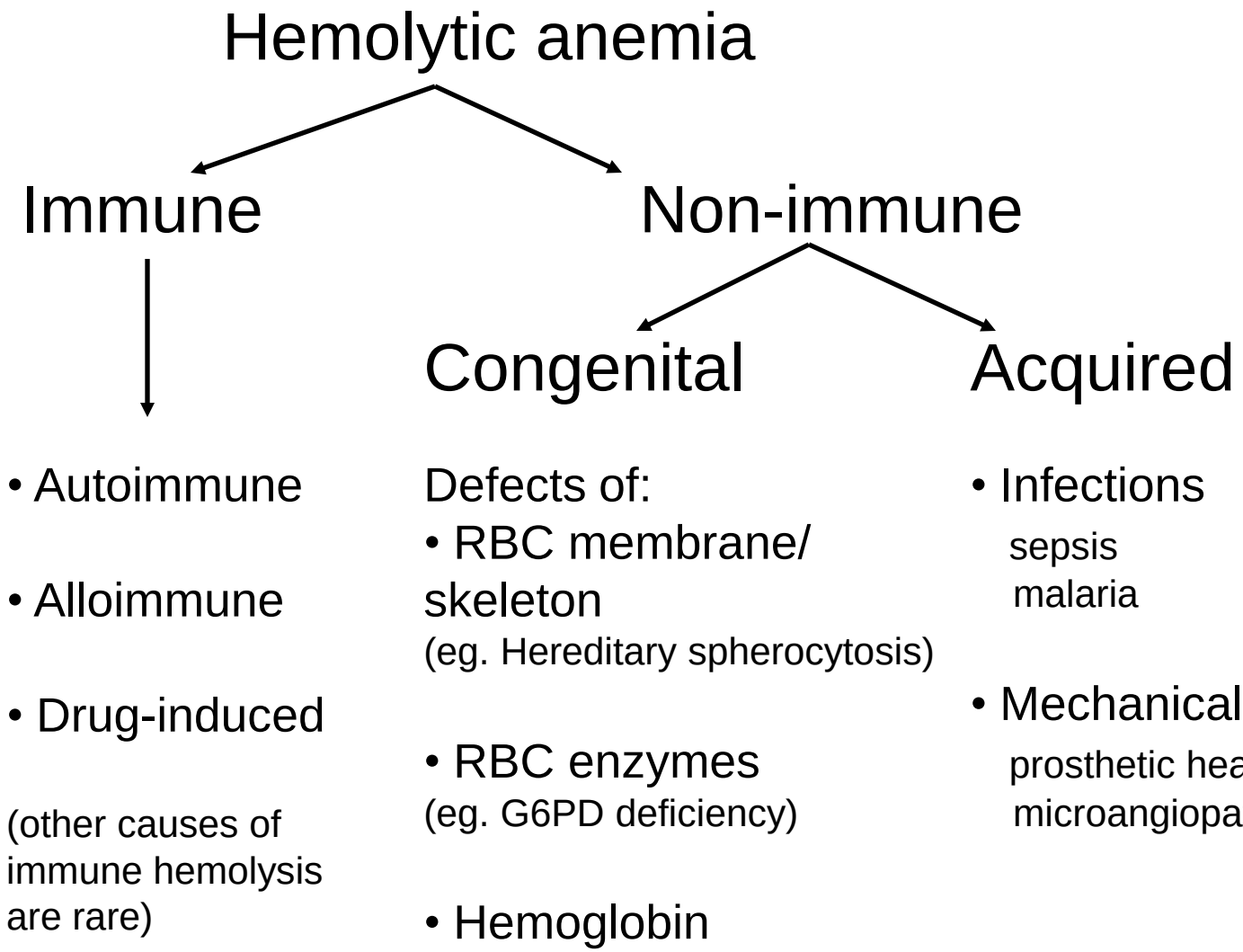


*MAHA: microangiopathic hemolytic anemia

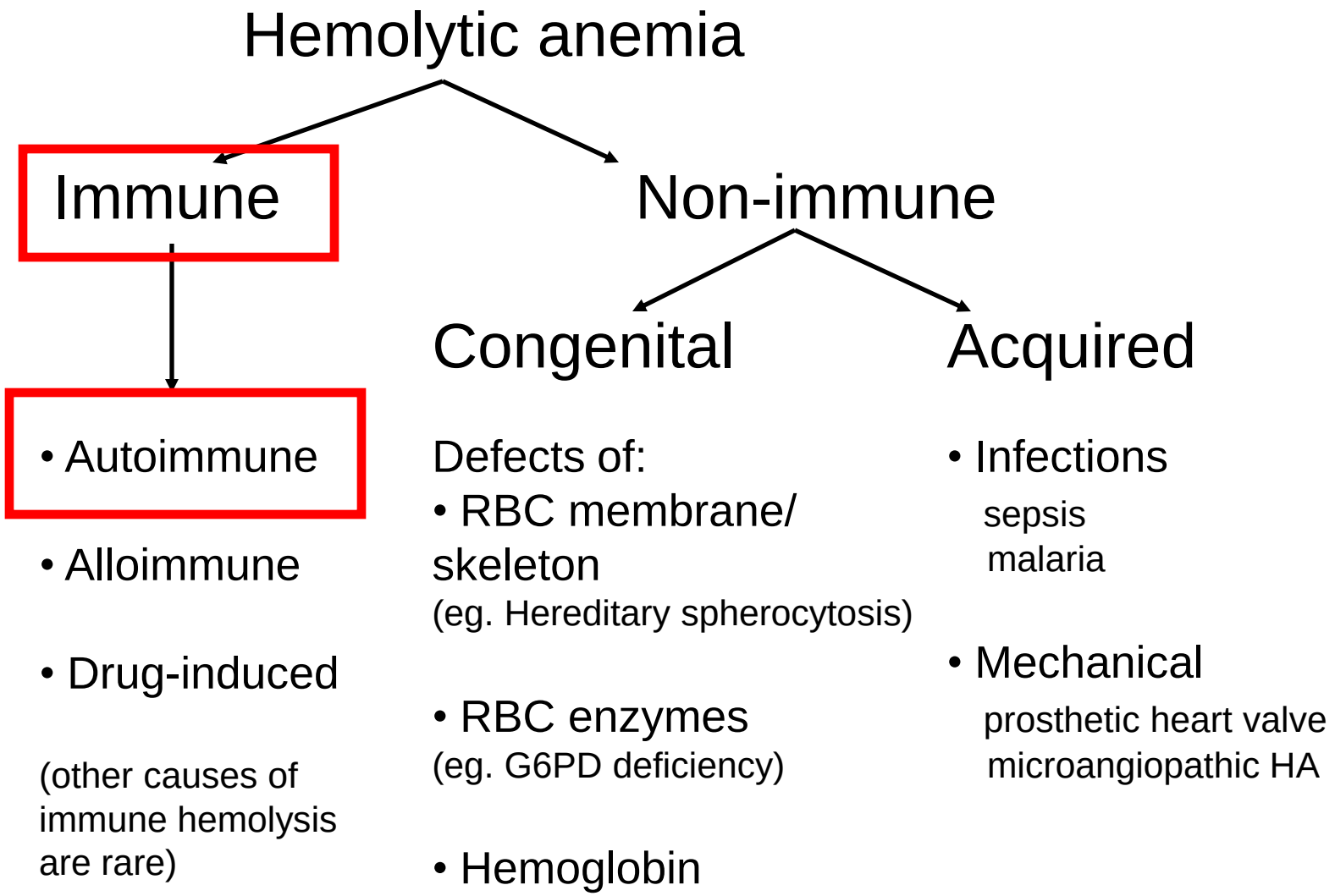
Extravascular vs Intravascular hemolysis

Test	Extravascular Hemolysis	Intravascular Hemolysis
LD	↑	↑↑
bilirubin	↑	↑
haptoglobin	N to absent	absent
hemoglobinuria	absent	present
free Hb in plasma	absent	present
urine hemosiderin	absent	present

An approach to hemolytic anemia



An approach to hemolytic anemia



Immune hemolysis

- most frequent cause of hemolysis
- due to IgG or complement on red cells
  - tags the red cell for phagocytosis
  - spherocytes if incomplete phagocytosis
  - lysis of RBC occurs if complement cascade goes to completion

Autoimmune hemolysis

- Most common type of immune hemolysis
- primary (idiopathic)
- secondary
  - autoimmune hemolysis secondary to:
    - autoimmune condition (such as SLE)
    - infection
    - lymphoma or CLL

Diagnosis of immune hemolytic anemia

- 1. Direct Antiglobulin Test (DAT or direct Coomb's test)
  - detects IgG or complement **on patient's red cells**
  - the vast majority of patients with active immune hemolysis will have a positive DAT.
- 2. Indirect Antiglobulin Test (IAT, indirect Coomb's test)
  - detects antibody **in patient's serum** against red cell antigens
  - A positive IAT does not necessarily mean hemolysis is occurring - It may simply mean allo-immunization due to previous exposure to "foreign" red cell antigens (past pregnancy or transfusion).
- 3. Peripheral Blood Film: spherocytes

Warm Auto Antibodies (IgG)

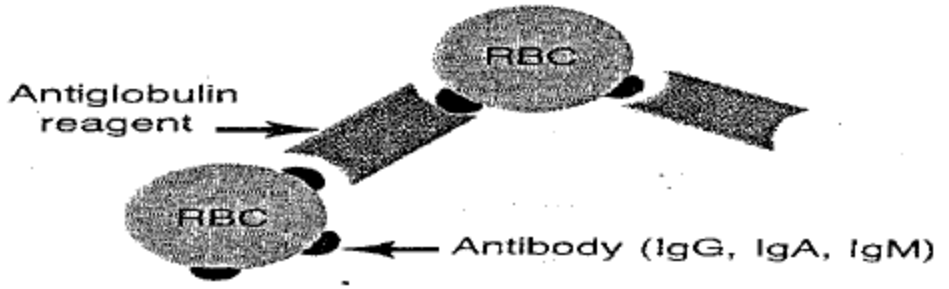
- Types of IgG
  - IgG1 → 80%
  - IgG2
  - IgG3
  - } 5%
  - IgG4 → 15%
- Affinity for complement
  - G3>G1>G2>G4

minimal

Cold Auto Antibodies (IgM)

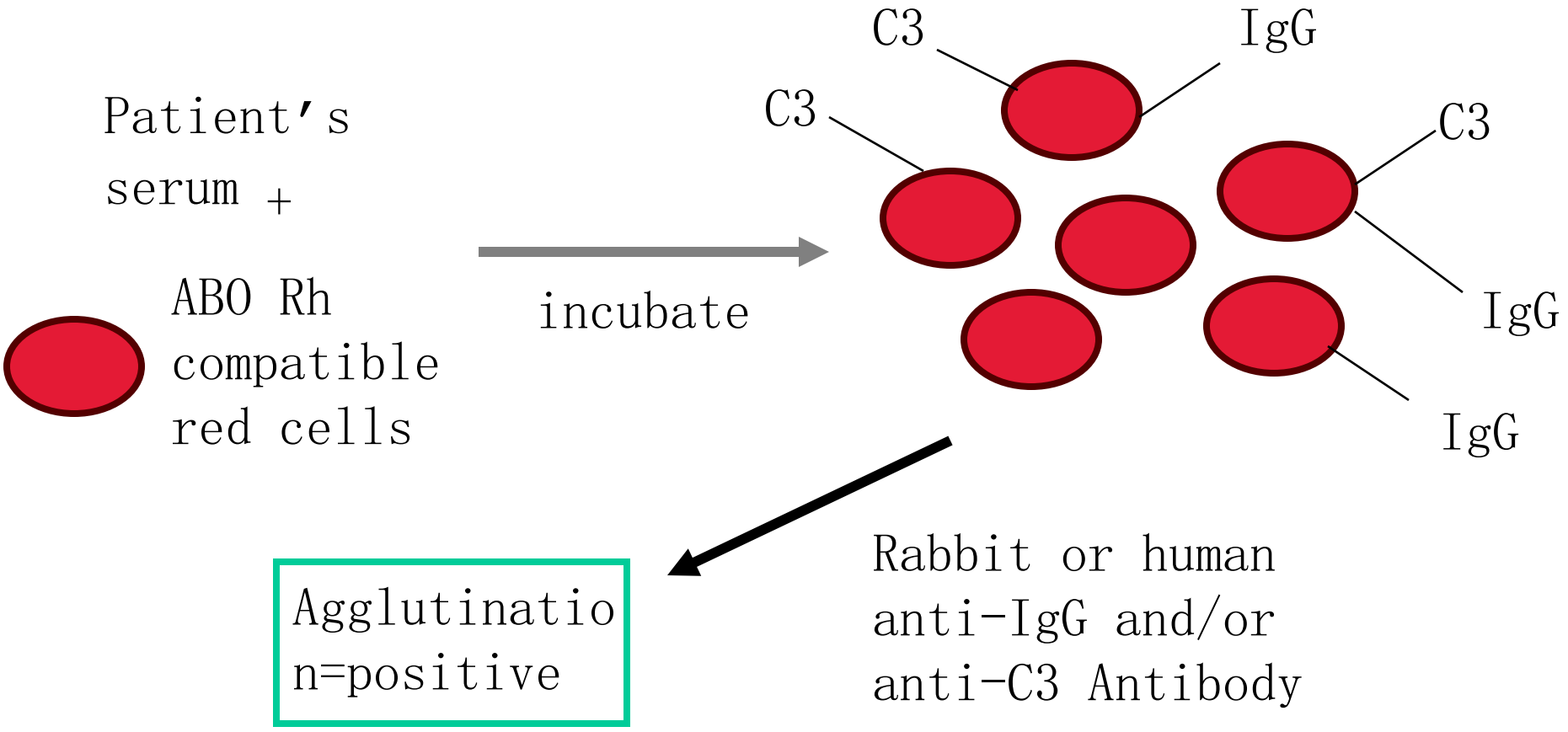
- IgM directed against 'Antigen I' on red cells
- Seen in
  - Elderly
  - Lymphomas
  - Infections - EBV, mycoplasma
- DAT (Coombs) - + for C3, negative for IgG
- Treat - stay warm

Coombs' or antiglobulin test



- Helps differentiate the cause of hemolysis
  - Hapten related: direct +, indirect –
  - Immune complext: direct +/ - (complement +)
  - Autoimmune: direct + (without hemolysis in some patients), indirect + in some, remains elevated for up to 2 years

Indirect antiglobulin test (aka Indirect Coombs Test)



IMMUNE HEMOLYSIS

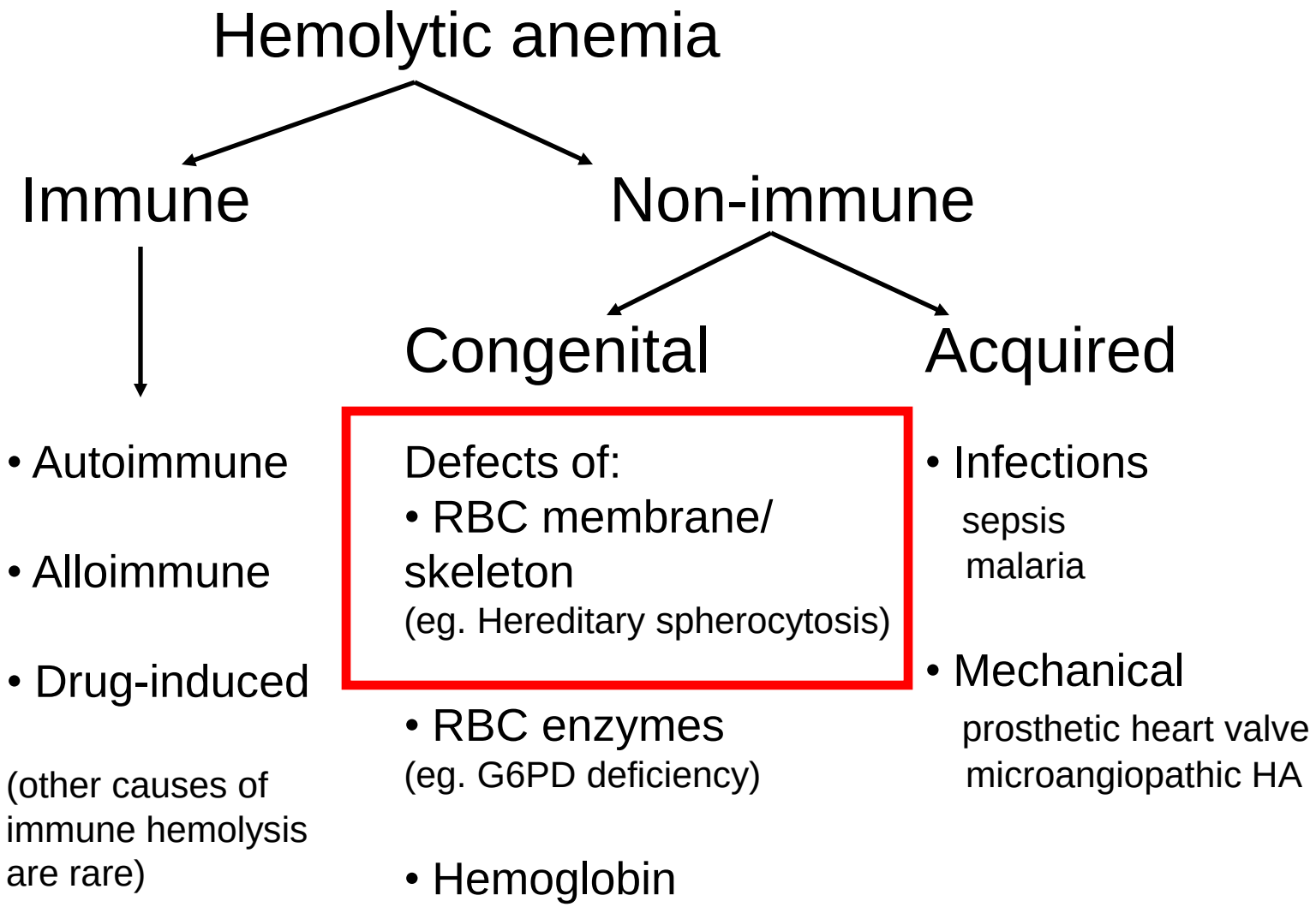
Drug-Related

- Immune Complex Mechanism
  - Quinidine, Quinine, Isoniazid
- "Haptenic" Immune Mechanism
  - Penicillins, Cephalosporins
- True Autoimmune Mechanism
  - Methyldopa, L-DOPA, Procaineamide, Ibuprofen

Treatment of autoimmune hemolysis

- treat the underlying cause, if there is one
- stop suspect drugs if possible
- prednisone
- transfuse RBC’s, if needed

An approach to hemolytic anemia



Membrane defects

- Hereditary vs acquired
  - Spherocytosis
  - Elliptocytosis
  - Stomatocytosis

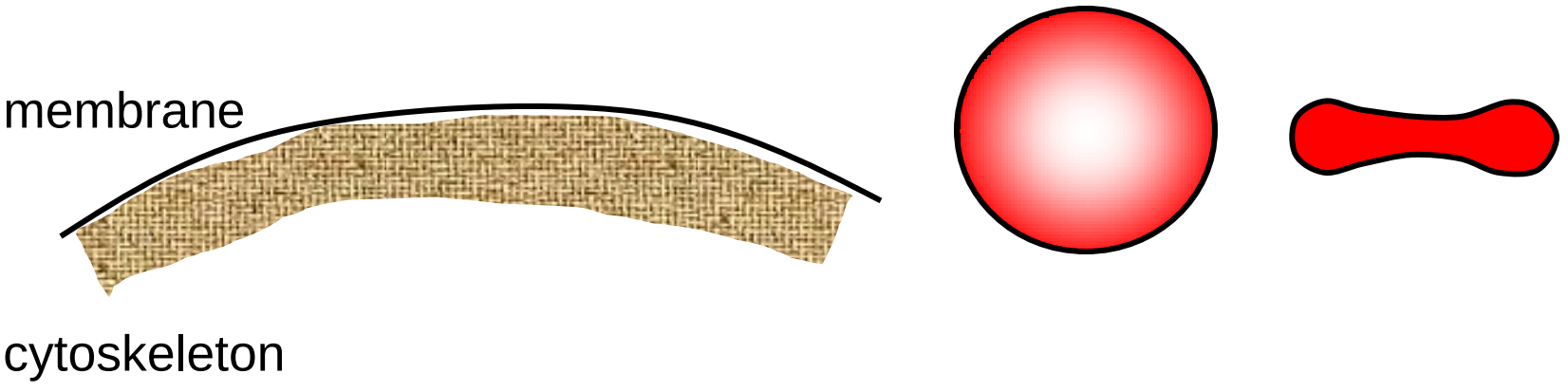
Membrane defects

- Abnormal shape of red cells
- Decreased flexibility of RBC membrane
- Inability to pass through spleen
  - “conditioned” by spleen
  - Membrane loss

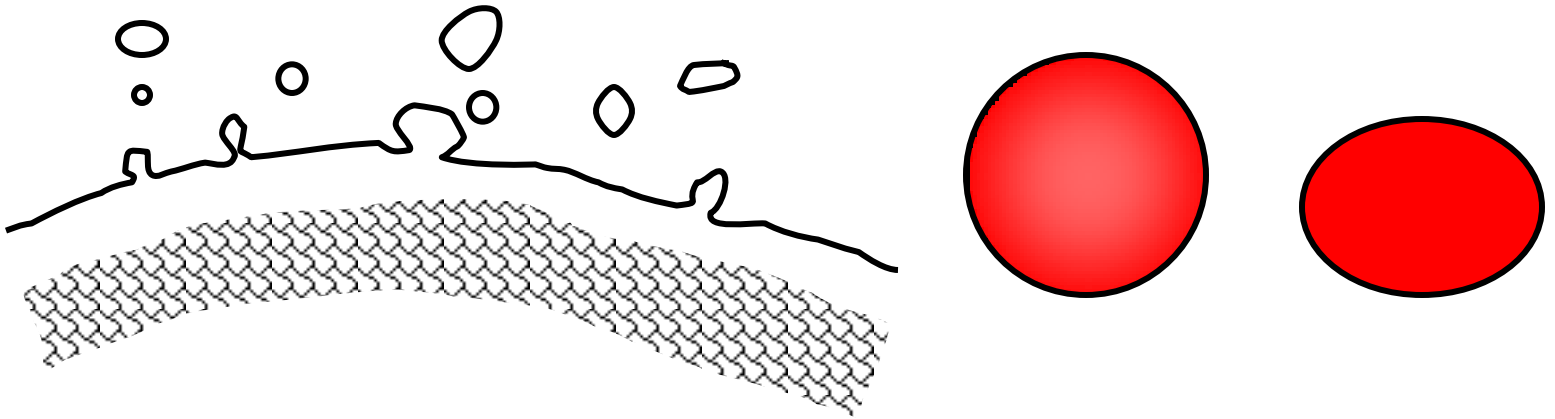
Hereditary spherocytosis

- most common inherited red cell membrane disorder
    - 1/5000 in northern European populations
- autosomal dominant
- caused by mutations in the genes that encode RBC membrane cytoskeleton proteins.

Normal

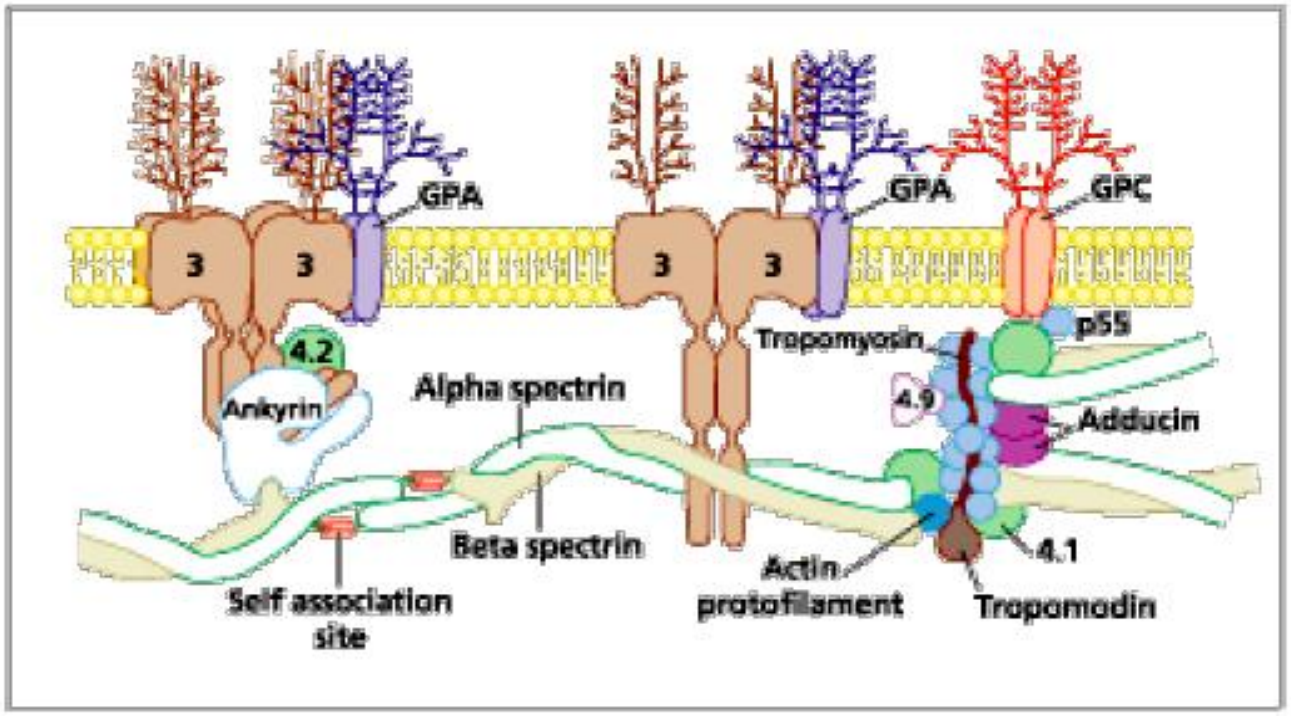


Hereditary spherocytosis



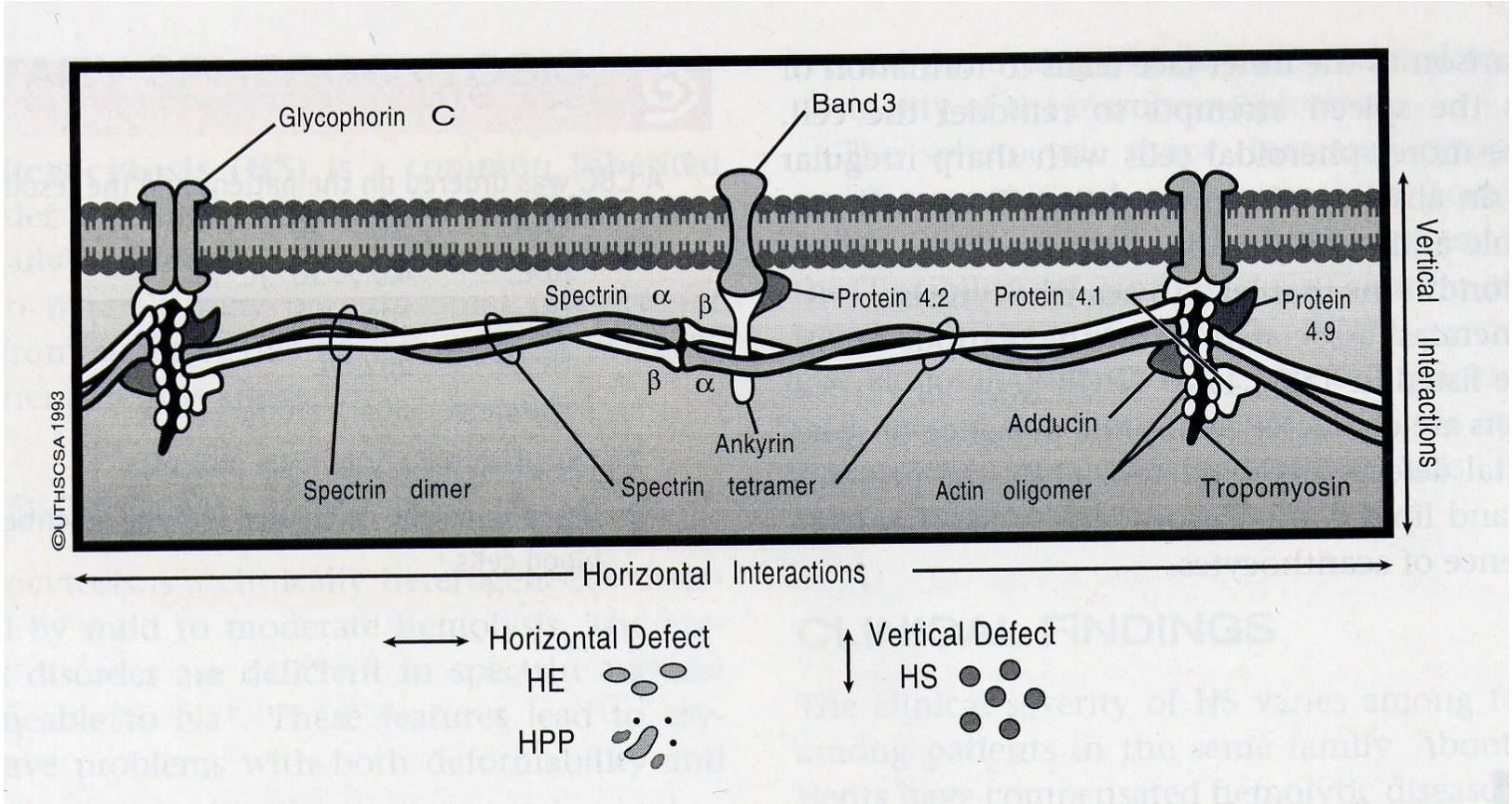
loss of membrane = loss of SA = loss of deformability = increased splenic clearance

Membrane defects



Model of RBC membrane

Membrane defect in HS



Hereditary spherocytosis

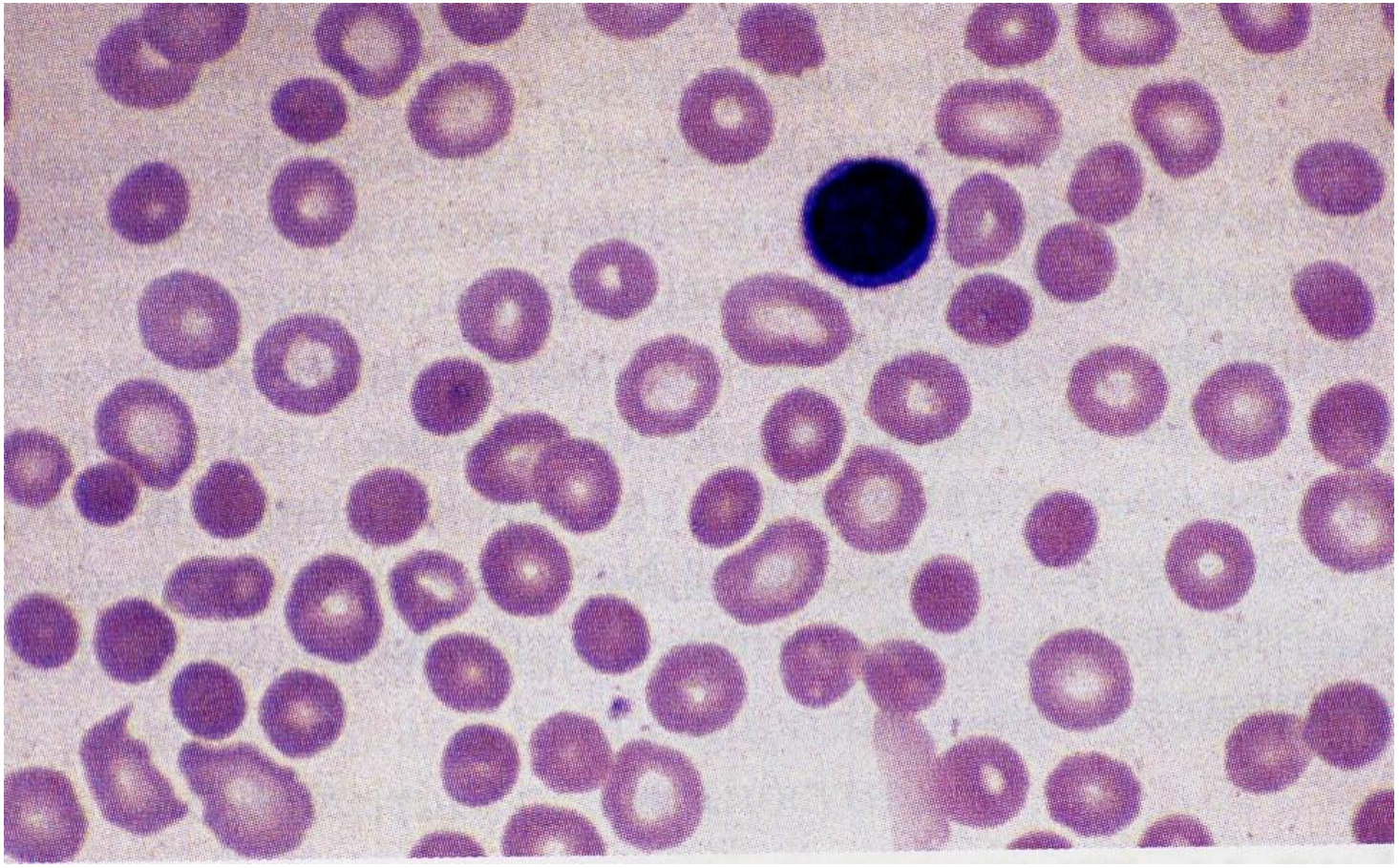
- Spherocytes are cleared by the spleen more rapidly
  - lack of deformability means they cannot squeeze through the sieve-like slits of the spleen.

Hereditary spherocytosis

- Clinical features:
  - clinical severity varies
  - most have mild to moderate anemia
  - splenomegaly, cholelithiasis, jaundice may occur
- Laboratory features
  - hemolytic anemia with spherocytes
  - osmotic fragility test
  - negative DAT

Blood Smear

Spherocytes



Osmotic Fragility

	•Normal	•HS
[NaCl]	***% Lysis***	***% Lysis***
1.0% NS	0	0
.75% NS	10	20
.50% NS	30	60
.25% NS	70	90
.00% NS	100	100

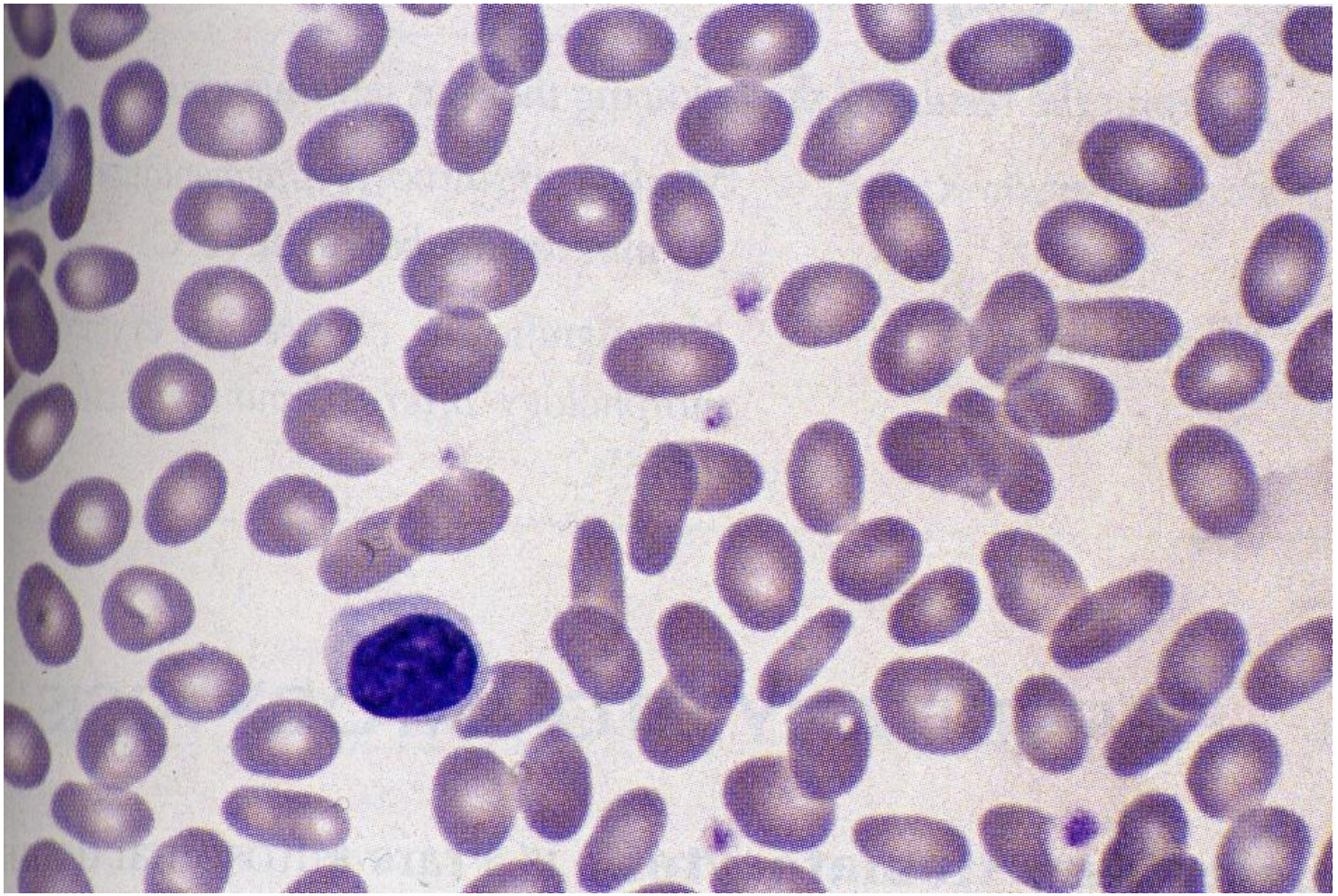
Hereditary spherocytosis

- Treatment
  - most patients do not need treatment
  - splenectomy
  - counsel patient and family about inheritance

Hereditary elliptocytosis

- Autosomal dominant
- Ddx
  - Iron deficiency
  - Thalassemia
  - Megaloblastic anemia

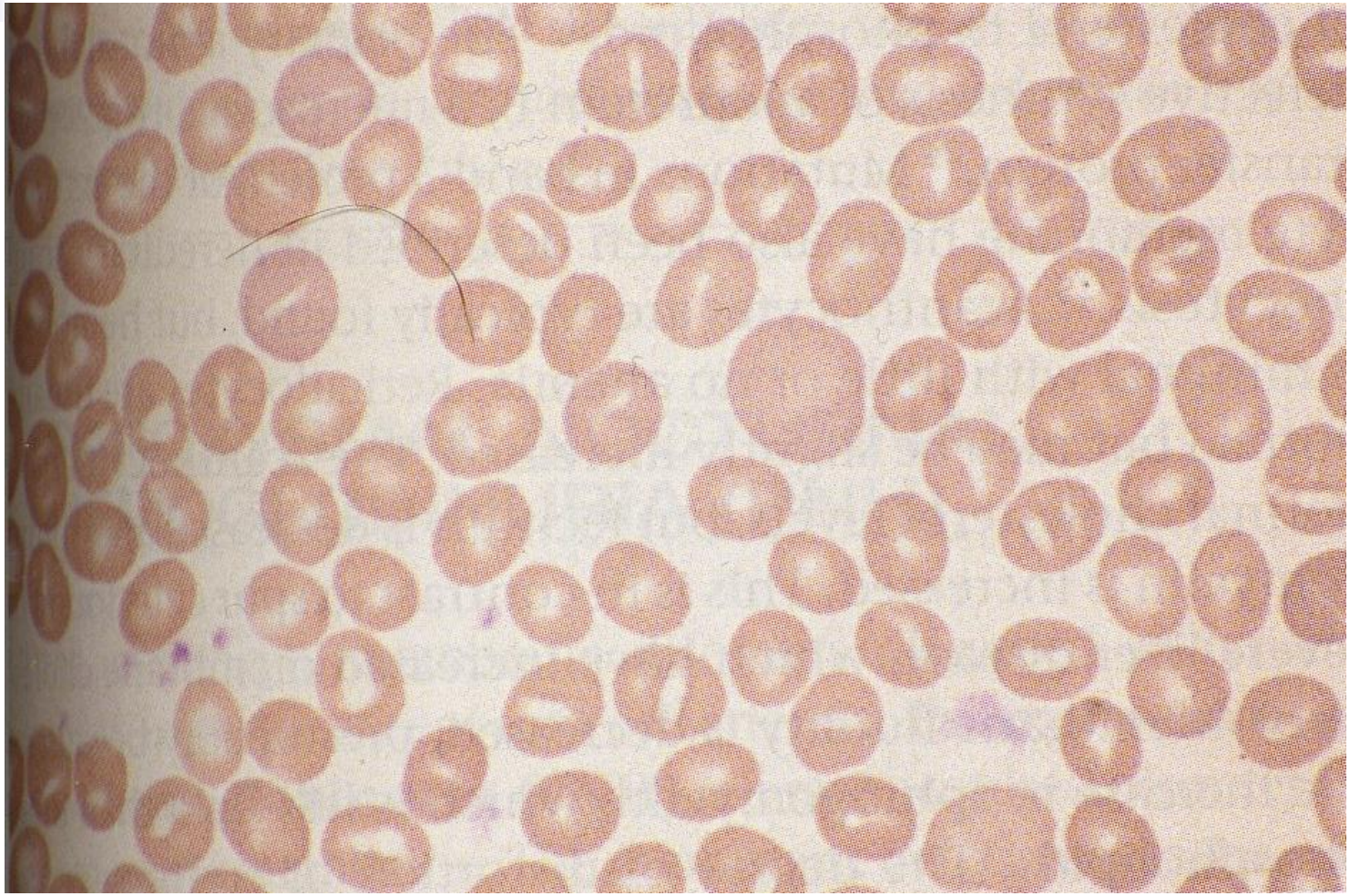
Hereditary elliptocytosis



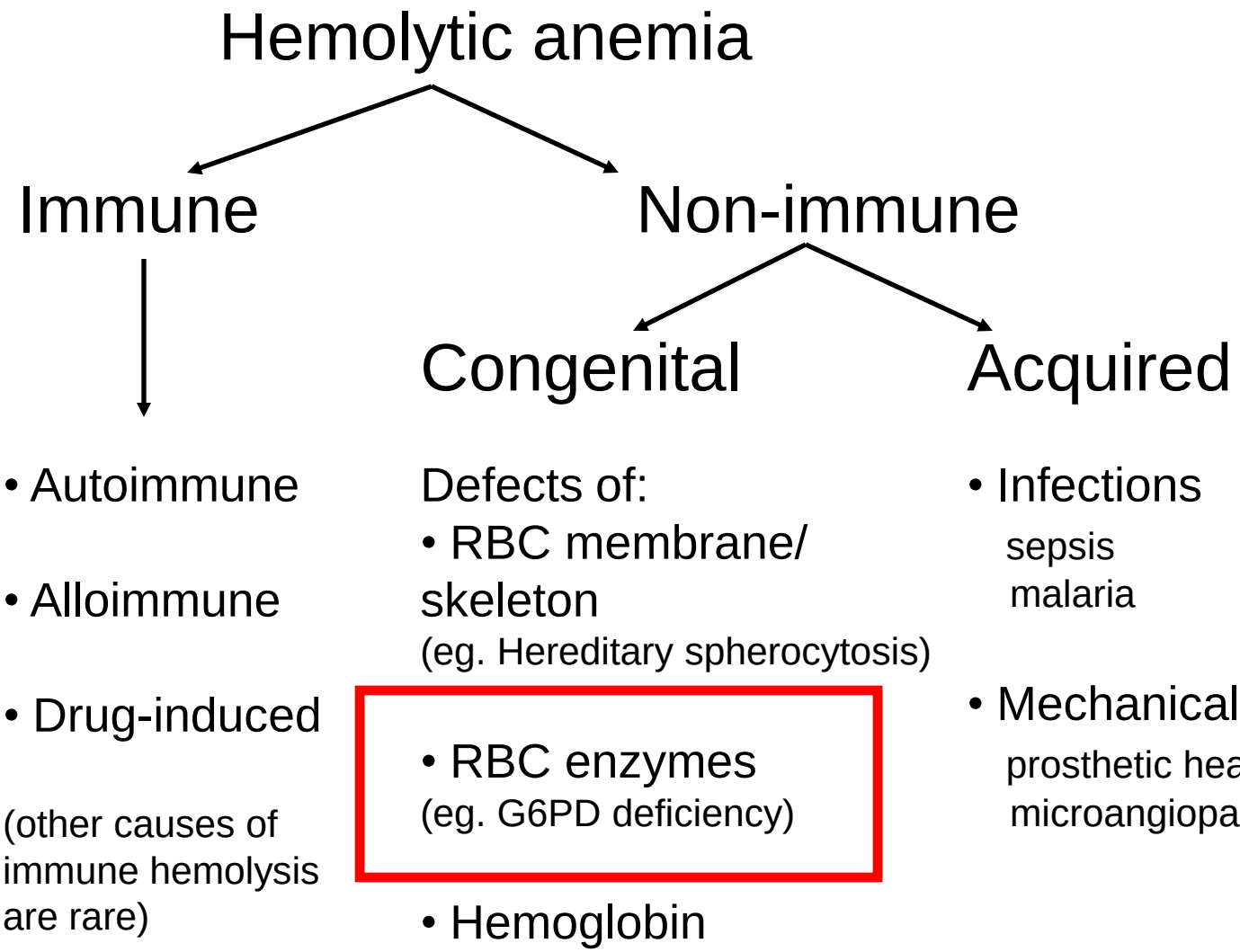
Hereditary stomatocytosis

- Wide transverse slit or stoma
- Few stomatocytes (3-5%) on smear can be normal

Hereditary stomatocytosis



An approach to hemolytic anemia



Enzyme deficiencies

- Glucose-6-phosphate dehydrogenase deficiency
- Pyruvate kinase deficiency

G6PD deficiency

- Most common inherited red cell enzymopathy
  - up to 10% of those with African and Mediterranean descent
- Over 200 million people worldwide
- ? Survival advantage with malaria infection
- X-linked -Extensive polymorphism
- hemolysis is due to increased oxidative damage to red cells

G6PD deficiency

- clinical severity highly variable
  - Most experience little or no anemia unless exposed to precipitating event or drug
  - precipitants:
    - infections
    - sulfa, primaquine, dapsone
    - fava beans

G6PD deficiency
Oxidative stresses

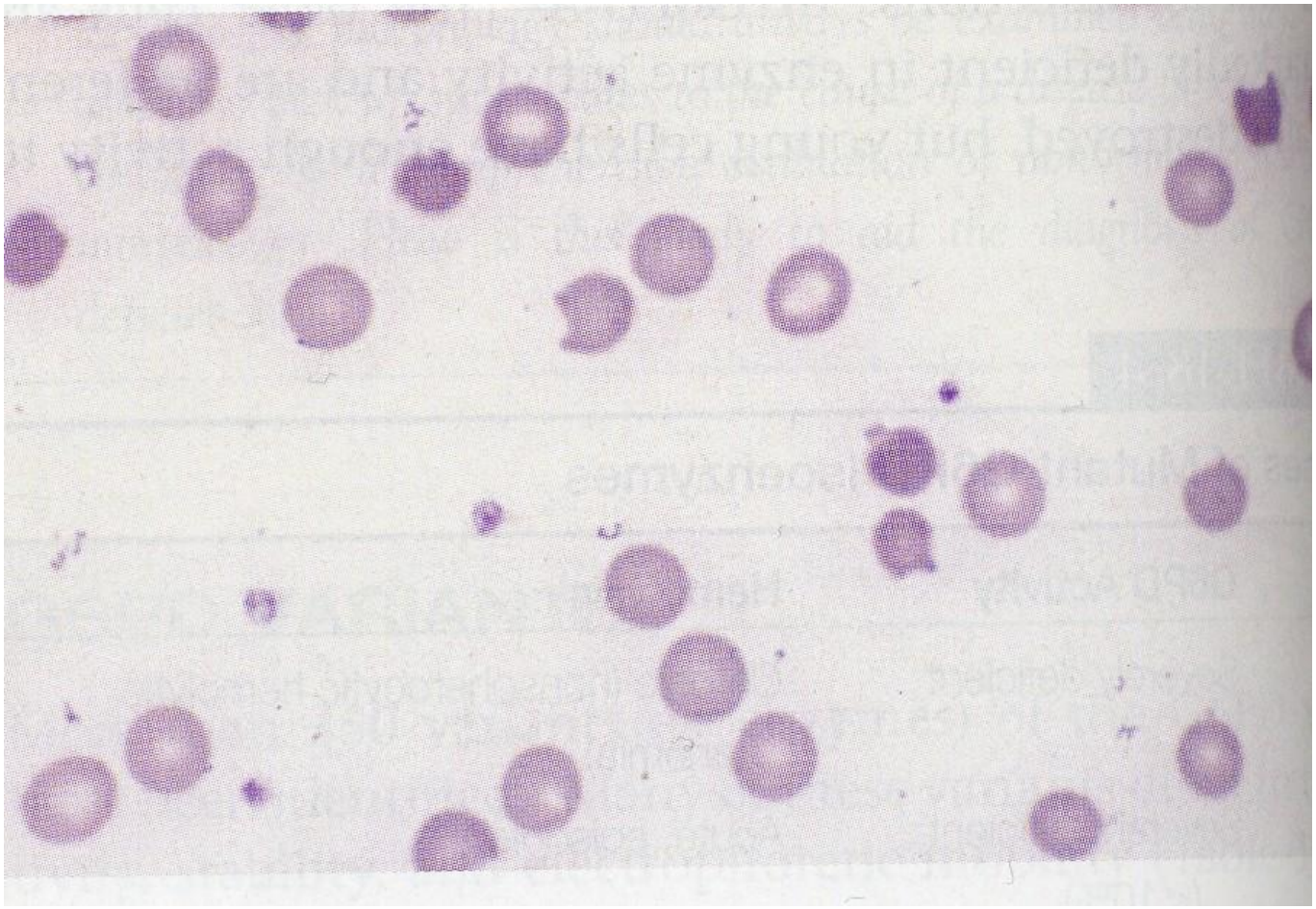
Acetamilide	Sulfamethoxazole
Methylene blue	Sulfamilamide
Napthalene	Sulfapyridine
Nitrofurantoin	Toluidine blue

- Severity of hemolytic anemia depends on
  - the type of defect
  - the level of enzyme activity
  - the severity of oxidant challenge

G6PD deficiency

- Laboratory diagnosis
  - bite cells
  - Heinz bodies
  - measure G6PD level
- Treatment
  - supportive
  - avoid precipitants
  - counsel patient/family

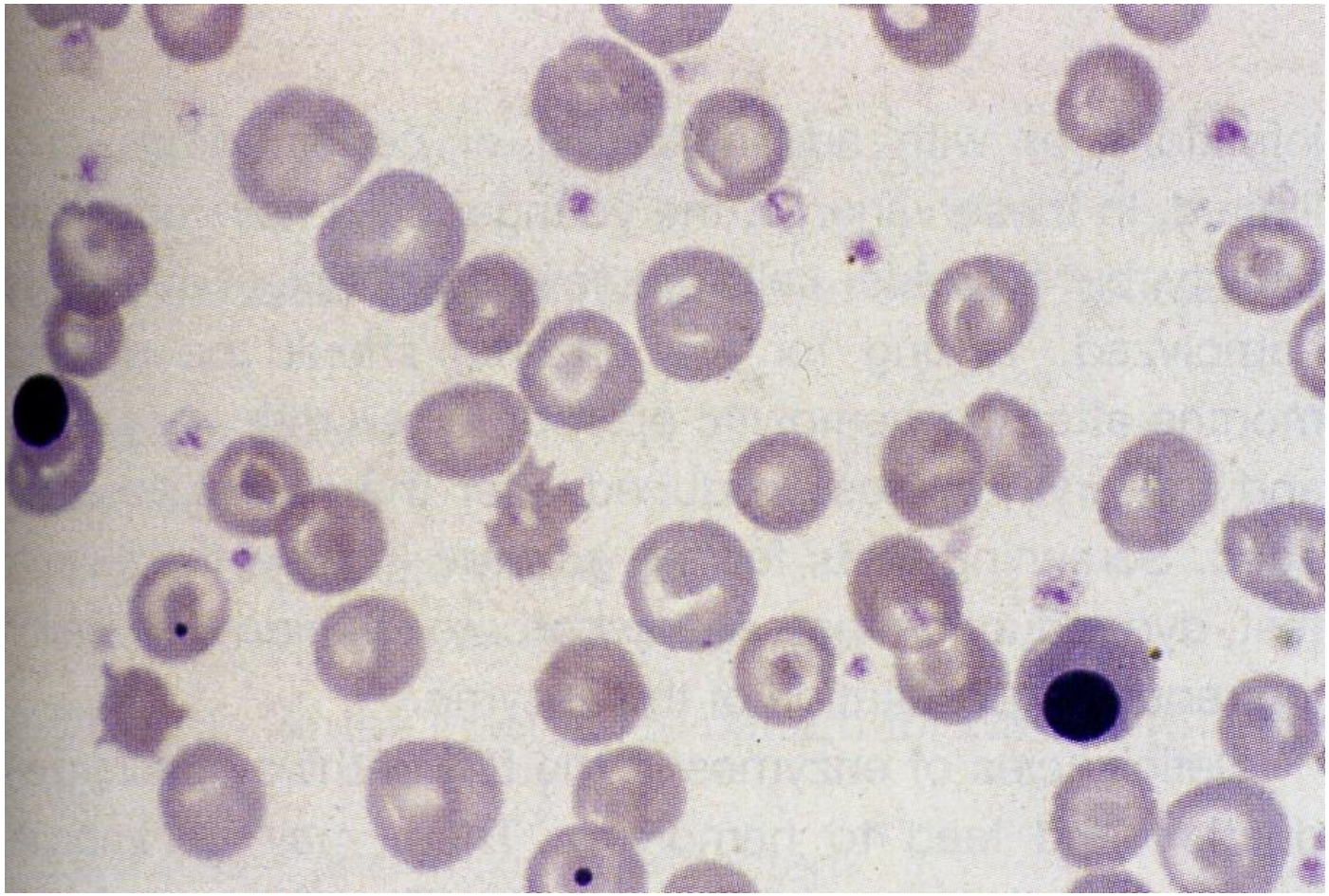
G6PD deficiency (Heinz body anemia)



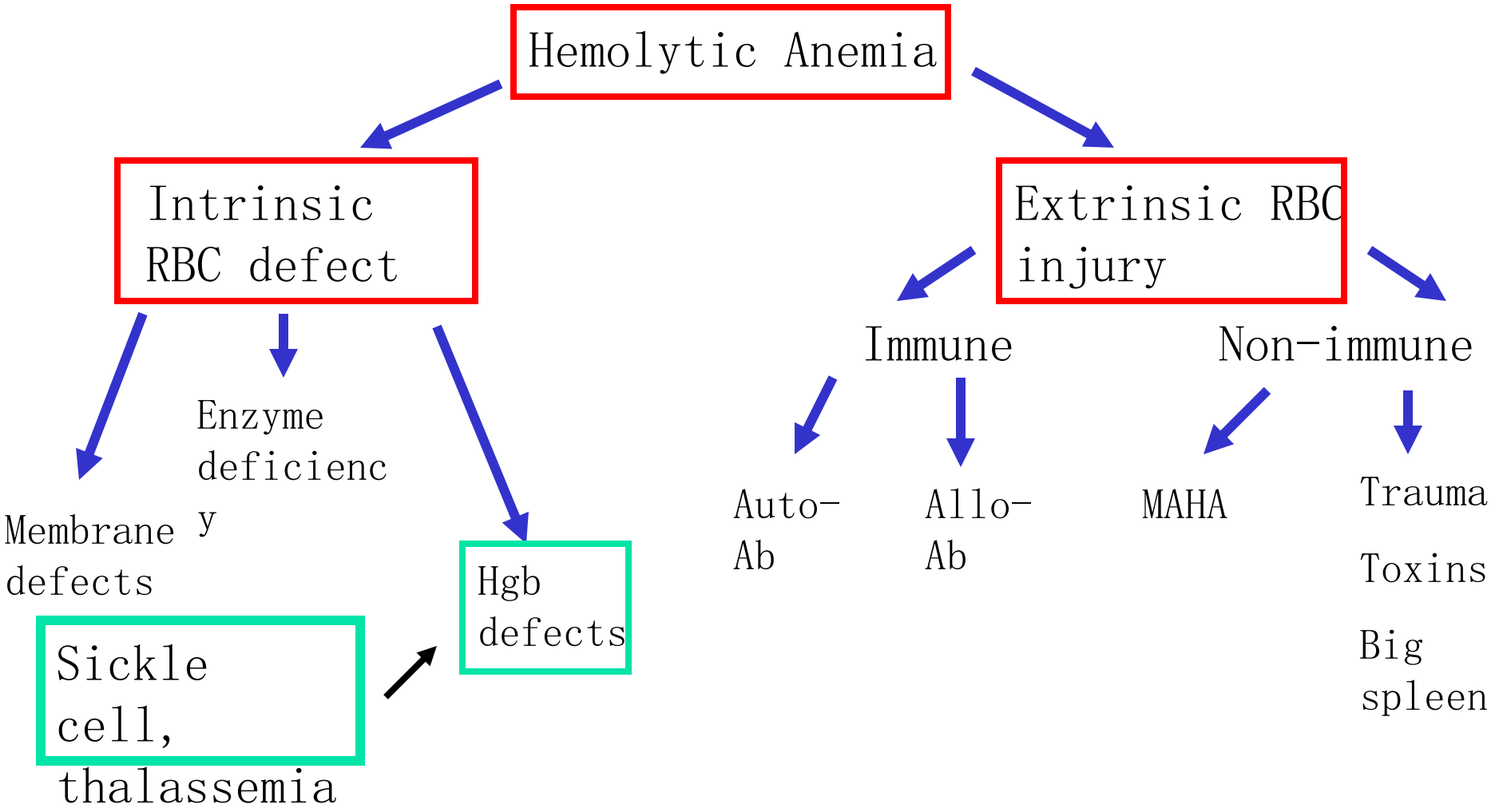
Pyruvate kinase deficiency

- Defects in glycolytic pathway
  - Decreased production of ATP
- Rare
- Autosomal recessive
- Heterogeneous disorder

PK deficiency



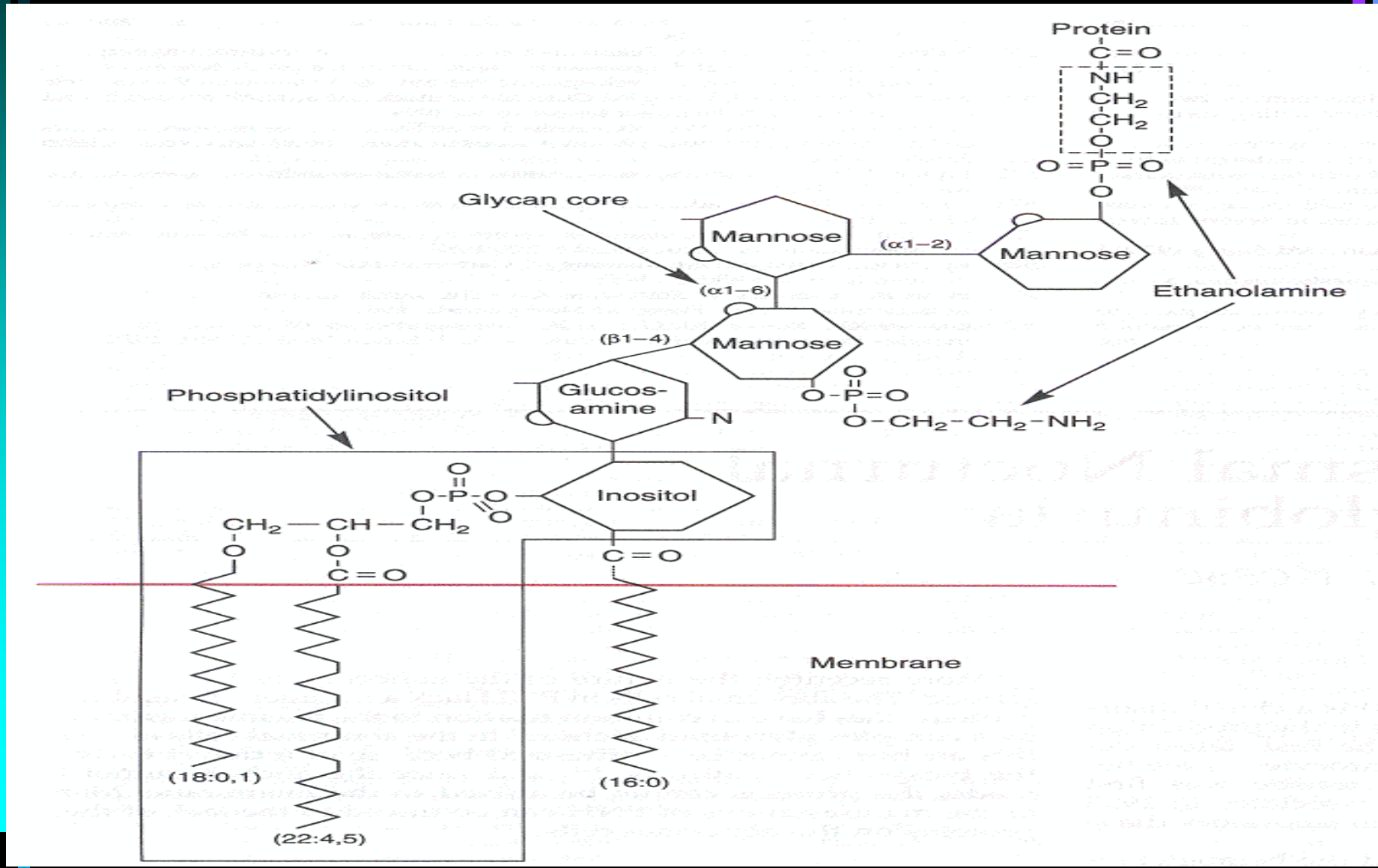
Differential diagnosis



Paroxysmal Nocturnal Hemoglobinuria

- Clonal cell disorder
- Ongoing Intra- & Extravascular hemolysis; classically at night
- Testing
  - Acid hemolysis (Ham test)
  - Sucrose hemolysis
  - CD-59 negative (Product of PIG-A gene)
- Acquired deficit of GPI-Associated proteins (including Decay Activating Factor)

GPI BRIDGE


Protein
C=O
NH
CH2
CH2
O
O=P=O
O
Glycan core
Mannose
(α1–2)
Mannose
(α1–6)
Ethanolamine
(β1–4)
Mannose
Phosphatidylinositol
Glucos-
amine
N
O-P=O
O-CH2-CH2-NH2
Inositol
O-P-O
O
CH2—CH—CH2
O
O
C=O
C=O
Membrane
(18:0,1)
(22:4,5)
(16:0)

Paroxysmal Nocturnal Hemoglobinuria

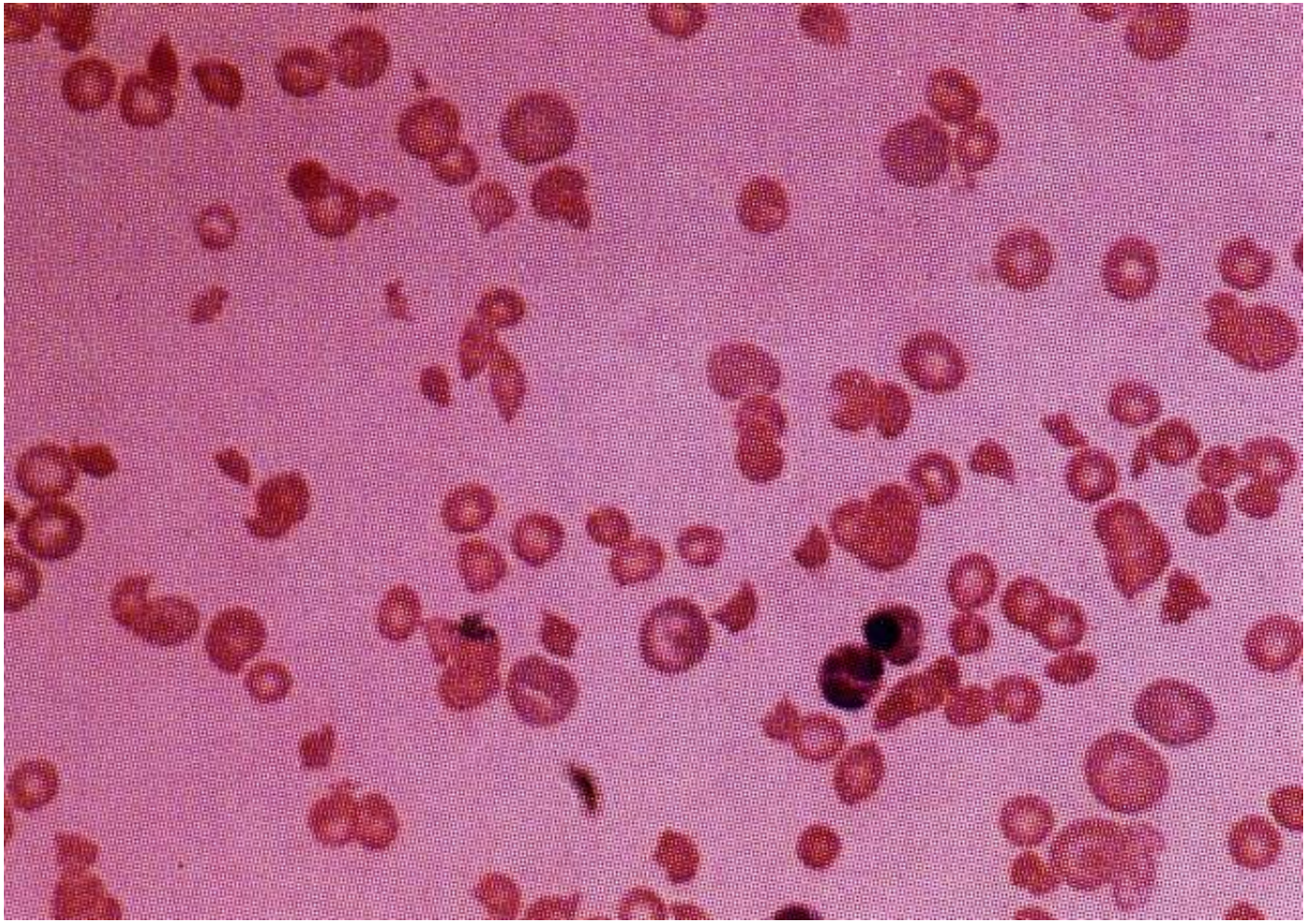
GPI Proteins

- GPI links a series of proteins to outer leaf of cell membrane via phosphatidyl inositol bridge, with membrane anchor via diacylglycerol bridge
- PIG-A gene, on X-chromosome, codes for synthesis of this bridge; multiple defects known to cause lack of this bridge
- Absence of decay accelerating factor leads to failure to inactivate complement & thereby to increased cell lysis

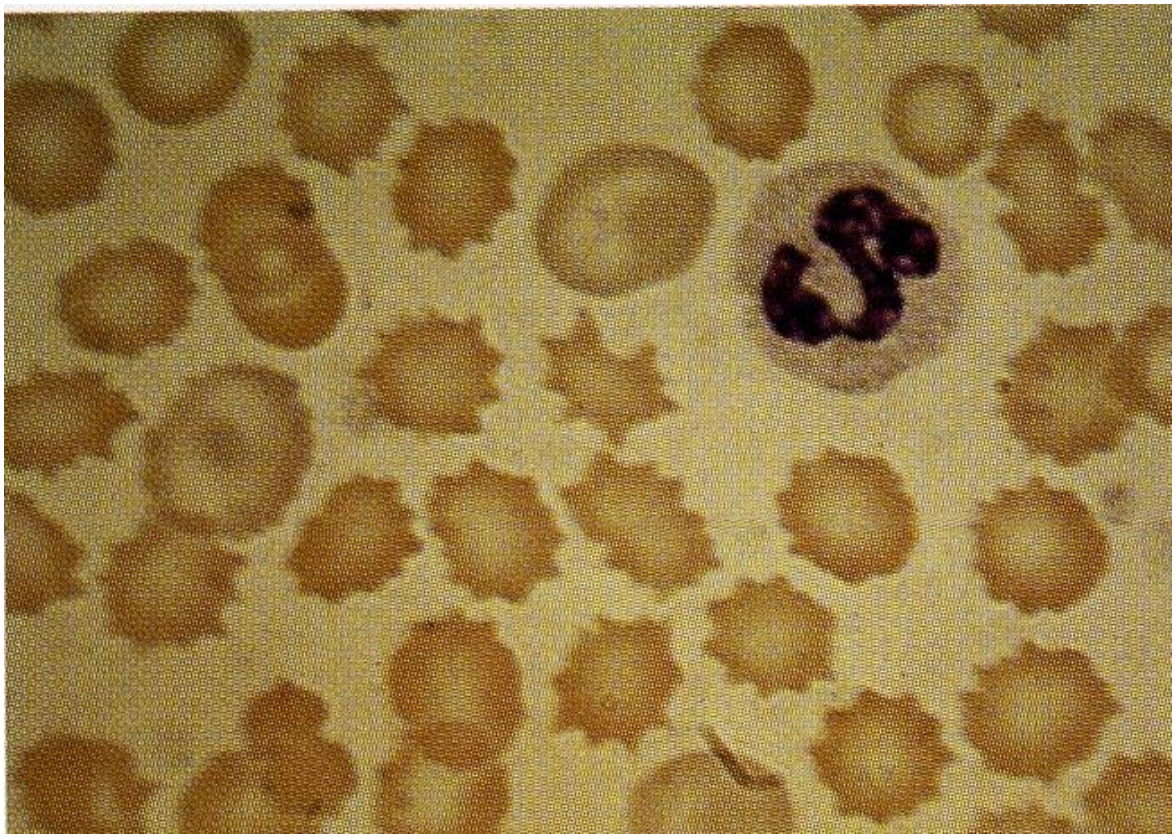
PNH

- Clinical findings:
  - The classic presentation is of hemoglobinuria in the first morning urine specimen.
  - The intravascular hemolysis that occurs during sleep may also be triggered by infection, surgery, or drugs.
  - Most patients also have hemosiderinuria.
  - Abdominal and back pain and headaches occur due to thrombosis of the abnormal platlets
- Lab findings:
  - The peripheral smear shows pancytopenia with a normochromic, normocytic anemia and increased reticulocytes.
  - The bone marrow is hyperplastic.
  - The sucrose hemolysis and Ham's tests are positive and there is decreased leukocyte alkaline phosphatase in the granulocytes

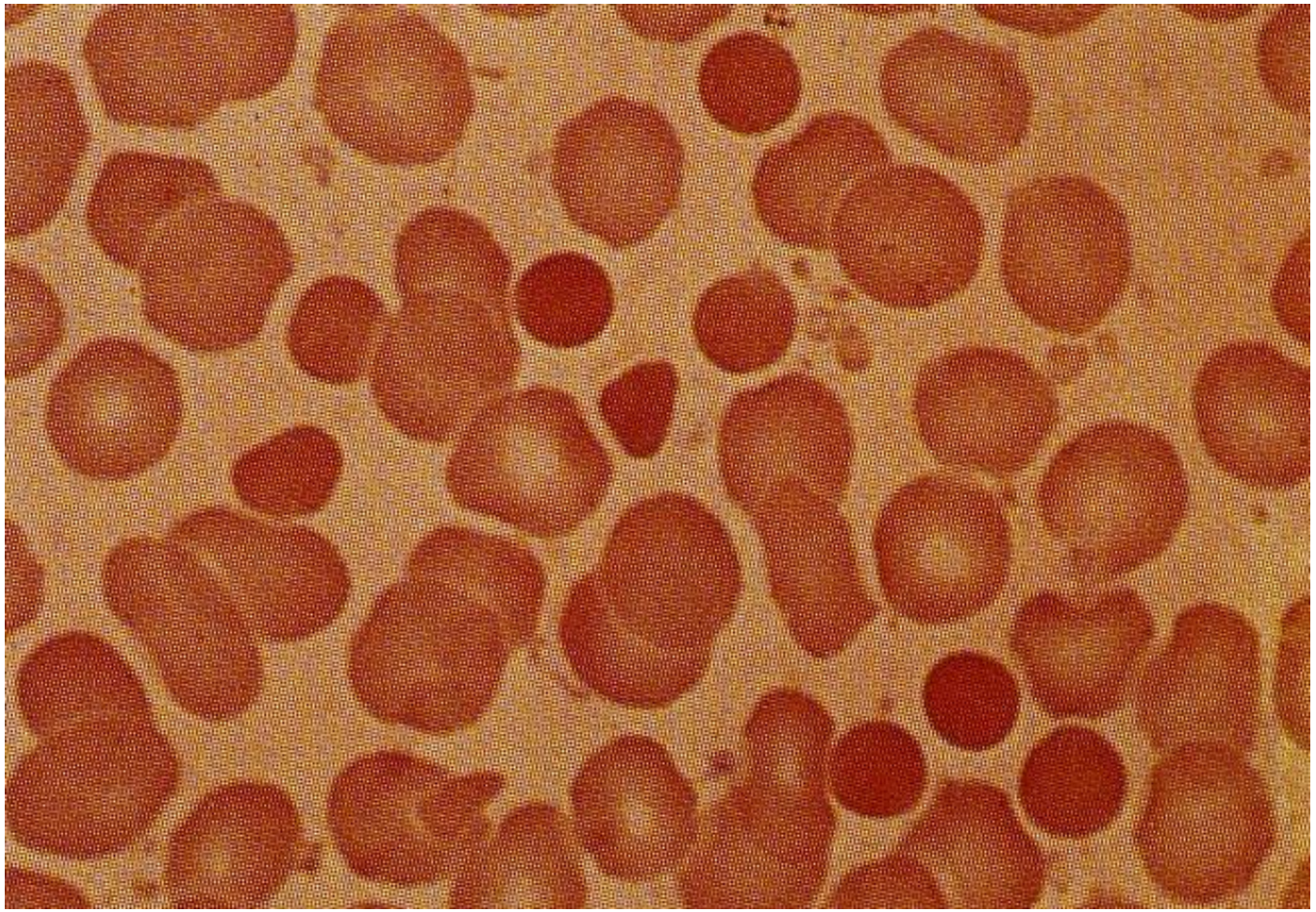
TTP



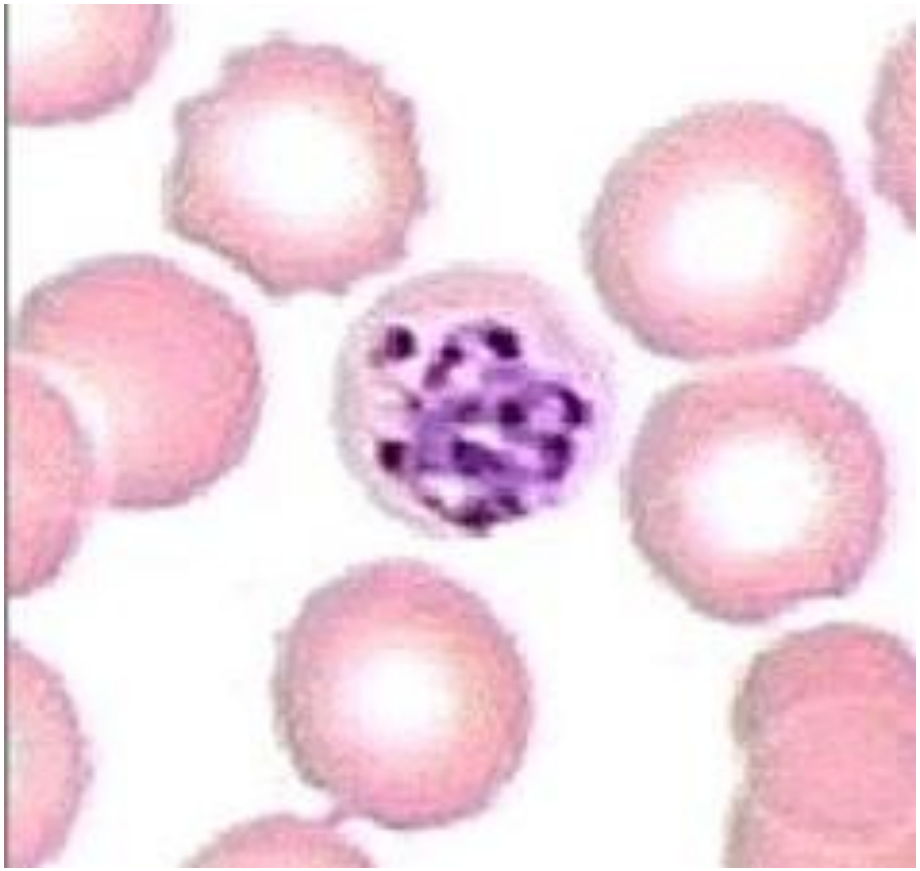
Spur cell anemia



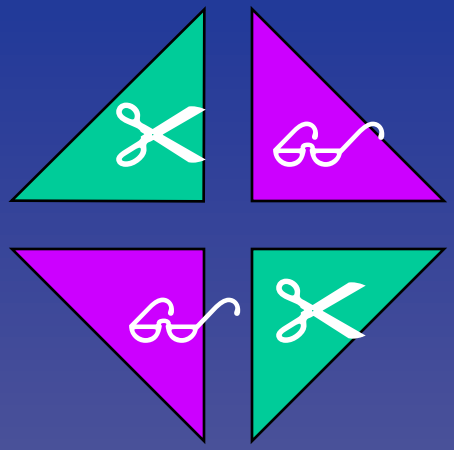
Thermal injury

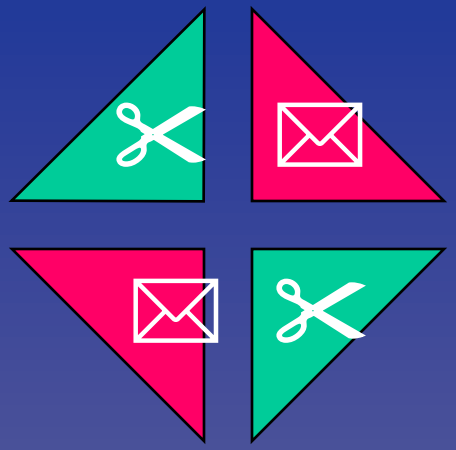


Plasmodium



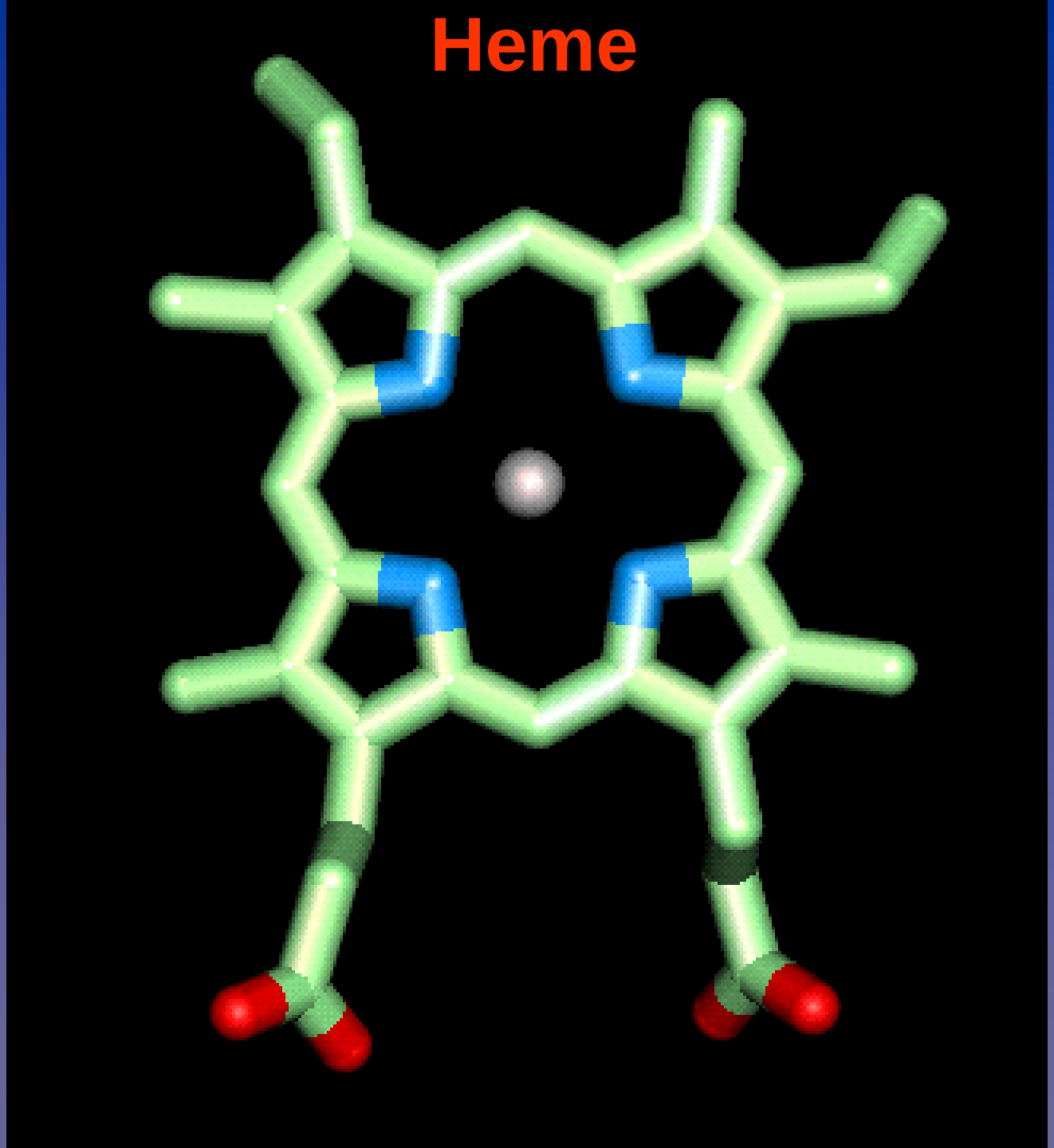
Hemoglobins in normal adults



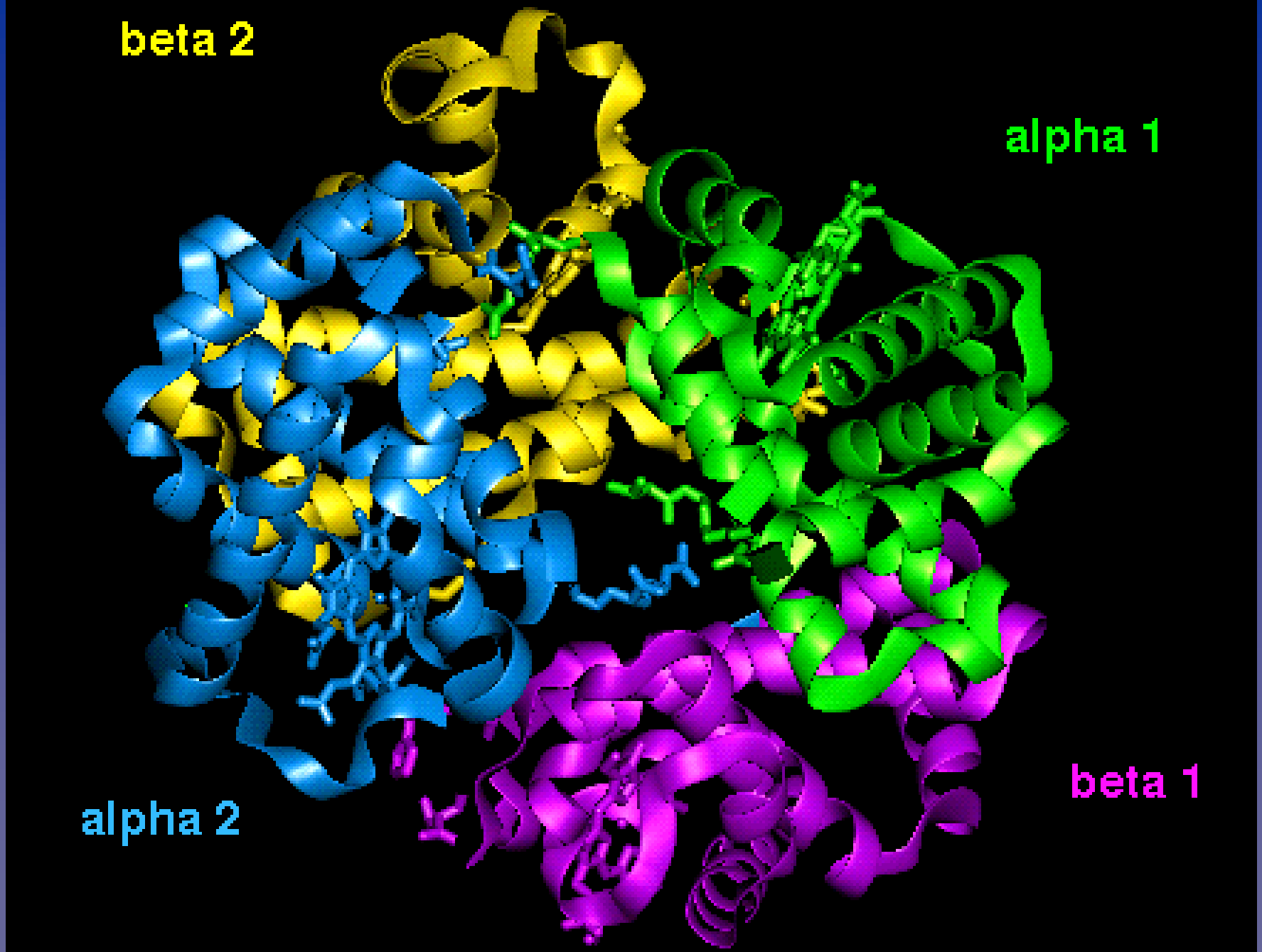


HbA	HbF	HbA_2
98%	~1%	<3.5%

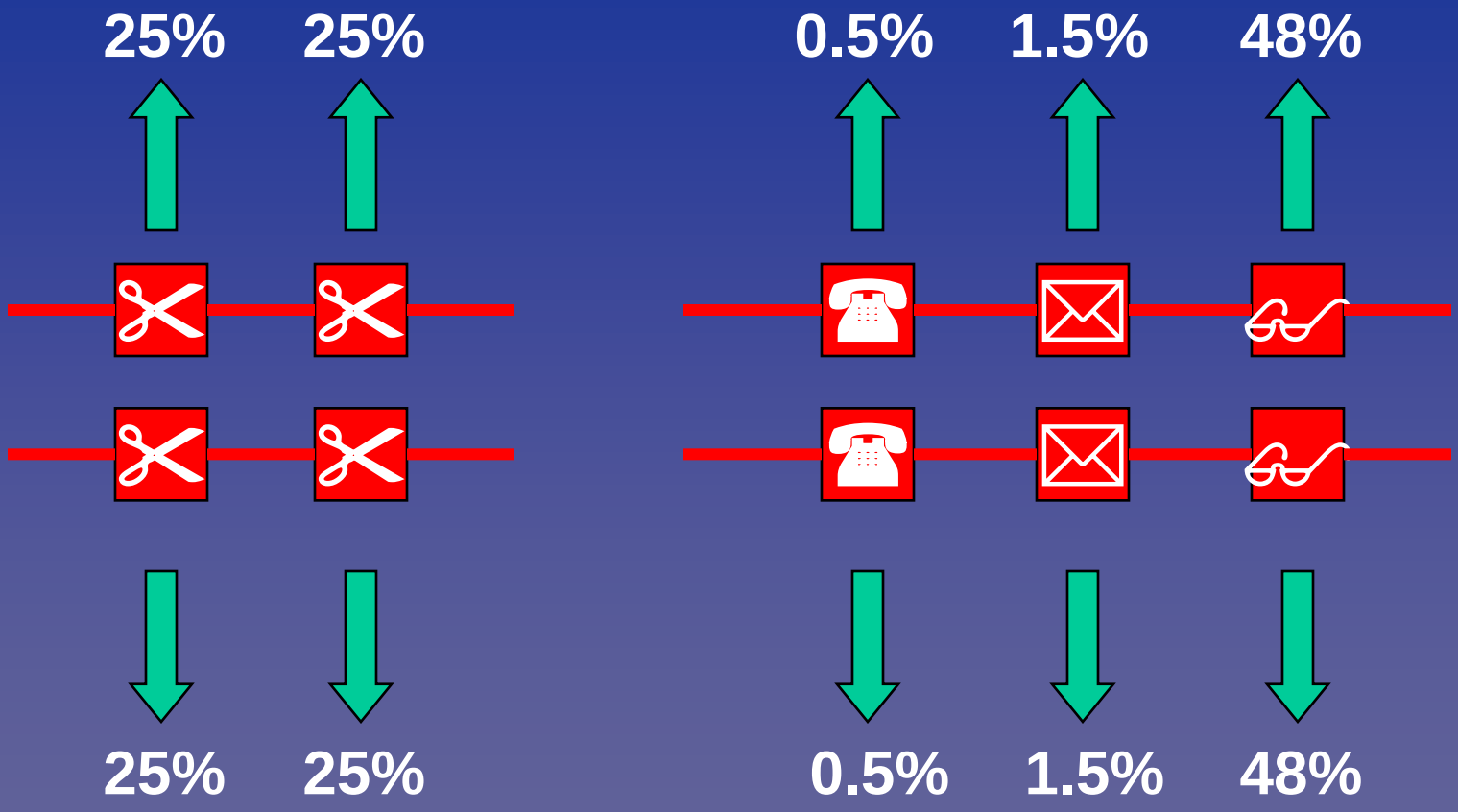
Heme



Globin


beta 2
alpha 1
alpha 2
beta 1

Hemoglobin synthesis



Chromosome 16

Chromosome 11

Hemoglobinopathy

definition

An inherited mutation of the globin genes leading to a qualitative or quantitative abnormality of globin synthesis

Structural hemoglobinopathy

Amino acid substitution in the globin chain e.g. sickle hemoglobin (HbS)

The Thalassemias

Syndromes in which the ***rate*** of synthesis of a globin chain is reduced

- ***beta*** thalassemia - reduced beta chain synthesis
- ***alpha*** thalassemia – reduced alpha chain synthesis

Hemoglobinopathies

Beta Chain Substitutions

Hgb S: Valine for glutamic acid (6th position, beta chain)

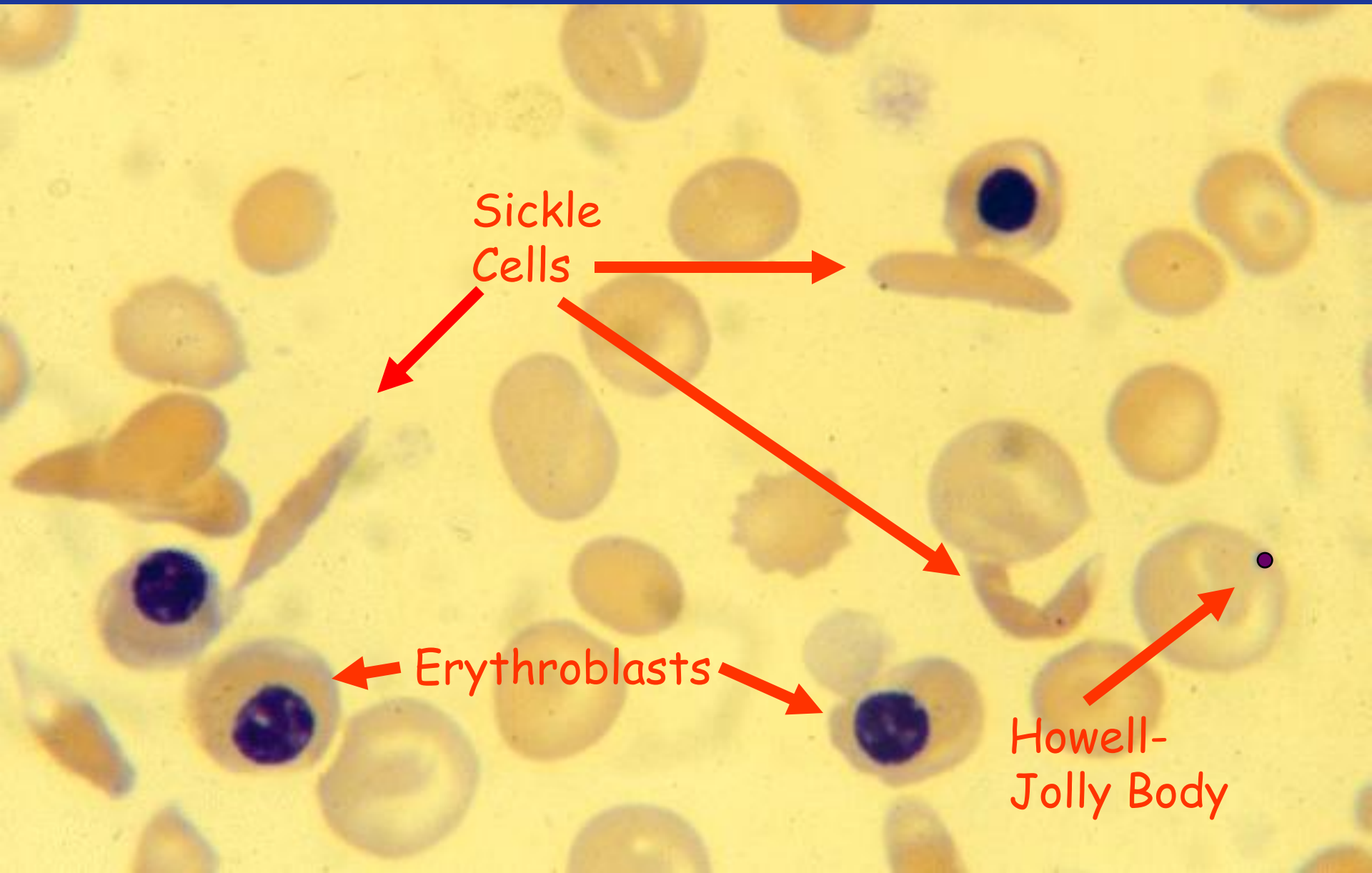
Hgb C: Lysine for glutamic acid (6th position, beta chain)

Hemoglobinopathies

Alkaline Electrophoresis

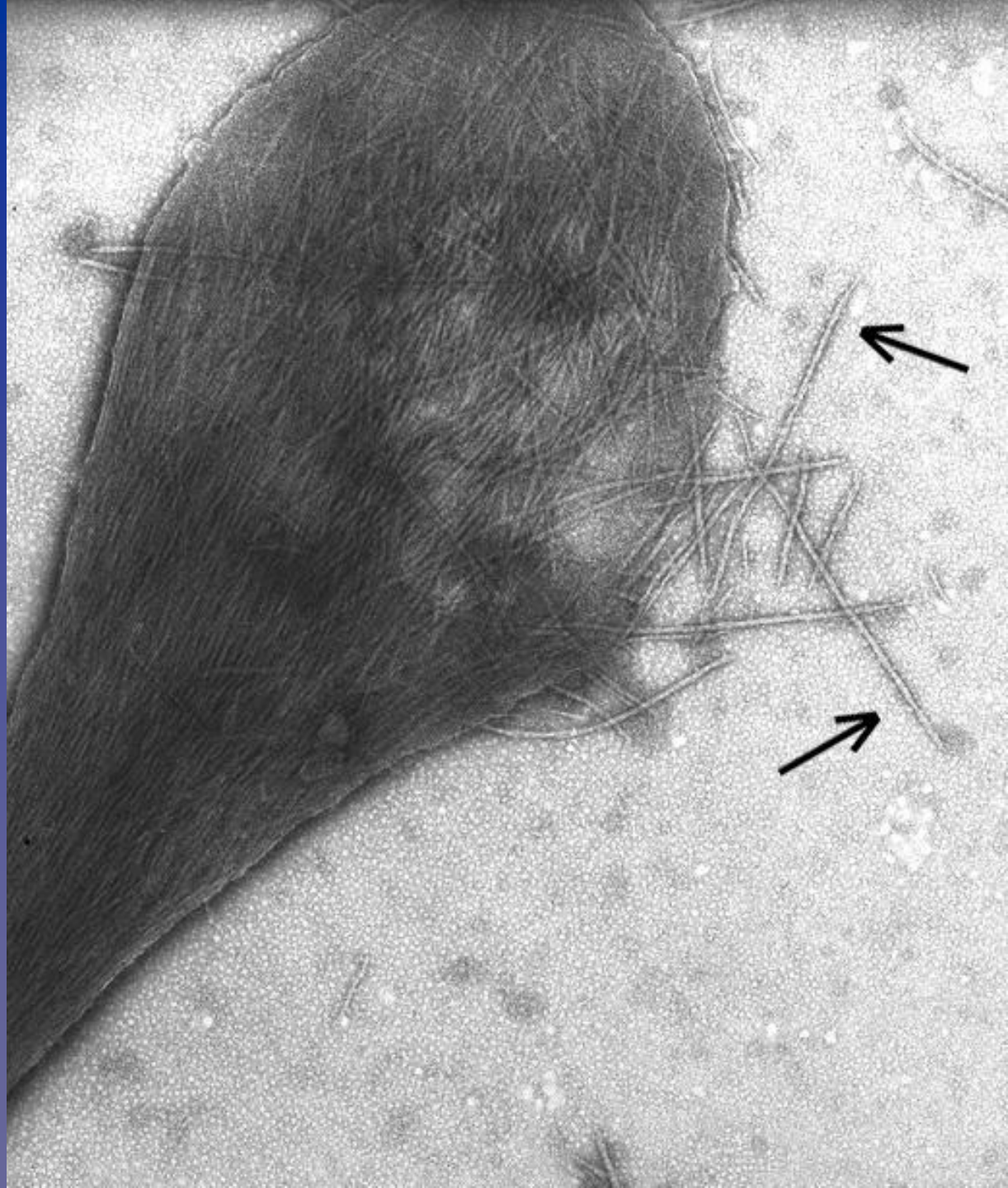
-					+
	C	S	F	A	
	A_2	D			
	E	G			
	O				

Sickle Cell Anemia – blood film

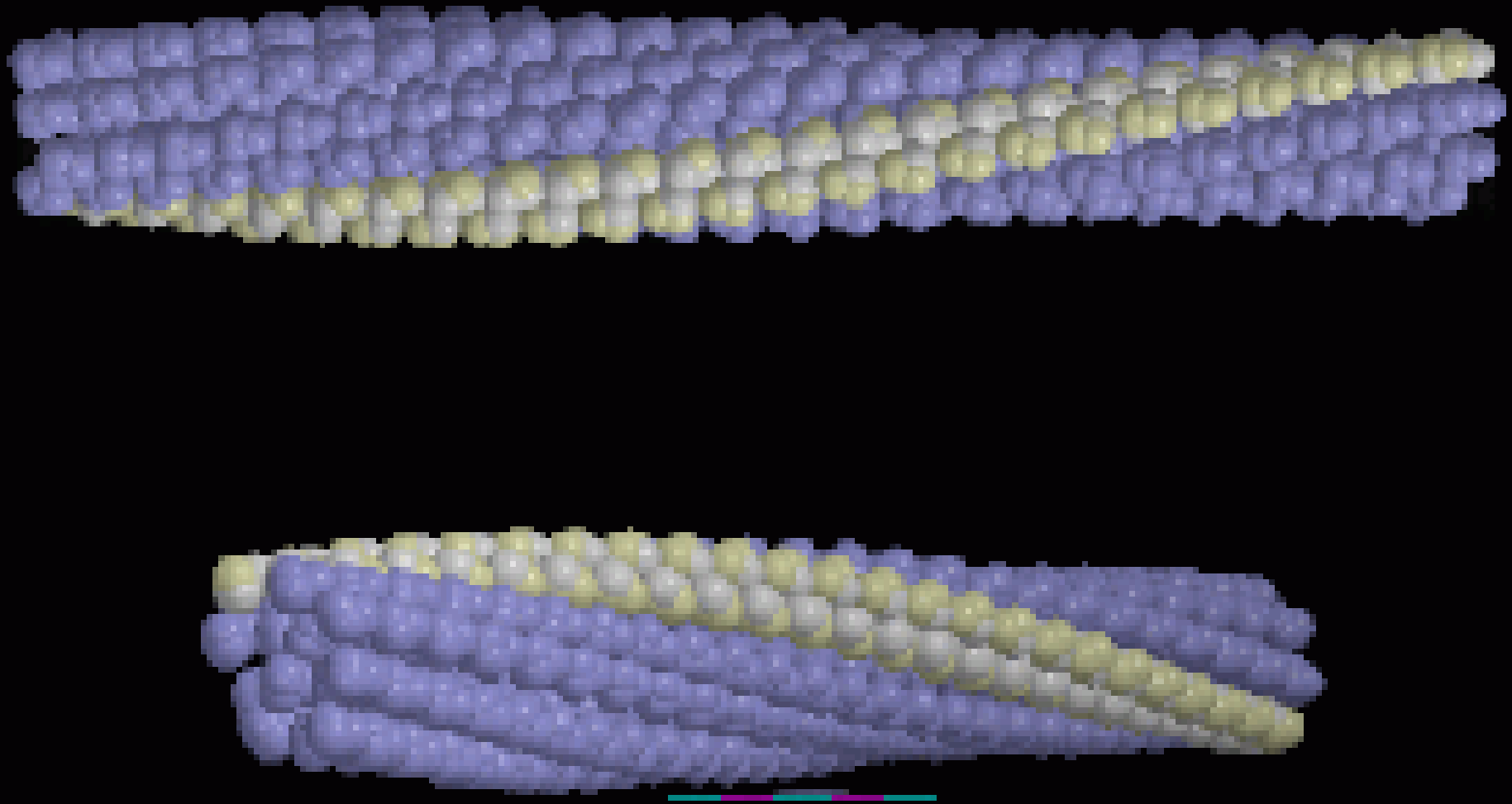


Sickle Cell Anemia –

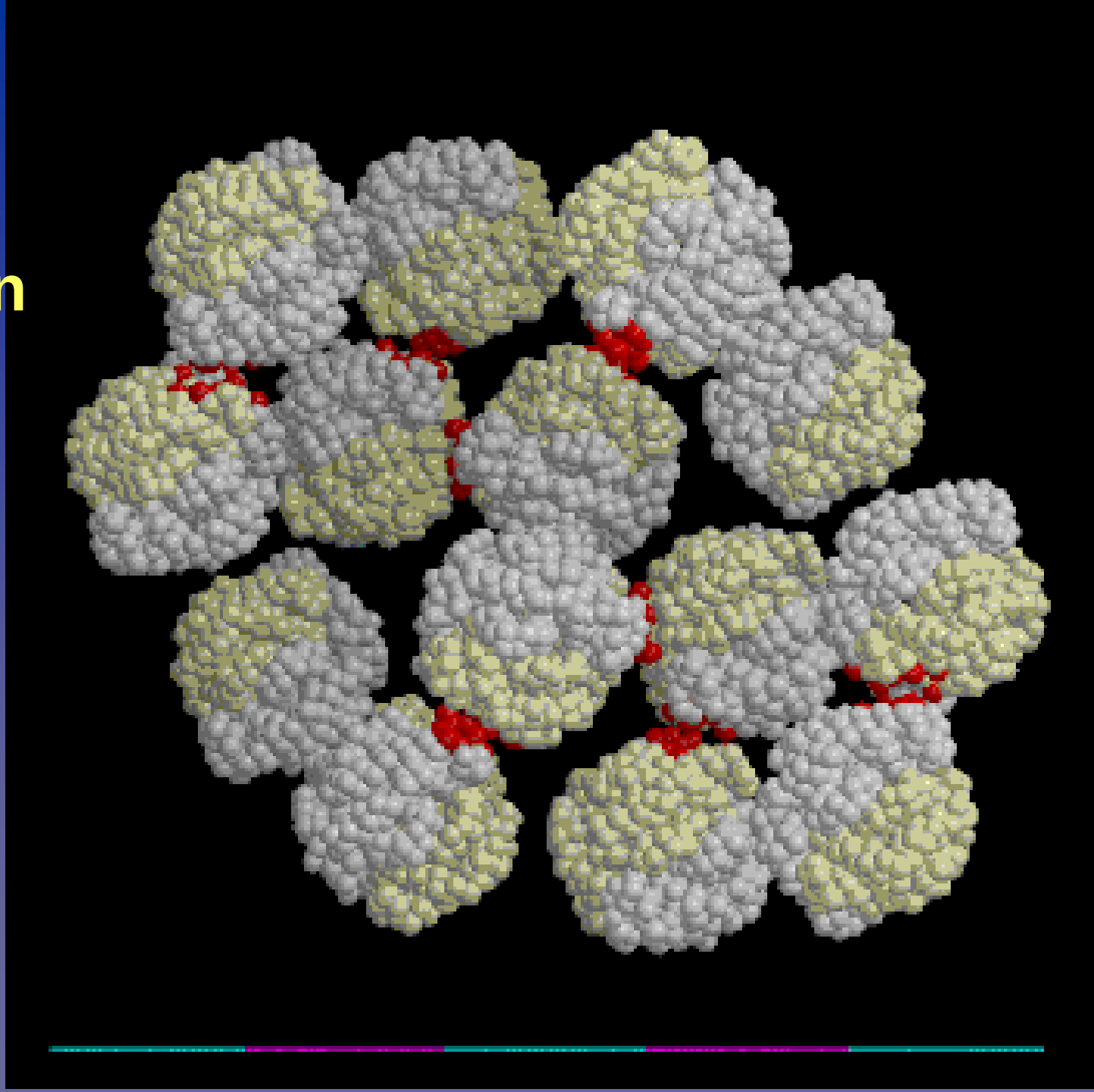
EM of red cell showing ‘tactoids’



Fibres of Sickle Hemoglobin



Fibres of Sickle Hemoglobin – cross section



Sickle cell anemia – clinical features

1. Hemolysis
2. Occlusion of blood vessels
    a. bone (‘painful crisis’)
    b. lung (‘acute chest syndrome’)
    c. brain
    d. heart
    e. spleen (‘Acute splenic sequestration’)
    f. hands (dactylitis in children)
    g. other

Sickle Cell Anemia - treatment

- Opiates and hydration for painful crises
- Pneumococcal vaccination
- Retinal surveillance
- Transfusion for serious manifestations (eg stroke)
- Hydroxyurea
- Stem cell transplant

Sickle Cell Trait

- Heterozygous state for HbS (HbAS)
- No serious clinical consequences
- Sudden death during intensive training
- Hematuria, isosthenuria (renal papillary necrosis)

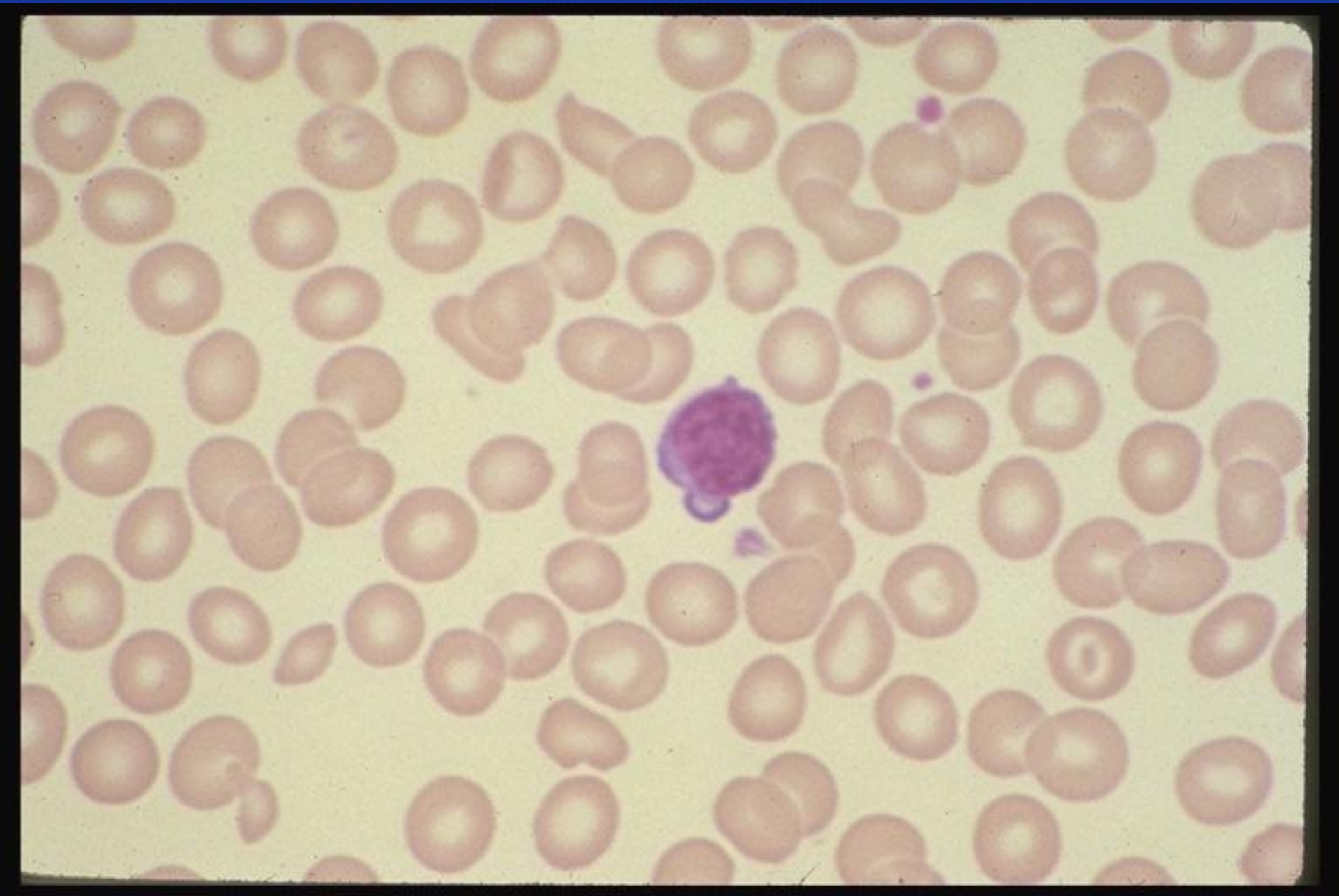
Beta thalassemia

- Impaired production of beta chain
- beta thalassemia minor – heterozygous (or trait)
- beta thalassemia major - homozygous

Beta thalassemia trait

- No symptoms
- Mild microcytic anemia

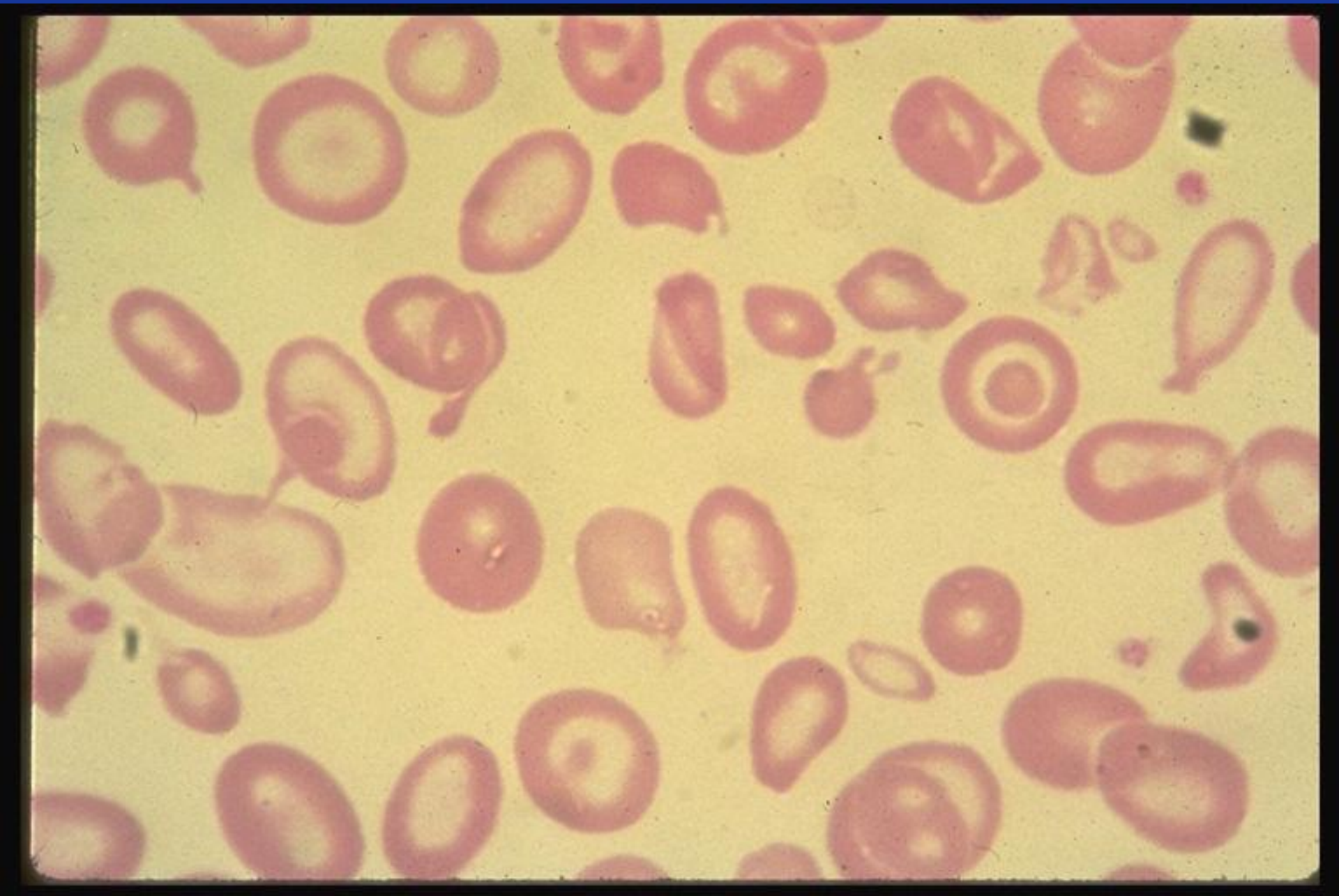
Beta thalassemia - heterozygous (minor or trait)

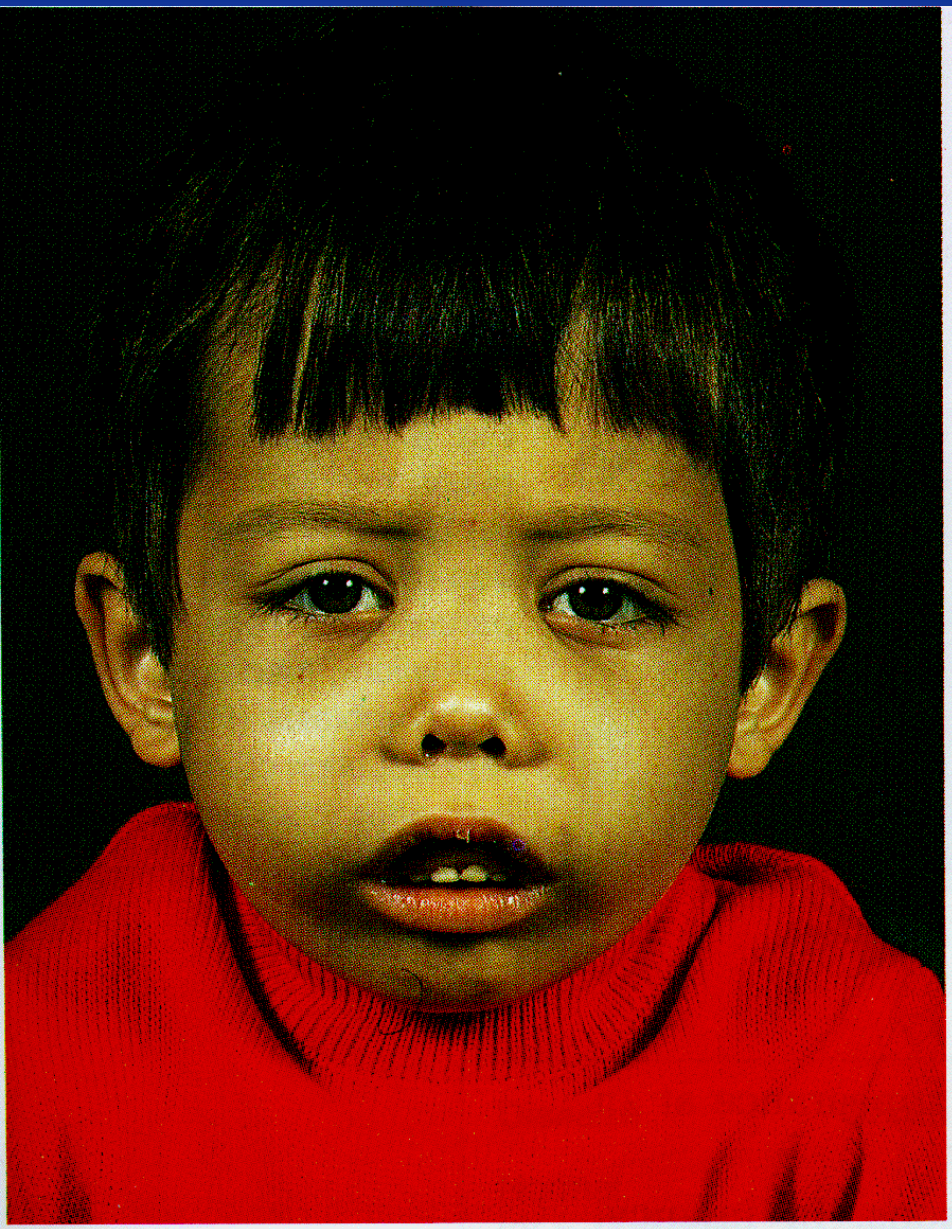


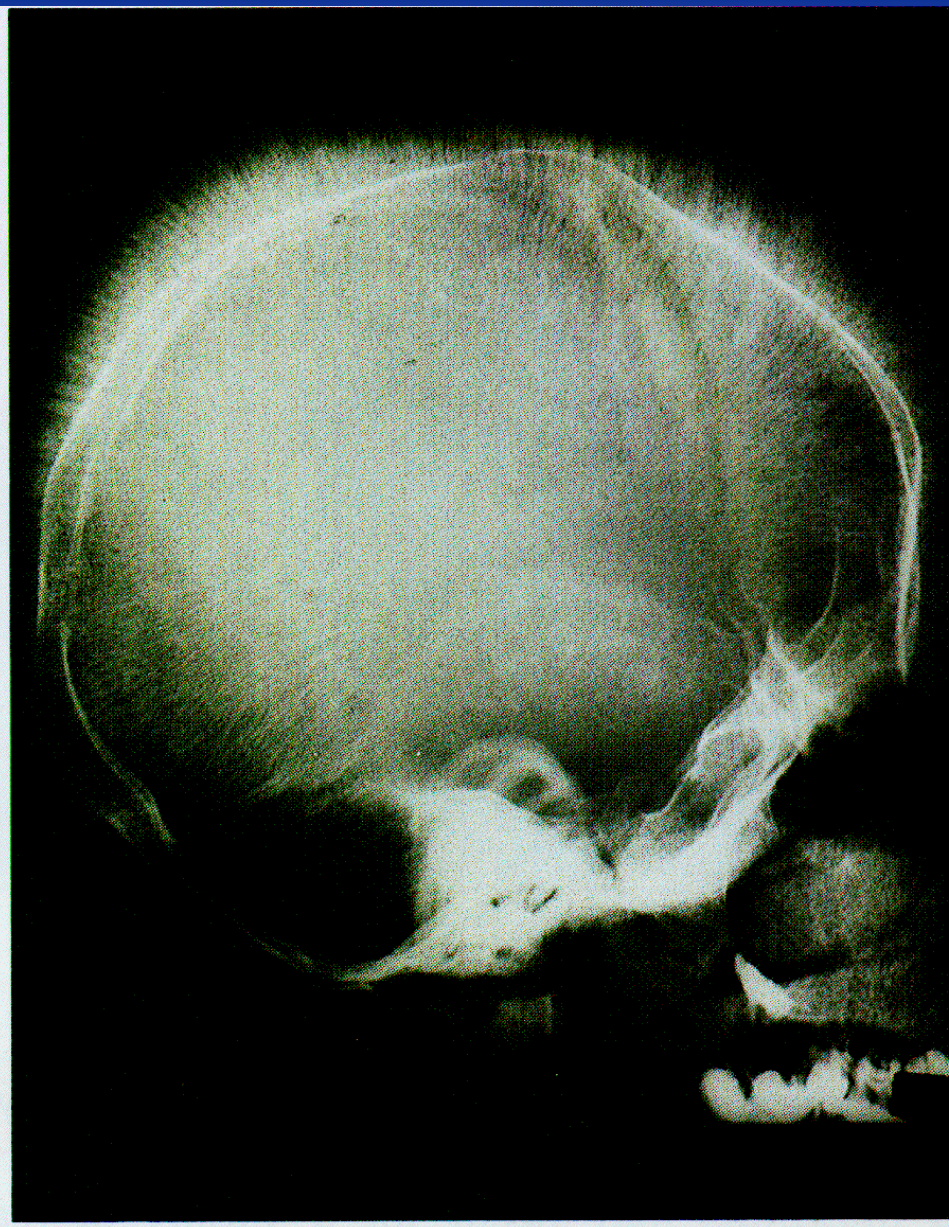
Beta thalassemia major

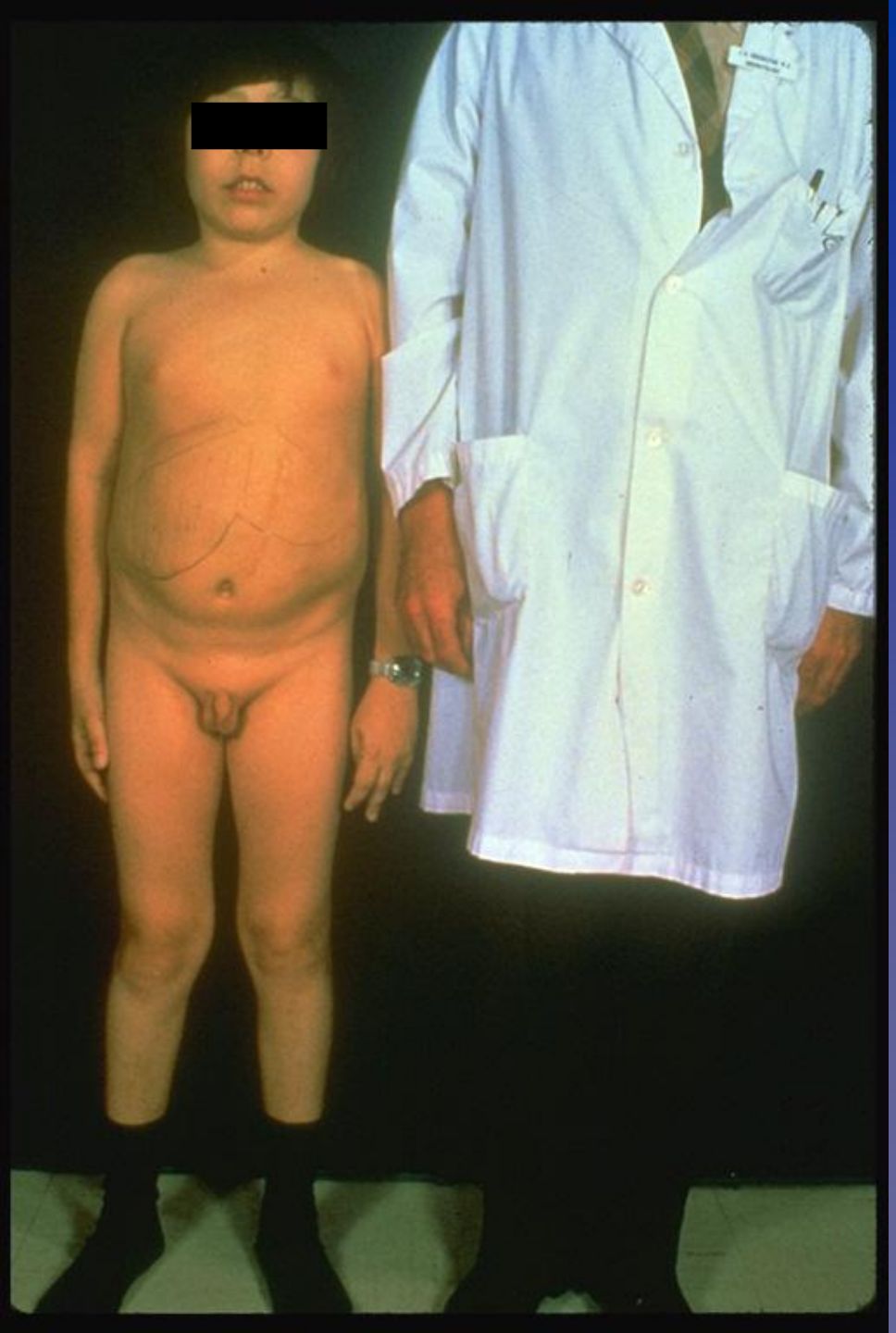
- No beta chain produced (no HbA)
- Severe microcytic anemia occurs gradually in the first year of life
- Marrow expansion
- Iron overload
- Growth failure and death

Beta thalassemia major









Beta thalassemia major
Male 18 years

Beta thalassemia major

treatment

- Transfusion
- Iron chelation
- stem cell transplant

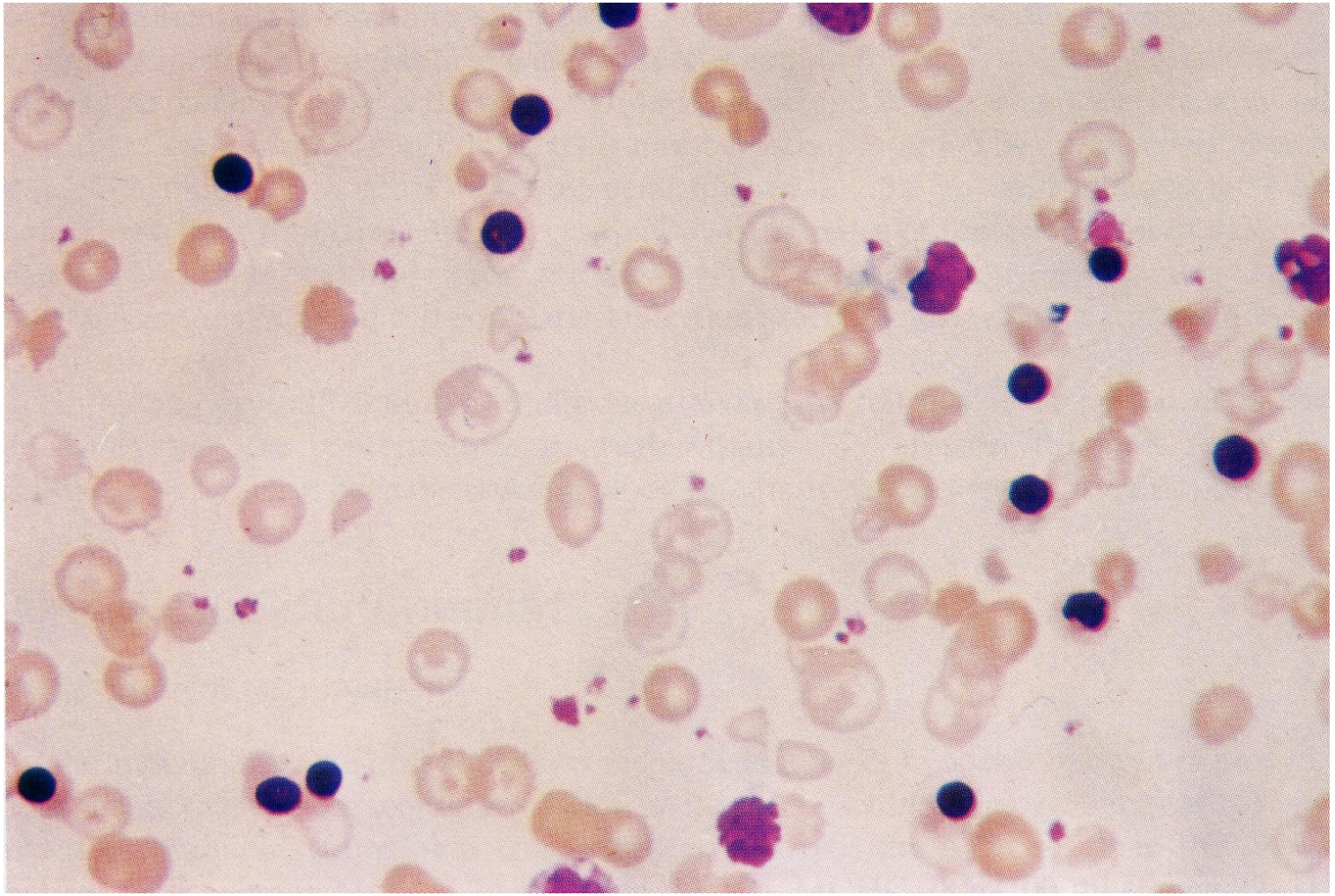
Alpha thalassemia

✂✂/ ✂✂	Normal
✂✂/ ✂-	Mild microcytosis
✂✂/- -	Mild microcytosis
✂-/- -	Hemoglobin H disease
- -/- -	Hemoglobin Barts – Hydrops Fetalis

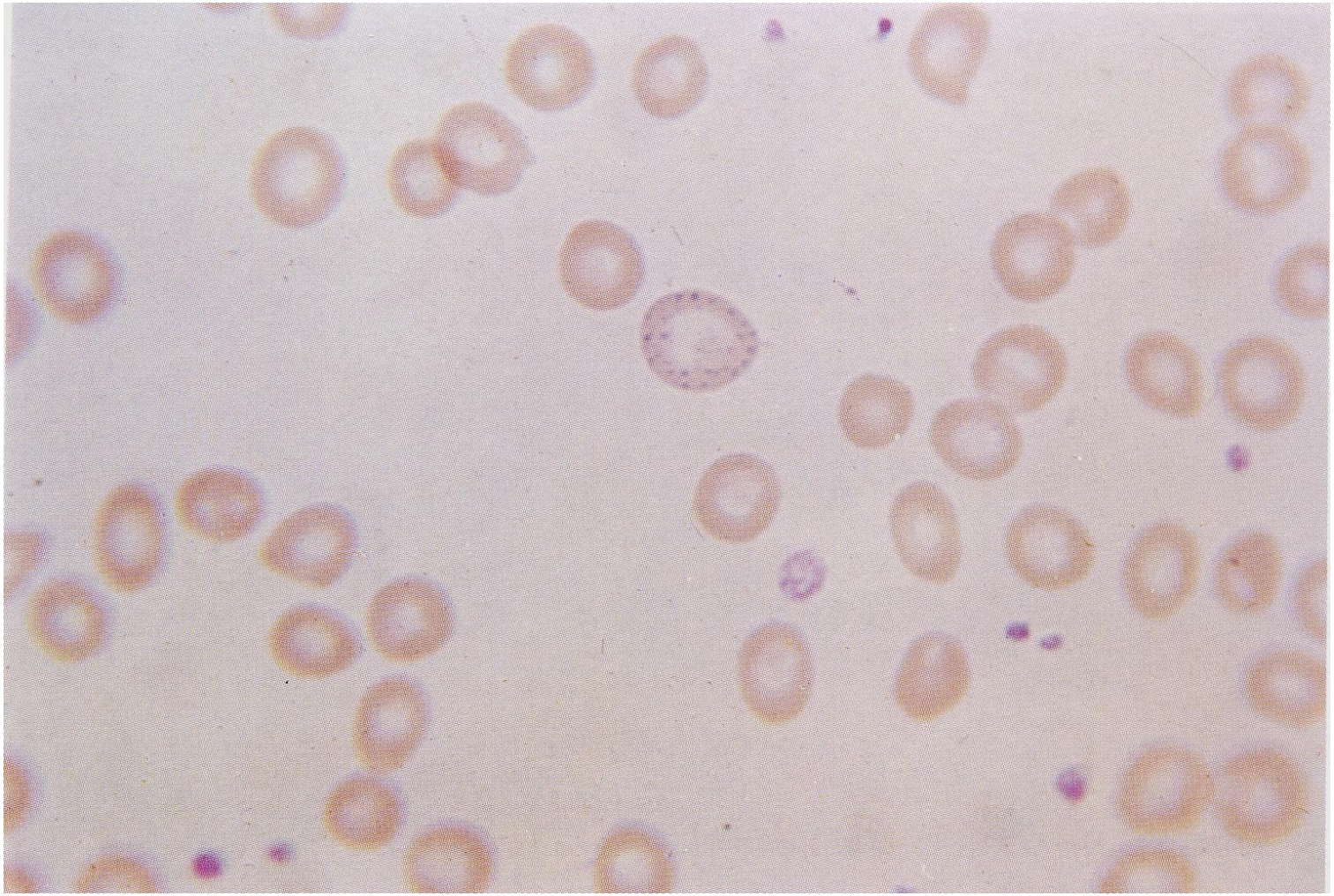
Hemoglobinopathy-antenatal diagnosis

- Test partners of heterozygous or affected individuals
- Antenatal diagnosis from DNA obtained by chorionic villus sampling, or by amniocentesis

Thalassemia major

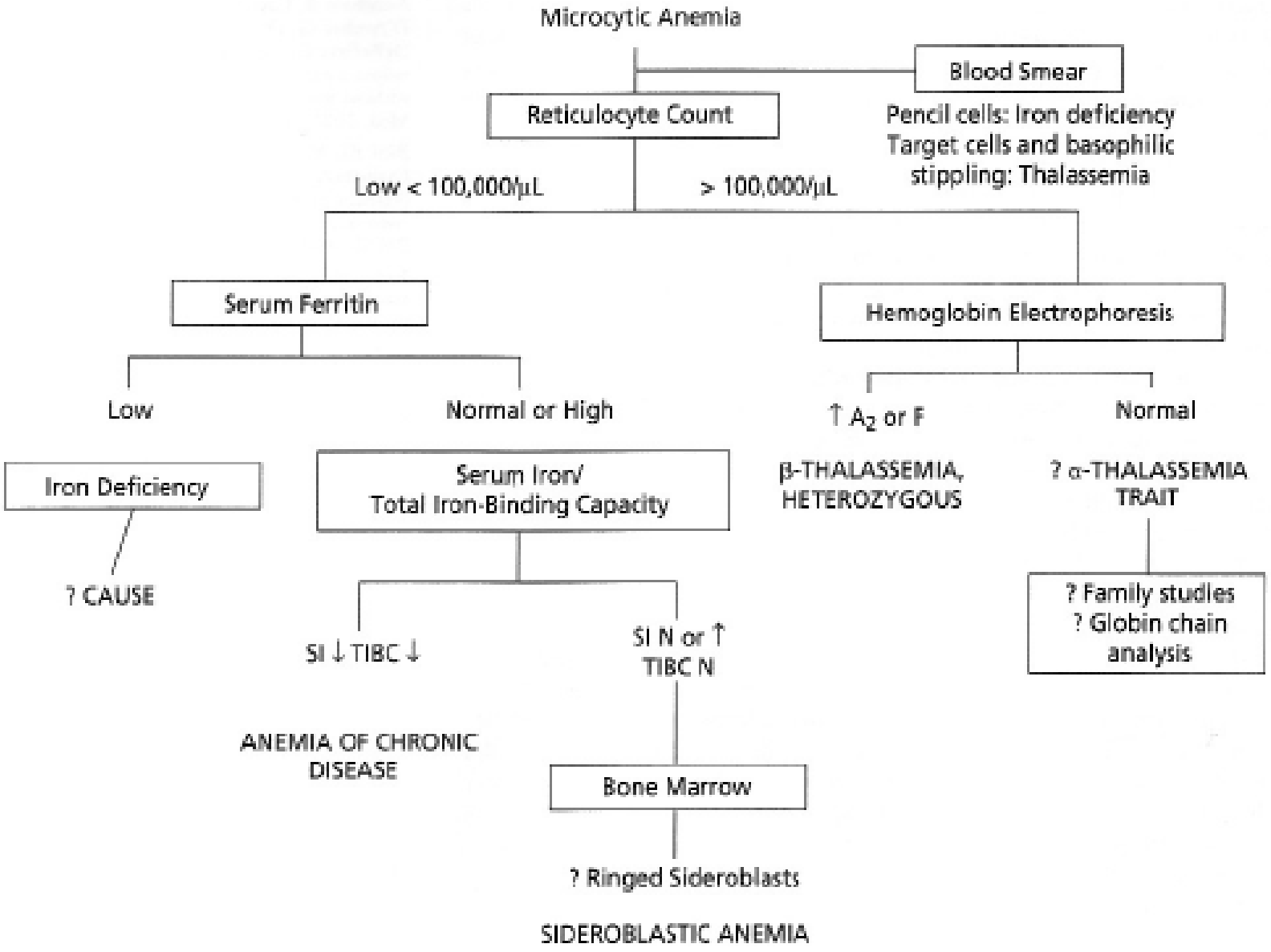


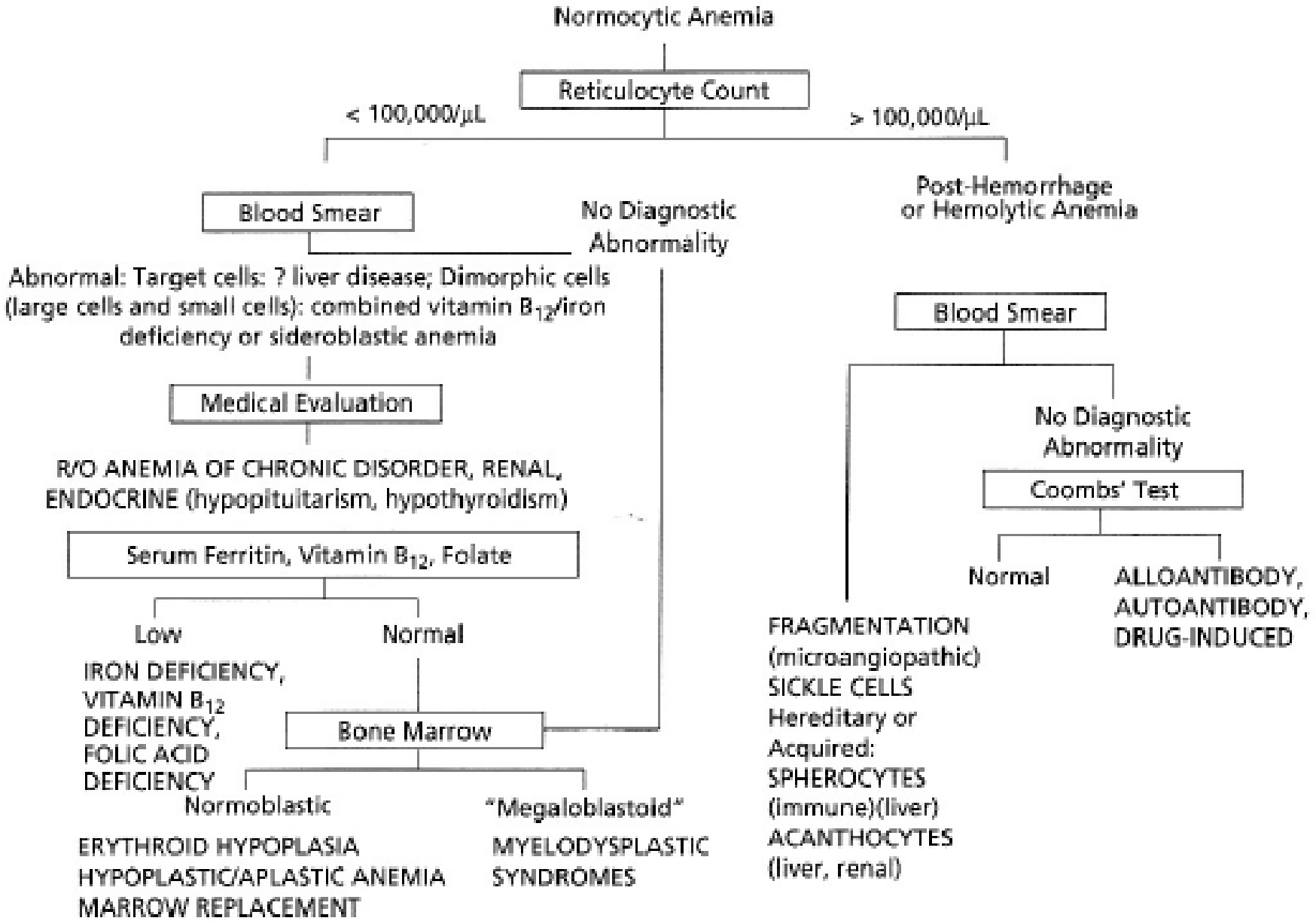
Thalassemia minor

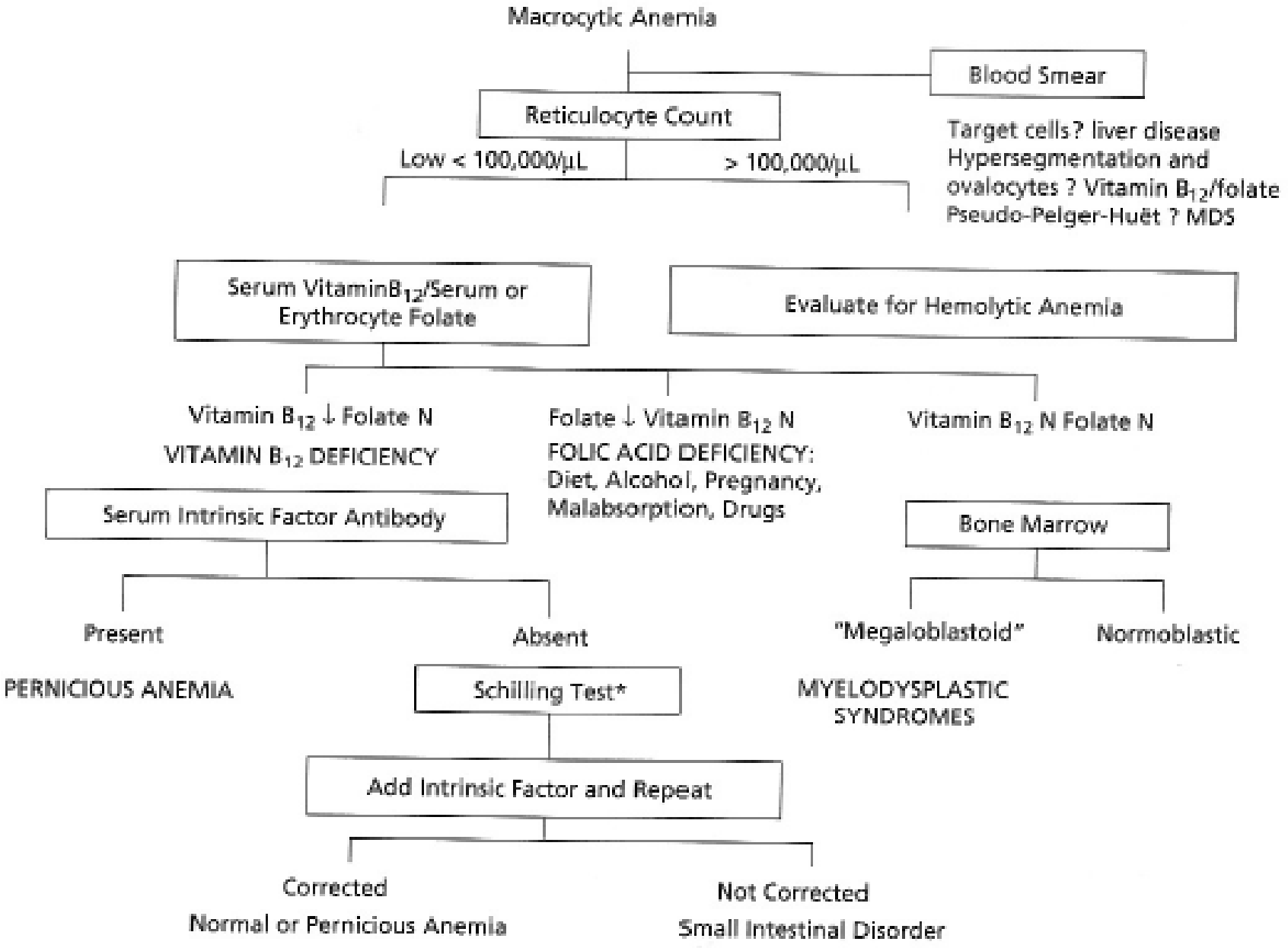


Thalassemias

- Hemoglobin Constant Spring – formed by a combination of two structurally abnormal α chains (each elongated by 31 amino acids at the COOH end) and two normal β chains.
  - The abnormal α chains are inefficiently synthesized resulting in an α thal 1 like phenotype (excess β chains)
  - Homozygous individuals have mild hypochromic, microcytic anemia similar to a mild a α thalassemia.
- Hemoglobin Lepore – a normal α chain plus a δ-β hybrid (N-terminal δ, and C-terminal β).
  - There is ineffective synthesis of the hybrid chain leading to α chain excess and the same problems seen in β thalassemia.


Microcytic Anemia
Blood Smear
Reticulocyte Count
Pencil cells: Iron deficiency
Target cells and basophilic stippling: Thalassemia
Low < 100,000/μL
> 100,000/μL
Serum Ferritin
Hemoglobin Electrophoresis
Low
Normal or High
↑ A2 or F
Normal
Iron Deficiency
Serum Iron/
Total Iron-Binding Capacity
β-THALASSEMIA, HETEROZYGOUS
? α-THALASSEMIA TRAIT
? CAUSE
SI ↓ TIBC ↓
SI N or ↑
TIBC N
? Family studies
? Globin chain analysis
ANEMIA OF CHRONIC DISEASE
Bone Marrow
? Ringed Sideroblasts
SIDEROBLASTIC ANEMIA


Normocytic Anemia
Reticulocyte Count
< 100,000/μL
> 100,000/μL
Blood Smear
No Diagnostic Abnormality
Post-Hemorrhage or Hemolytic Anemia
Abnormal: Target cells: ? liver disease; Dimorphic cells (large cells and small cells): combined vitamin B12/iron deficiency or sideroblastic anemia
Medical Evaluation
R/O ANEMIA OF CHRONIC DISORDER, RENAL, ENDOCRINE (hypopituitarism, hypothyroidism)
Serum Ferritin, Vitamin B12, Folate
Low
Normal
IRON DEFICIENCY, VITAMIN B12 DEFICIENCY, FOLIC ACID DEFICIENCY
Bone Marrow
Normoblastic
"Megaloblastoid"
ERYTHROID HYPOPLASIA HYPOPLASTIC/APLASTIC ANEMIA MARROW REPLACEMENT
MYELODYSPLASTIC SYNDROMES
Blood Smear
No Diagnostic Abnormality
Coombs' Test
Normal
ALLOANTIBODY, AUTOANTIBODY, DRUG-INDUCED
FRAGMENTATION (microangiopathic)
SICKLE CELLS
Hereditary or Acquired:
SPHEROCYTES (immune)(liver)
ACANTHOCYTES (liver, renal)


Macrocytic Anemia
Blood Smear
Reticulocyte Count
Target cells? liver disease
Hypersegmentation and ovalocytes ? Vitamin B_{12}/folate
Pseudo-Pelger-Huët ? MDS
Low < 100,000/μL
> 100,000/μL
Serum VitaminB_{12}/Serum or Erythrocyte Folate
Evaluate for Hemolytic Anemia
Vitamin B_{12} ↓ Folate N
VITAMIN B_{12} DEFICIENCY
Folate ↓ Vitamin B_{12} N
FOLIC ACID DEFICIENCY:
Diet, Alcohol, Pregnancy, Malabsorption, Drugs
Vitamin B_{12} N Folate N
Serum Intrinsic Factor Antibody
Bone Marrow
Present
Absent
"Megaloblastoid"
Normoblastic
PERNICIOUS ANEMIA
Schilling Test*
MYELODYSPLASTIC SYNDROMES
Add Intrinsic Factor and Repeat
Corrected
Normal or Pernicious Anemia
Not Corrected
Small Intestinal Disorder