Interpretation of Diagnostic Tests

Hemogram / Coagulation Studies

How to Approach Anemia ?

1. Hb: make sure anemia
2. MCV: morphology approach
3. Reticulocyte: kinetic approach

Definition of Anemia for Chinese

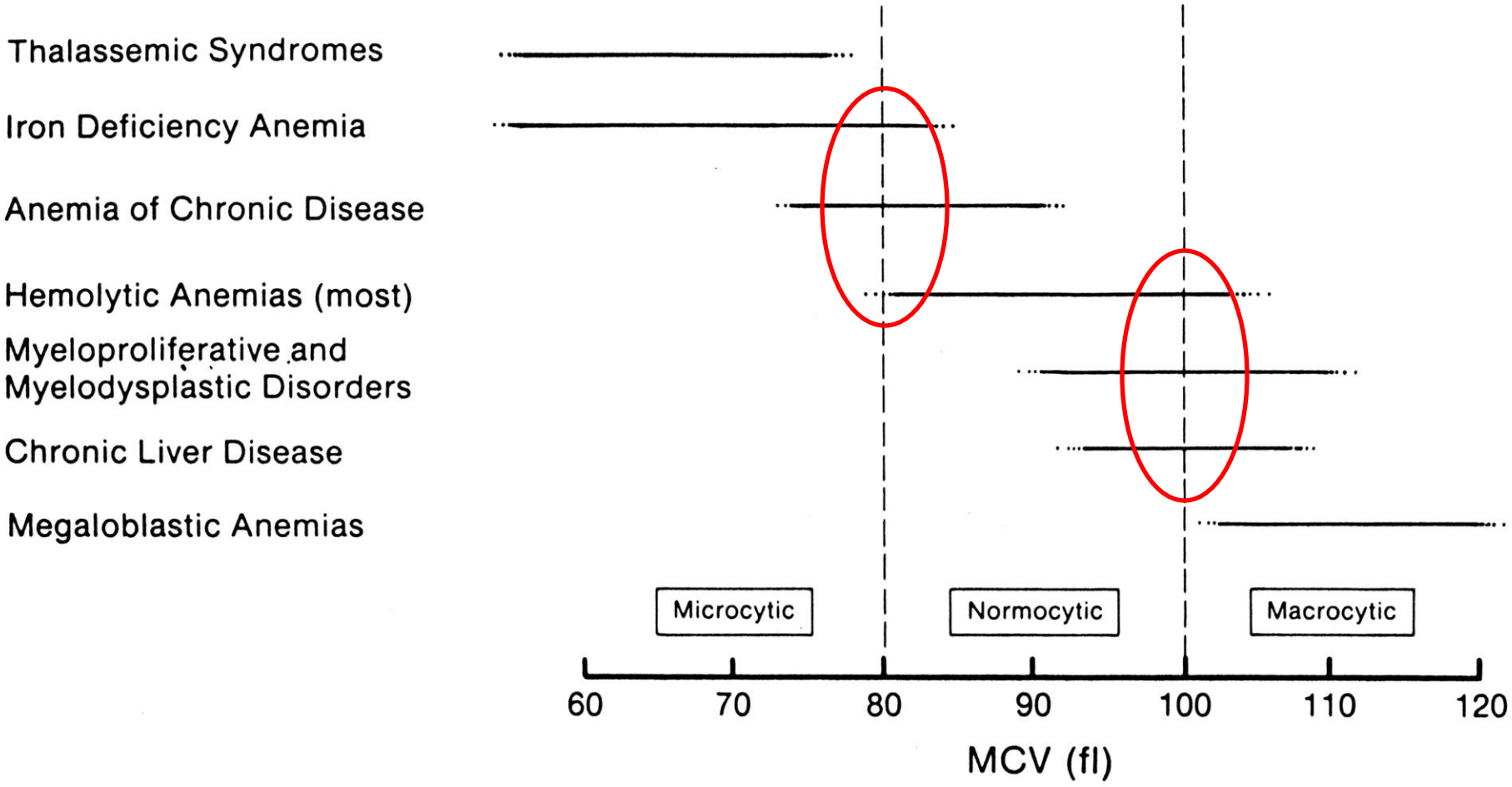
	Hemoglobin
Adult male	< 13.0 g/dL
Adult femal	< 11.0 g/dL
Children	< 11 g/dL
Newborn	< 15 g/dL

註：MCV = Hct / RBC, fL: femtoliter 10^{-15} liter

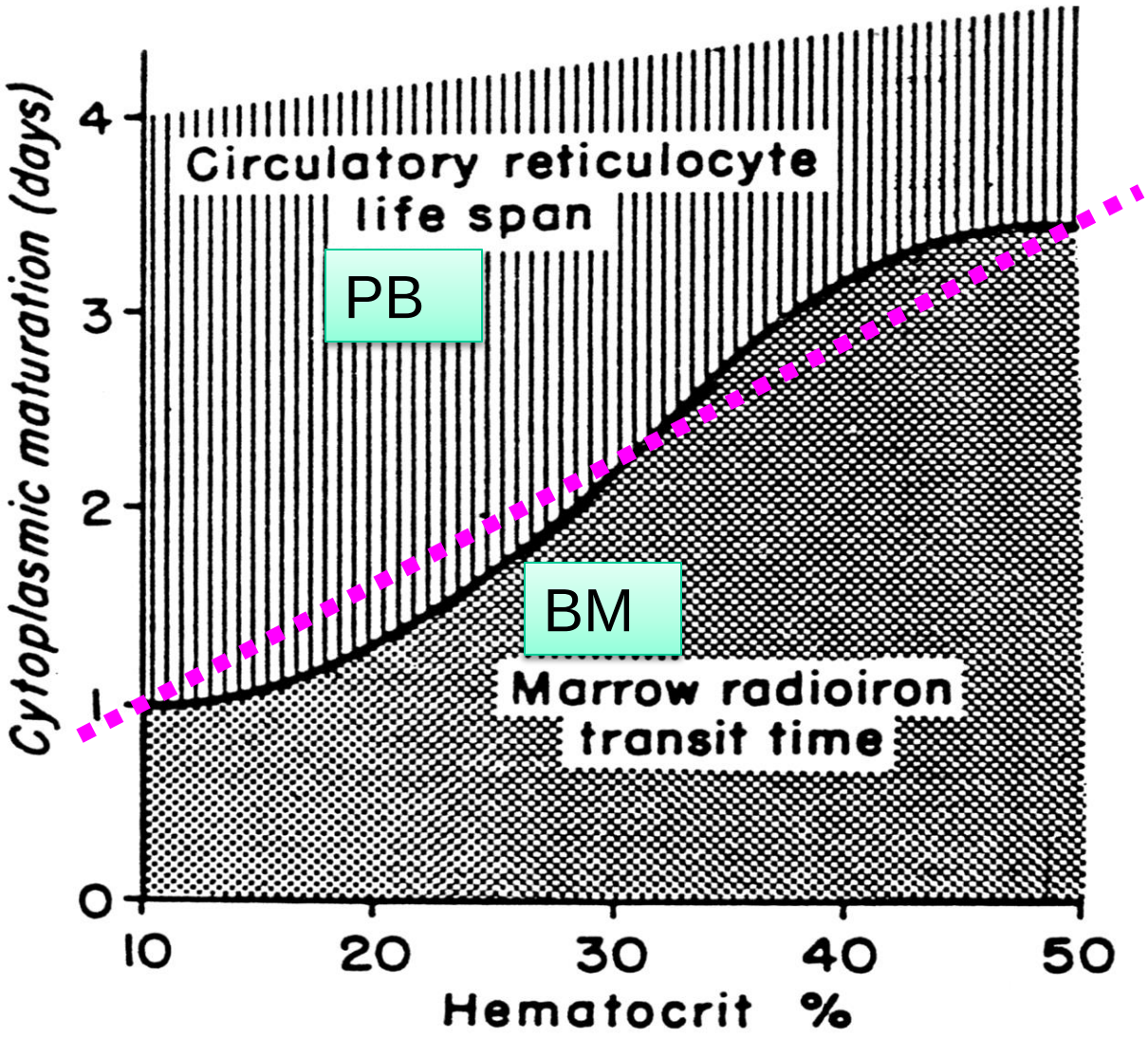
貧血分類	MCV (fl)	原因
大細胞貧血 Macrocytic anemia	100~160	
正常細胞貧血 Normocytic anemia	80~100	
小細胞貧血 Microcytic anemia	55~80	

原則：
- Disturb DNA synthesis → MCV ↑
- Disturb Hemoglobin synthesis → MCV ↓

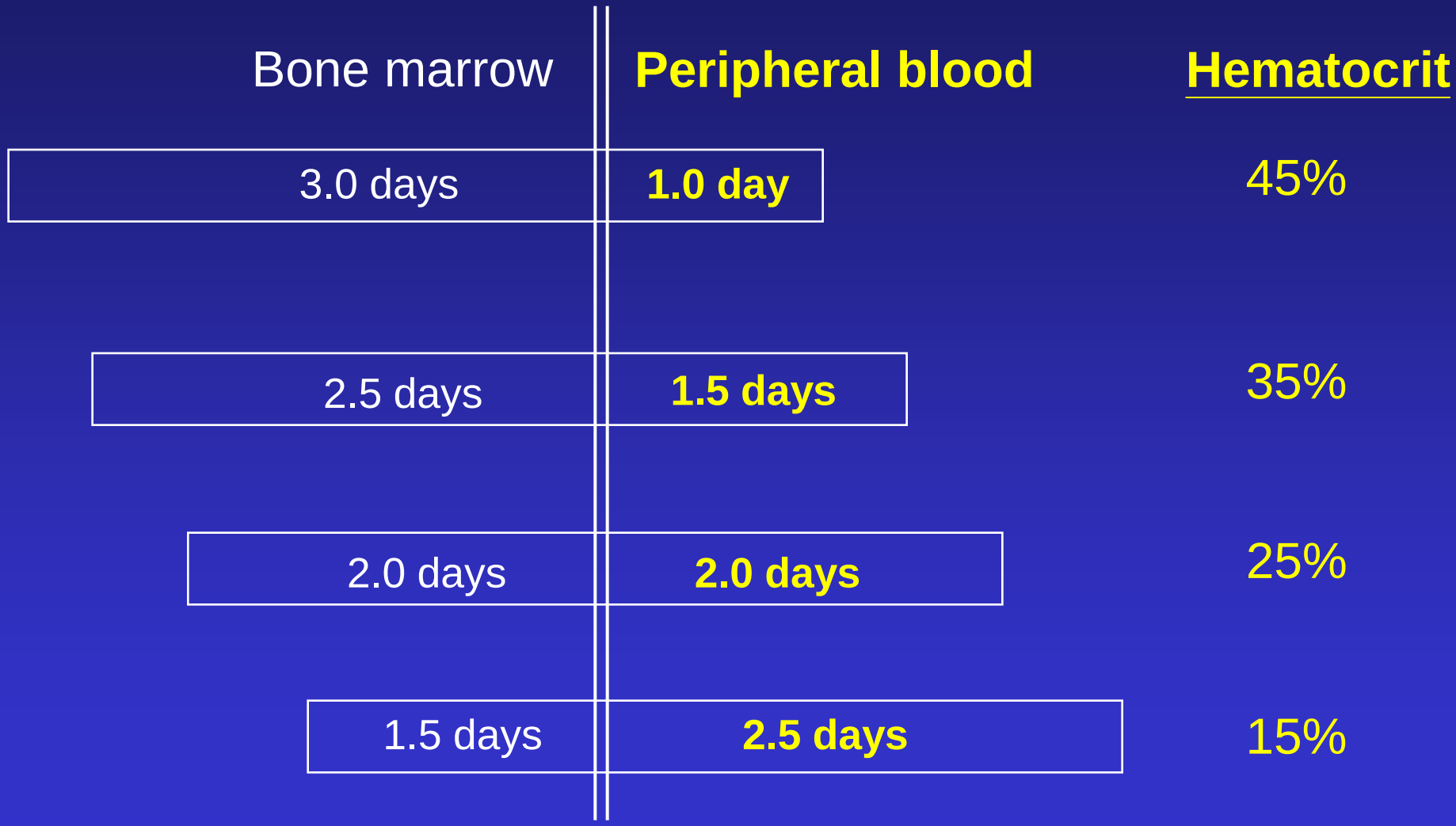
MCV Distribution in Different Anemia



Distribution of Reticulocyte vs Degree of Anemia



Reticulocyte Maturation Time in P.B.



Reticulocyte Production Index

an estimate of marrow production relative to normal

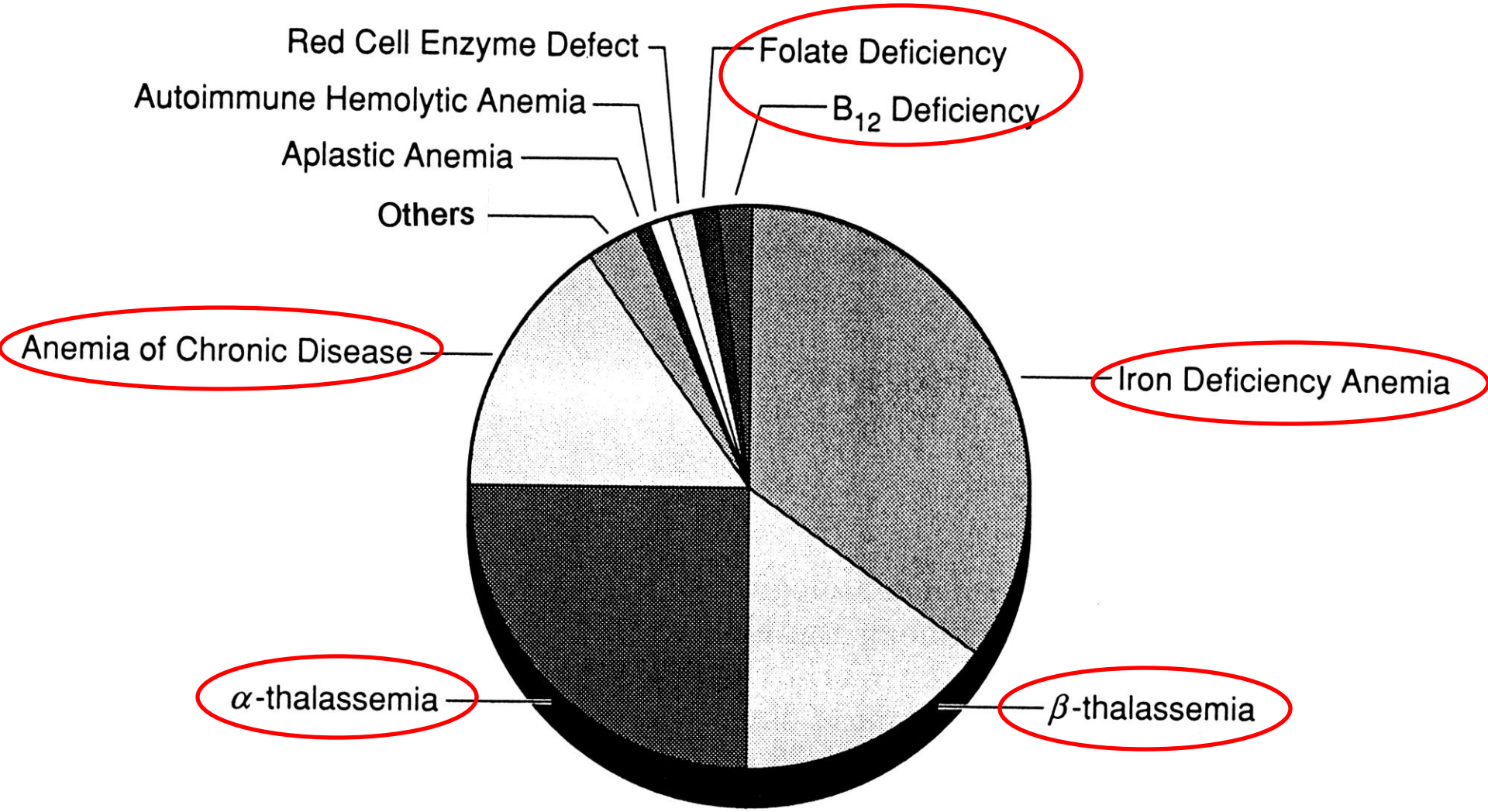
$$\text{Corrected Ret} = \text{Ret(\%)} \times \frac{\text{Hct}}{45}$$

$$\text{RPI} = \frac{\text{Corrected Ret}}{\text{Maturation index}}$$

舉例：某一貧血病患 Hct = 25%, reticulocyte count = 20%

計算 RPI= 20% X (25 / 45) ÷2.0 = 5.5

- RPI > 3 (2.5): hemolytic anemia
- Normal: about 0.5~1.5
- RPI < 0.5: ineffective erythropoiesis or marrow hypofunction


Red Cell Enzyme Defect
Folate Deficiency
Autoimmune Hemolytic Anemia
B_{12} Deficiency
Aplastic Anemia
Others
Anemia of Chronic Disease
Iron Deficiency Anemia
α-thalassemia
β-thalassemia

Stages in Development of Iron Deficiency

I	II	III	IV
Loss of storage iron	Loss of circulating iron	Decrease of Hb production	Decrease of tissuse iron

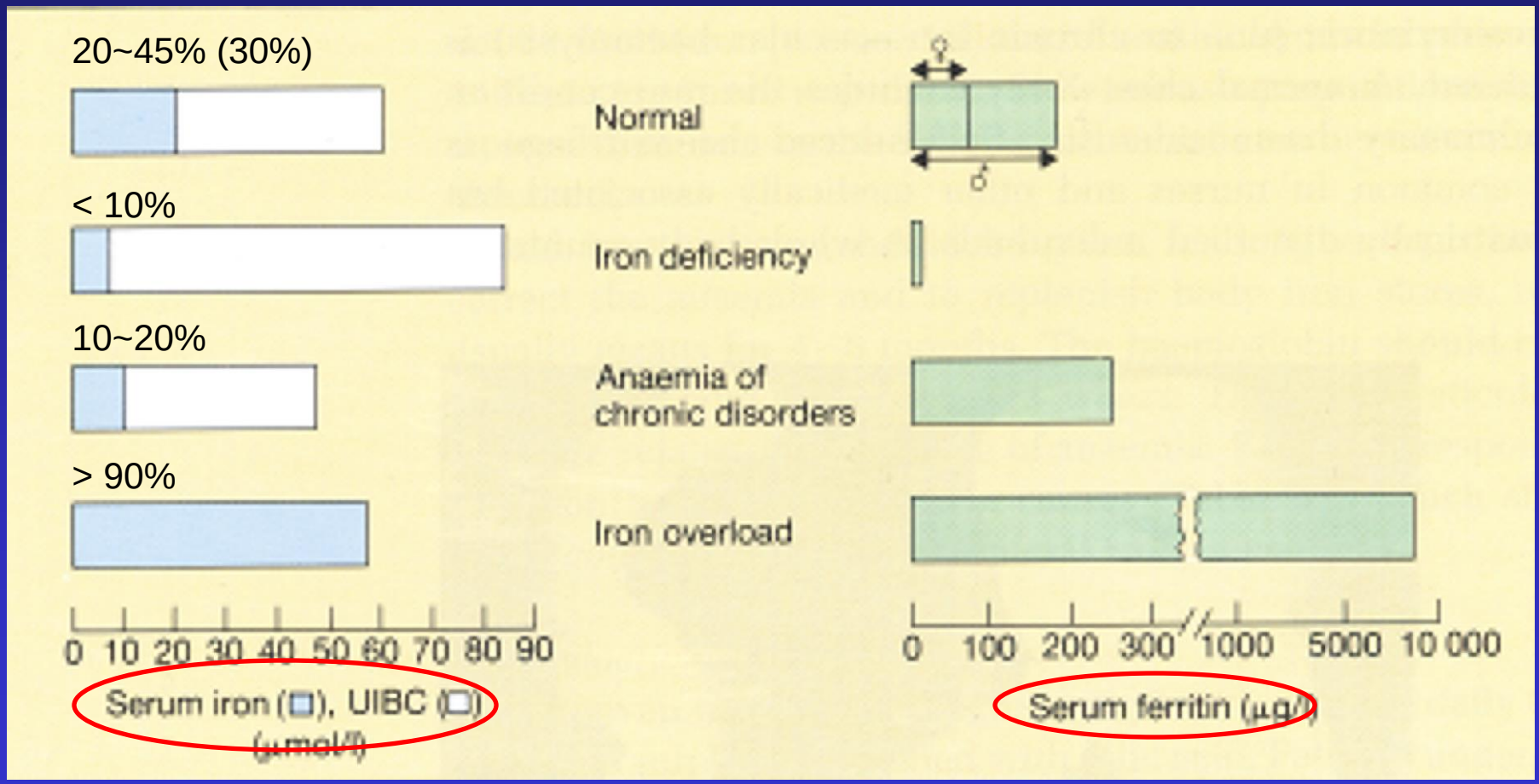
↓ serum ferritin →

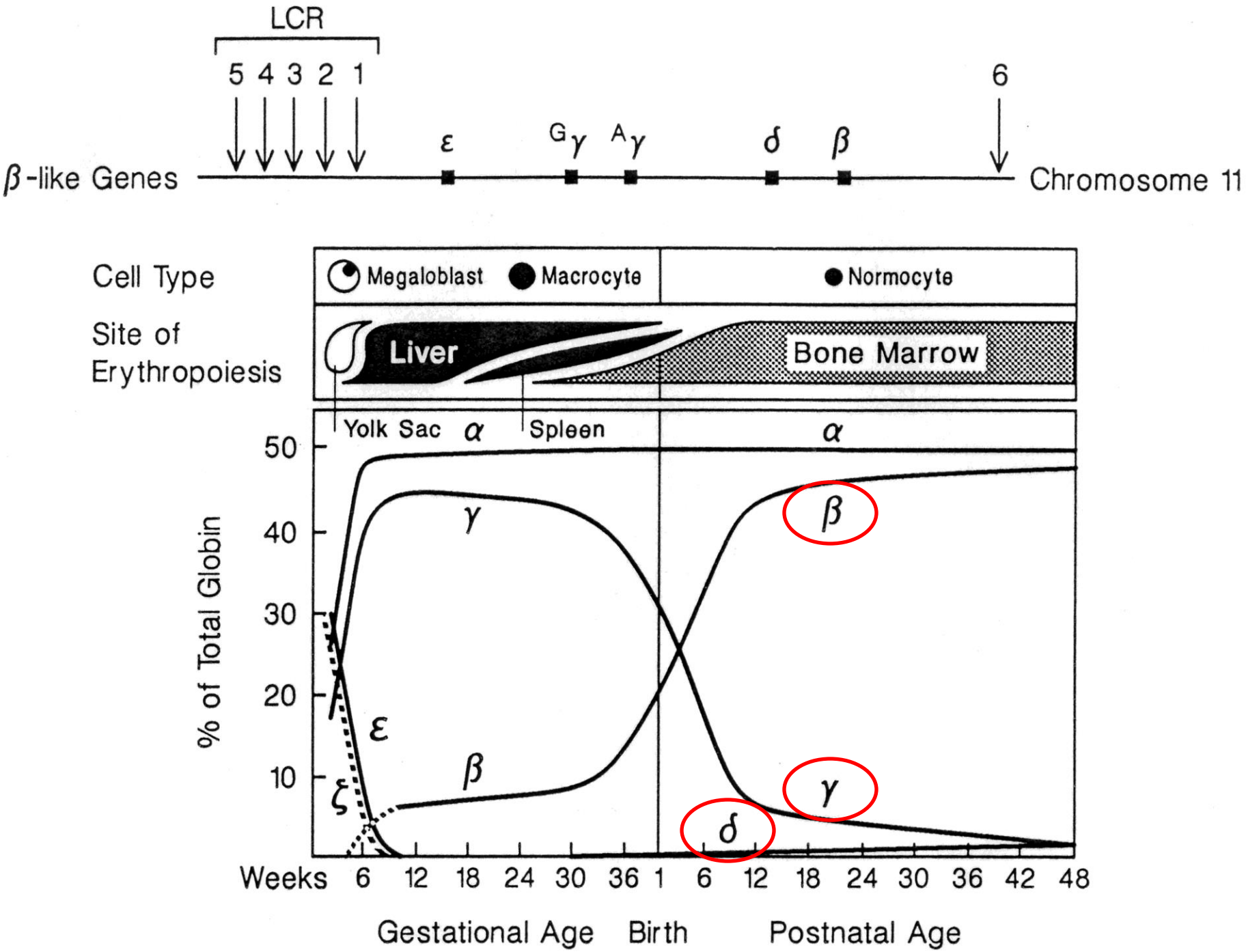
↓ transferrin saturation →

anemia & ↓ MCV →

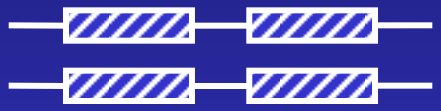
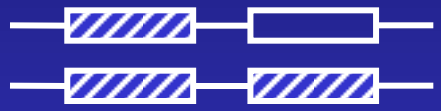
atrophic tongue
nail deformity
pica behavior

Transferrin saturation (%) = serum iron / total iron binding capacity




LCR
5 4 3 2 1
6
ε
Gγ
Aγ
δ
β
β-like Genes
Chromosome 11
Cell Type
Megaloblast
Macrocyte
Normocyte
Site of Erythropoiesis
Liver
Bone Marrow
Yolk Sac
α
Spleen
α
% of Total Globin
50
40
30
20
10
γ
ε
ζ
β
β
γ
δ
Weeks
6
12
18
24
30
36
1
6
12
18
24
30
36
42
48
Gestational Age
Birth
Postnatal Age

Thalassemia Major or Intermediate

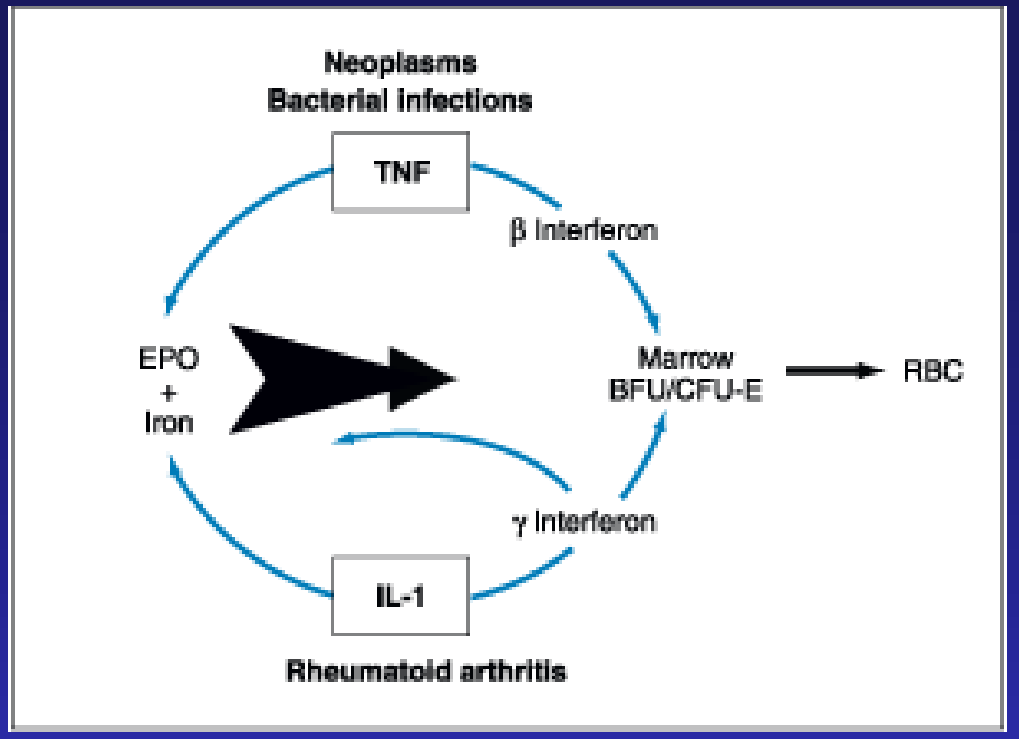
	Fetus	Adult
Normal	$\alpha_2\gamma_2$ (Hb F)	$\alpha_2\beta_2$ (Hb A)
α-thalassemia	γ_4 (Hb Bart's)  high O_2 affinity, unable to transport O_2 → hydrops fetalis	β4 (Hb H)  β4 is unstable, intracellular precipitation (inclusion bodies) → hemolysis (major)
β-thalassemia	$\alpha_2\gamma_2$ (Hb F) (health fetus)	$\alpha_2\gamma_2$ (Hb F) ↑ $\alpha_2\delta_2$ ($Hb A_2$) ↑ accumulation of α-chain in RBC precursor → ineffective erythropoiesis (major)

Anemia of Chronic Diseases

Incidence: 15~20% of anemia

Causes:

neoplastic disorders
chronic infectious disorders
chronic inflammatory disorders



Mechanisms:

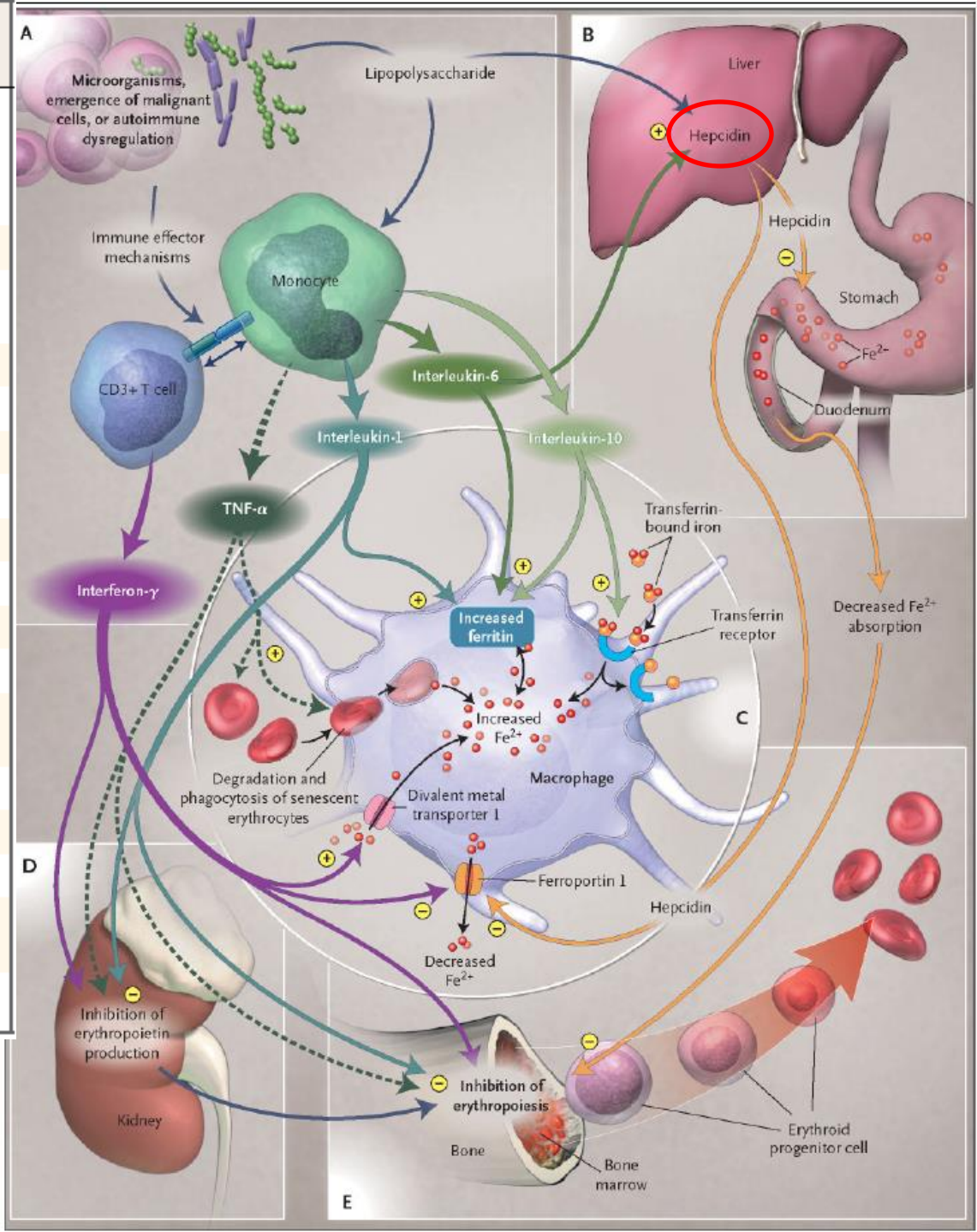
Multiple cytokines (TNF, IL-1, IL-6, βorγ-IFN, etc.) synergistically inhibit (partially mediated by excess hepcidin production from liver) erythropoiesis, EPO production & reticulendothelial iron release.

Characteristics:

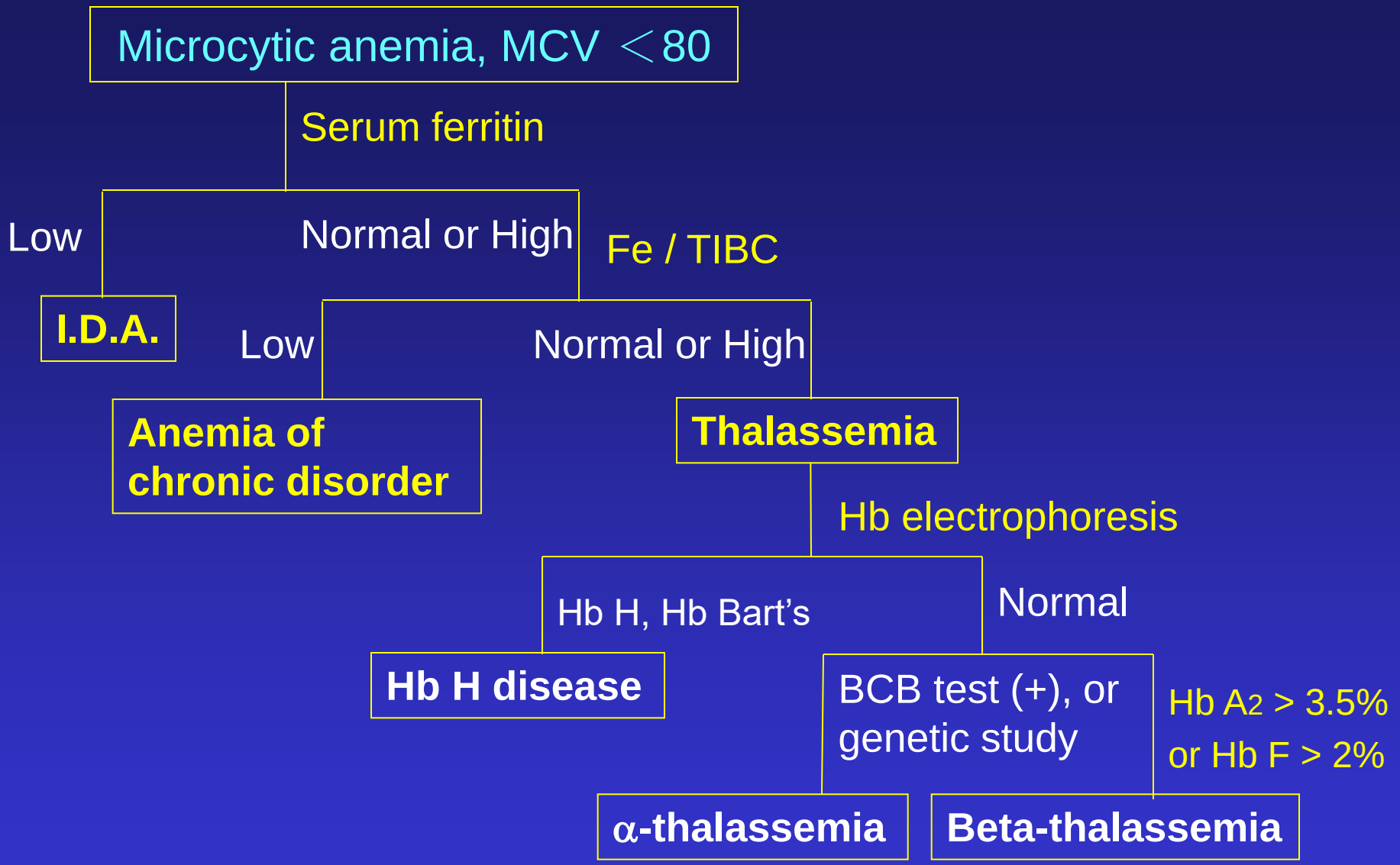
1. Hb level: ~7 to 10 g%; low reticulocyte count
2. normal or ↑ serum ferritin, and ↑ bone marrow iron storage
3. ↓serum iron and ↓TIBC
   transferrin saturation: 10~20%

Table 1. Underlying Causes of Anemia of Chronic Disease.

Associated Diseases	Estimated Prevalence*
	percent
Infections (acute and chronic)	18–95[8-10]
Viral infections, including human immunodeficiency virus infection	
Bacterial	
Parasitic	
Fungal	
Cancer†	30–77[9,12-14]
Hematologic	
Solid tumor	
Autoimmune	8–71[5,9,15,16]
Rheumatoid arthritis	
Systemic lupus erythematosus and connective-tissue diseases	
Vasculitis	
Sarcoidosis	
Inflammatory bowel disease	
Chronic rejection after solid-organ transplantation	8–70[17-19]
Chronic kidney disease and inflammation	23–50[20-22]



From: NEJM 352:1011, 2005


Microcytic anemia, MCV ＜80
Serum ferritin
Low
Normal or High
Fe / TIBC
I.D.A.
Low
Normal or High
Anemia of chronic disorder
Thalassemia
Hb electrophoresis
Hb H, Hb Bart's
Normal
Hb H disease
BCB test (+), or genetic study
Hb A2 > 3.5%
or Hb F > 2%
α-thalassemia
Beta-thalassemia

Mathematical Indices for Discriminating IDA from Thalassemia

	Indices	Formula	Cut-off value	Sensitivity	Specificity	Youden's index
◉	RBC	RBC	5 million	84~96%	84~96%	74~82
	RDW index	MCV × RDW / RBC	220	75~100%	75~100%	63~80
	Green & King index	$(MCV^2 \times RDW)/100 \times RBC$	65	70~97%	70~97%	67
◉	Mentzer index	MCV / RBC	13	62~89%	62~89%	48~65
	Srivastava index	MCH / RBC	3.8	60~91%	60~91%	37~50
	Shine & Lal index	$(MCV^2) \times (MCH/100)$	1530	11~100%	11~100%	0~11

Pediatrics International 2002;44:612; Europ J Haematol 2007;78:524

Laboratory Signs of Hemolysis

1. unconjugated hyperbilirubinemia
   ↑ indirect bilirubin
2. relatively increased serum AST (GOT)
   ↑ AST >> ALT
3. ↑↑ serum LDH
4. ↑↑ corrected reticulocyte count
   reticulocyte production index > 3
5. ↓↓ serum haptoglobin

Conditions influencing Haptoglobin Level

Low haptoglobinemia

1. Hemolytic anemia (either intra- or extra-vascular)
2. Ineffective erythropoiesis, megaloblastic anemia
3. Hemorrhage into tissue
4. Severe liver disease and cirrhosis of liver
5. Congenital deficiency (about 2% in normal Causasians)
6. Newborn

High haptoglobinemia

1. Chronic infection
2. Trauma, tissue damage
3. Surgery
4. Malignancy
5. Collagen diseases (RA, SLE)
6. Steroid or estrogen therapy, or oral pills
7. Pregnancy

Intravascular vs Extravascular Hemolysis

	Intravascular	Extravascular
↑serum bilirubin (T/I)		
↑serum LDH		
↓serum haptoglobin		
↑urine urobilinogen	++	++
Hemoglobinuria	+++	-
Hemosiderinuria	+++	-
Fragmented RBC	++	-
Spherocytes	-	+

Urine routine:

Occult blood (++)~(++++)

but, Sediment RBC 0-5/HPF

Classification of Hemolytic Anemia

Extravascular Hemolysis

1. Autoimmune HA
2. Hereditary spherocytosis
3. Pyruvate kinase deficiency

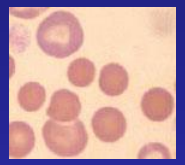
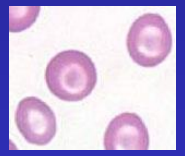
Intravascular Hemolysis

1. ABO mismatched blood transfusion
2. Paroxysmal nocturnal hemoglobinuria
3. G6PD deficiency with oxidant stress
4. Microangiopathic HA (TTP, DIC, etc.)
5. Some autoimmune HA
6. Some drug- or infection-induced HA

Laboratory Tests to Find the Cause of Hemolytic Anemia

1. Peripheral blood smear:
   polychromatic, poikilocytes, normoblast, Heinz bodies, spherocyte, elliptocytes, fragmented RBC
2. Coombs' test (direct): Immune mechanism
   Cryoglobulin, cold hemagglutinin: mycoplasma infection,
   Antinuclear factor, complement C3, C4: SLE
   Immunofixation electrophoresis / immunoelectrophoresis
3. Signs of **intravascular** hemolysis:
   Hemoglobinuria
4. Sugar water test (screening): PNH
   acid-Ham test, anti-CD55, CD59, CD16, CD66 (confirmatory)
5. Osmotic fragility test: Spherocytosis
6. G6PD level
7. Hb electrophoresis, BCB (Hb H) test

RBC morphology in Diagnosis of HA

Morphology		Cause	Syndrome
Spherocytes		Loss of membrane	Hereditary spherocytosis, Autoimmune warm HA, Burns, Chemical injury to RBC
Target cells		↑ surface/volume	Thalassemia, Liver disease
Schistocytes (fragmented)		Traumatic disruption of membrane	Microangiopathic anemia (DIC, TTP), Prosthetic valve
Heinz bodies	 	Precipitated abnormal Hb	Unstable Hb

Causes of Macrocytic Anemia

I. MCV > 115

Megaloblastic Anemia

Vitamin B_{12} or Folic acid deficiency

II. MCV 100 - 110

Reticulocytosis: Hemolysis or Blood loss

Increased Membrane Surface

Liver disease or Postsplenectomy

Alcoholism (90% of patients)

Hypothyroidism (50% of patients)

Drugs: 6-MP, methotrexate, etc.

Primary Hematological Disorders

Aplastic anemia, Myelodysplastic syndrome, Leukemia, Myeloproliferative disease, Multiple myeloma, etc.

Megaloblastic Anemia

Mechanism: impaired DNA Synthesis

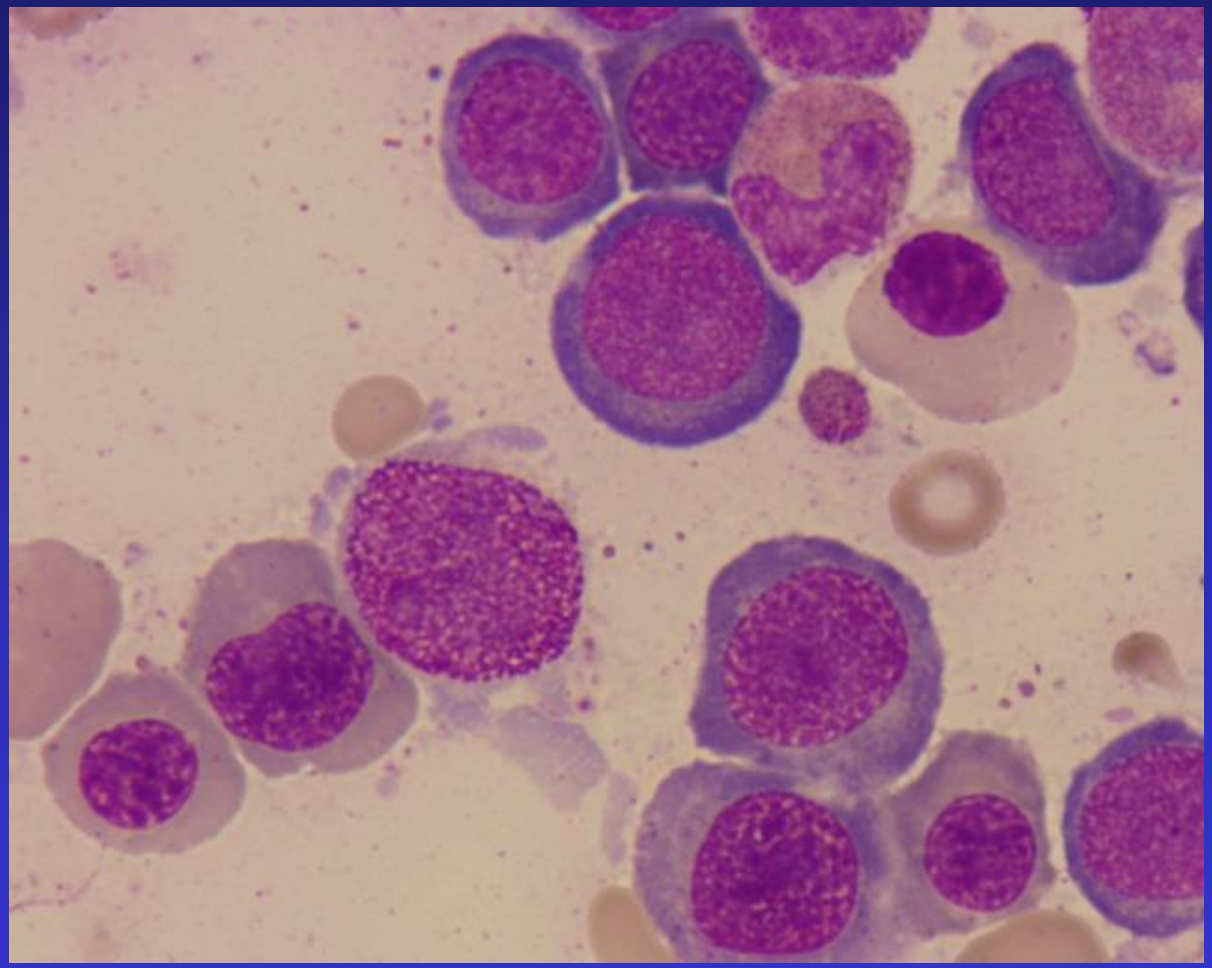
Laboratory findings

1. PB：
   pancytopenia, reticulocyte↓,
   MCV↑, ovalocyte,
   hypersegmented neutrophils
2. Serum indirect bilirubin↑, LDH↑, haptoglobin↓
3. BM：Megaloblastic hyperplasia

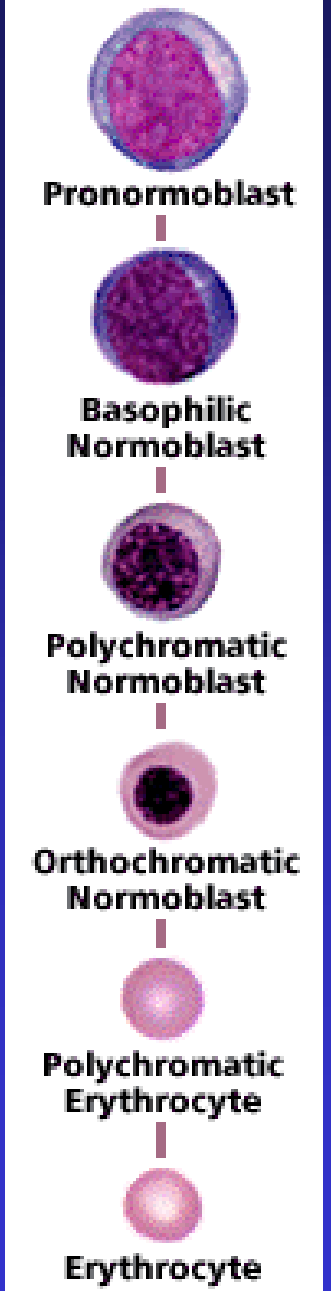
Causes:

1. Vit. B_{12} deficiency：
   pernicious anemia, gastrectomy, vegetarian
2. Folate deficiency：poor dietary intake
3. (Drugs：methotrexate, other chemotherapy agents)

Megaloblastic Change



Normal



Cause of Pancytopenia

- P: PNH
- A: Aplastic anemia
- N: Neoplasm / Near neoplasm (including MDS)
- C: Cirrhosis / Connective tissue disease
- Y: Vit B_{12} / Folic acid
- T: Toxin / Drug
- O: Overwhelming sepsis (Hemophagocytic syndrome) / Others

Aplastic Anemia

	P.B.			B.M.
	RBC	Neutrophil	Platelet	
Aplastic anemia	Hb< 10	< 1500	< 50K	
Severe aplastic anemia	Reticulo-cyte < 20K	< 500	< 20K	Cellularity < 25%, or 25-50% with < 30% residual hemopoietic cells
Very severe aplastic anemia	Reticulo-cyte < 20K	< 200	< 20K	Cellularity < 25%, or 25-50% with < 30% residual hemopoietic cells

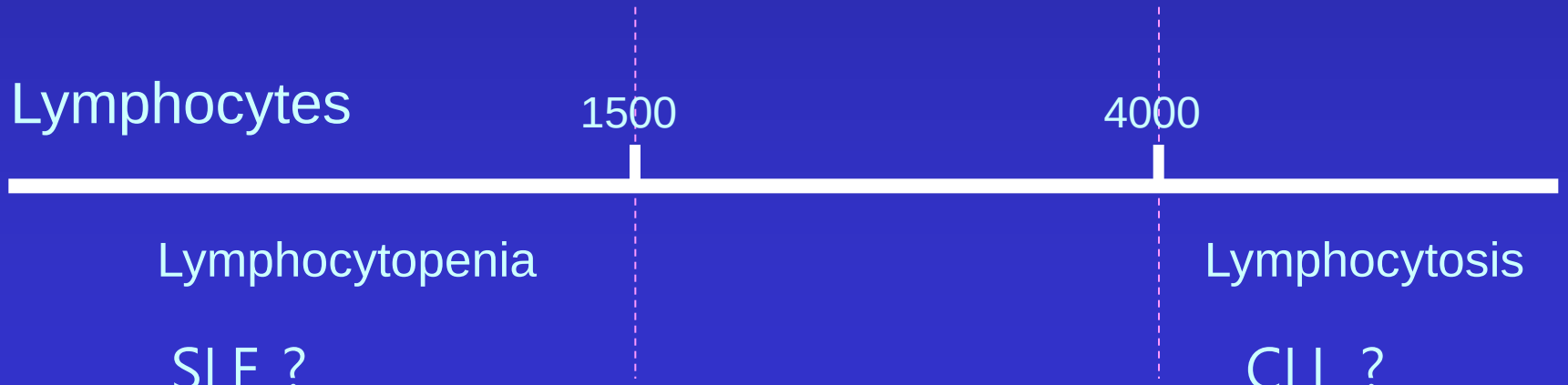
WBC

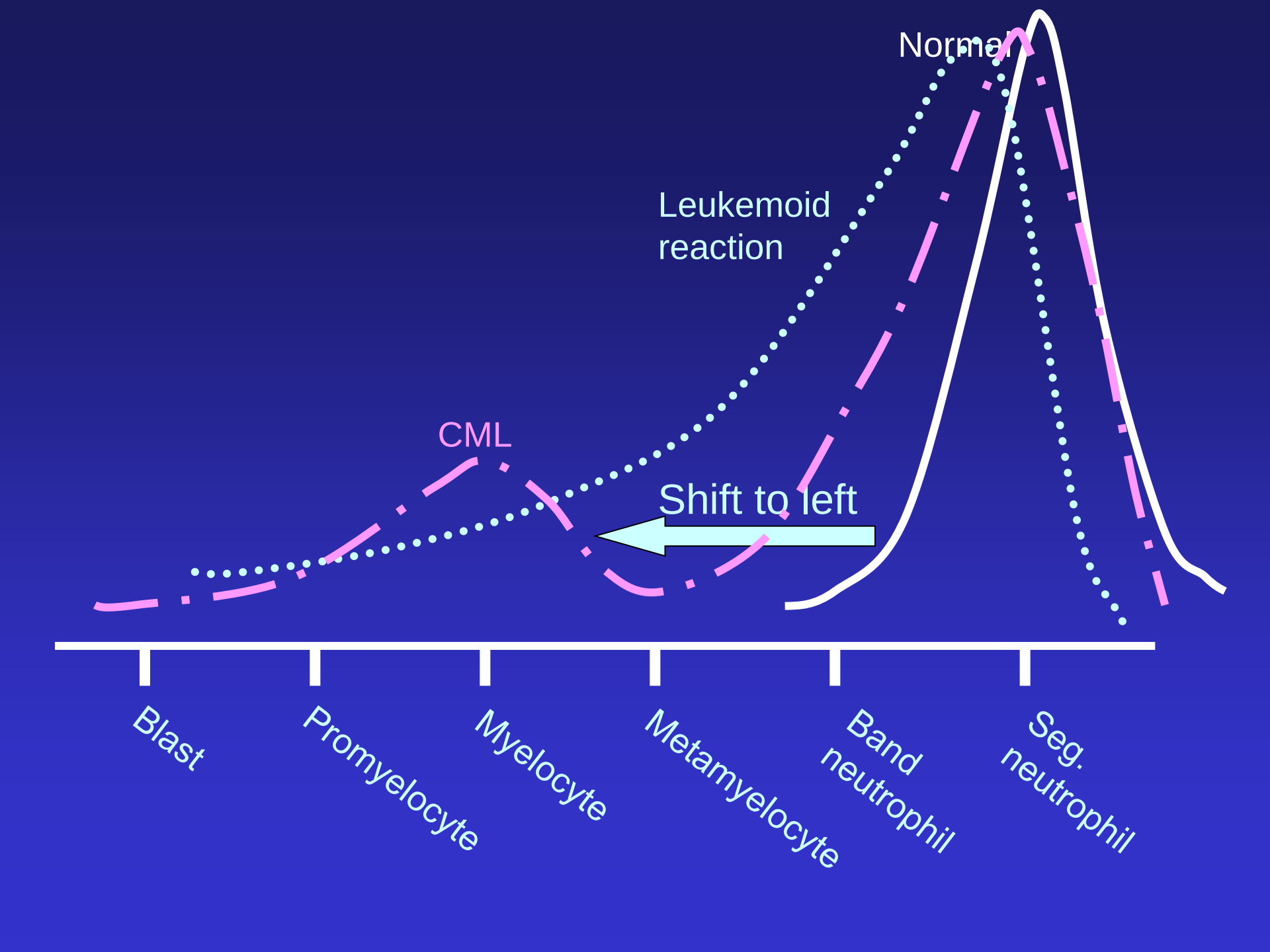
Absolute Count

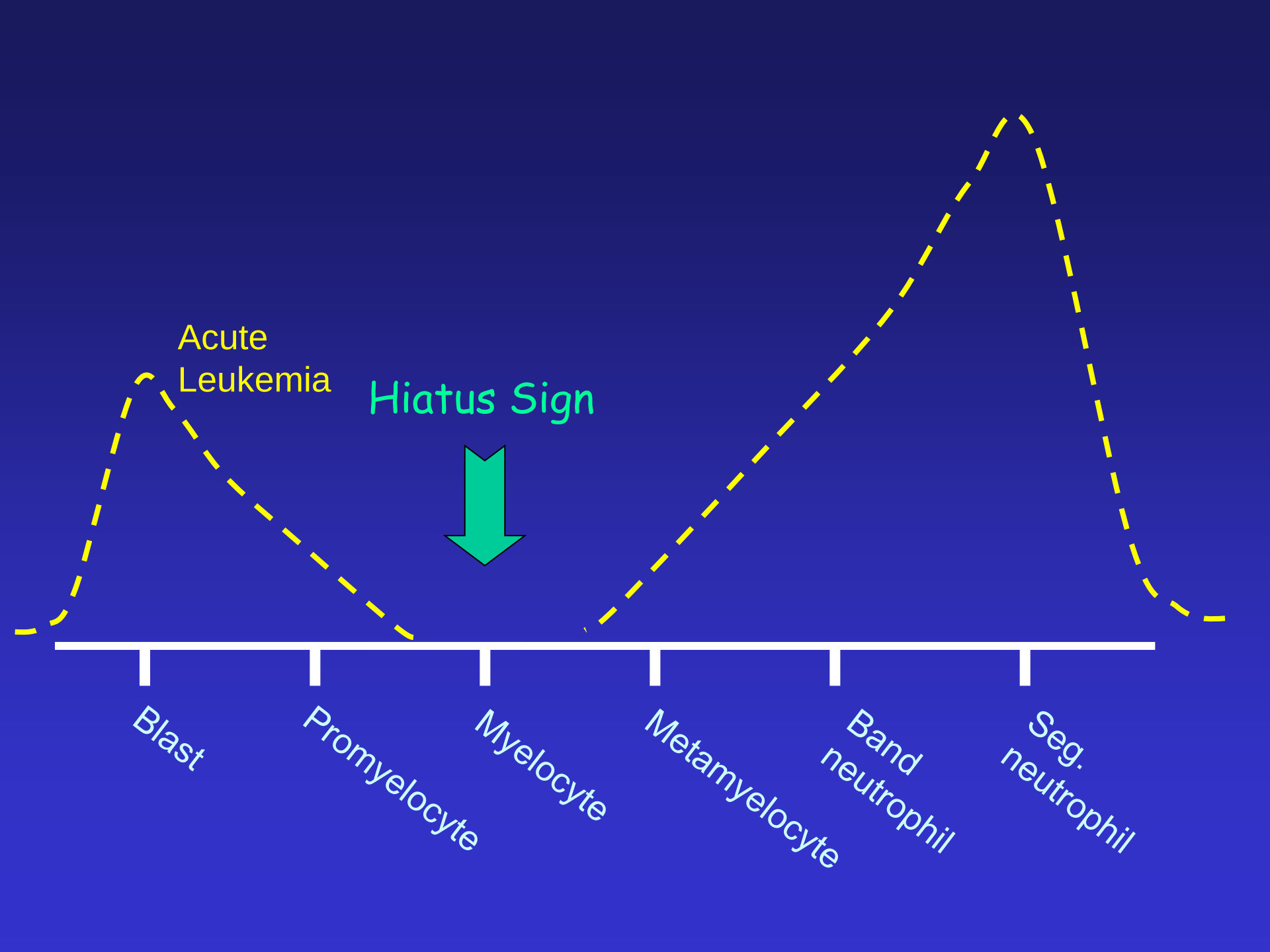
Neutrophils

ANC = WBC × (Seg% + Band%)

Lymphocytes




Normal
Leukemoid reaction
CML
Shift to left
Blast
Promyelocyte
Myelocyte
Metamyelocyte
Band neutrophil
Seg. neutrophil


Acute
Leukemia
Hiatus Sign
Blast
Promyelocyte
Myelocyte
Metamyelocyte
Band
neutrophil
Seg.
neutrophil

Leuko-erythroblastosis (myelophthisic sign)

Definition: anemia with space-occupying lesions of the bone marrow that cause bone marrow suppression with immature cells of the erythrocytic and myeloid series in the circulation.

P.B. smear findings:

1. Shifting to left (WBC)
2. Normoblasts

Pathophysiology:

Infiltration of marrow by ectopic cells$\rightarrow$ disruption of marrow / blood barrier$\rightarrow$ immature, differentiating marrow cells into P.B.

Causes:

1. Metastatic cancers
2. Hematological maligancies
3. Myelofibrosis
4. Disseminated TB, or granulomatosis

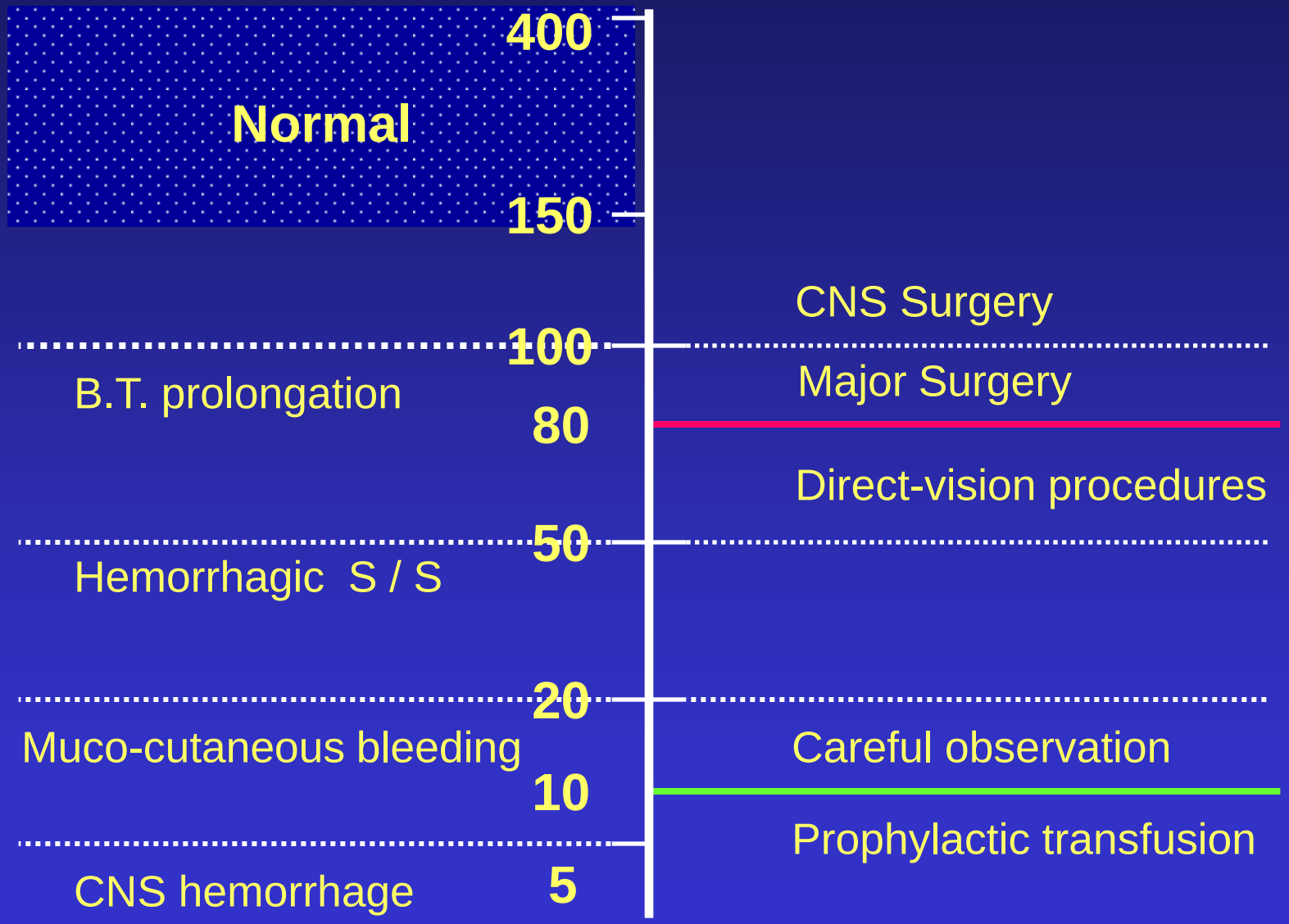
Bleeding Manifestations

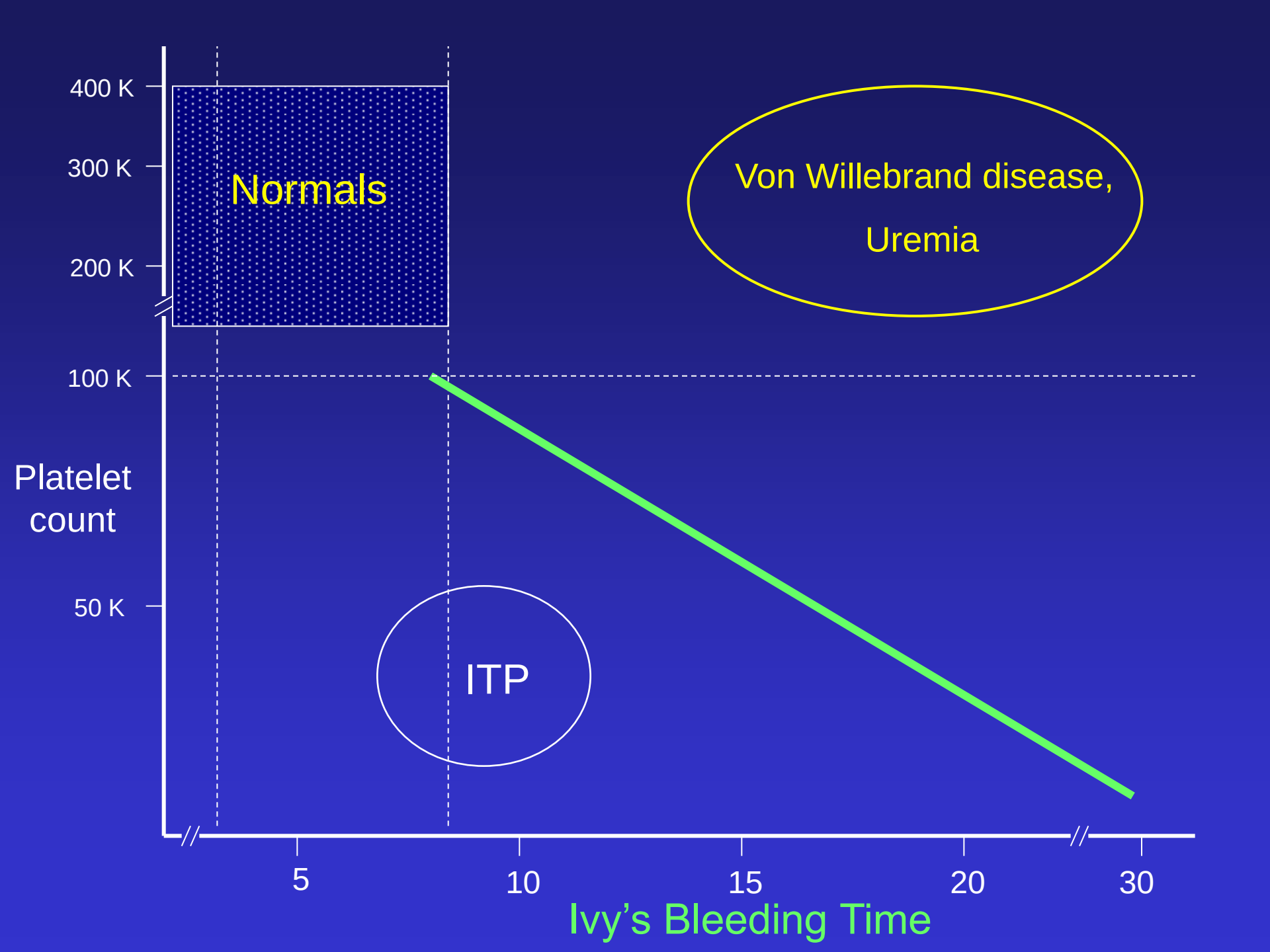
Clinical features	Platelet disorders	Coagulation disorders
Petechia	Characteristic	Rare
Superficial ecchymosis	Small & multiple	Large & solitary
Deep dissecting hematoma	Rare	Characteristic
Mucosal bleeding	Common, Spontaneous	Rare, except trauma or disease
Hemarthrosis	No	Yes (common in hemophilia, rare in other factor deficiency)
Onset of bleeding after trauma/surgery	Immediate	Delayed, or re-bleeding

Screening Tests for Bleeding Patients

1. Platelet count $\rightarrow$ thrombocytopenia
2. Template Bleeding time or PFA-100 $\rightarrow$ vWD or platelet dysfunction
3. PT $\rightarrow$ extrinsic or common pathway defect
4. aPTT $\rightarrow$ intrinsic or common pathway defect
5. (TT and Fibrinogen) $\rightarrow$ hypo- or dys-fibrinogenemia, inhibitors: FDP, heparin

Platelet Counts (K/mm^3)

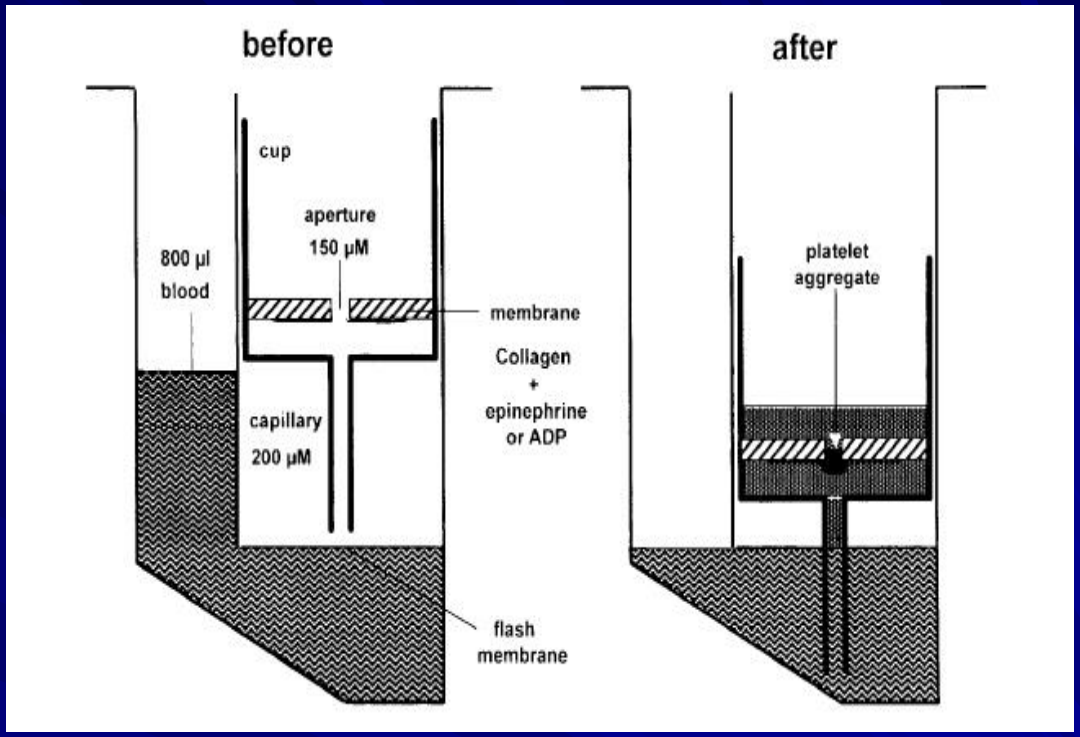



400 K
300 K
200 K
100 K
50 K
Platelet count
Normals
Von Willebrand disease,
Uremia
ITP
5
10
15
20
30
Ivy's Bleeding Time

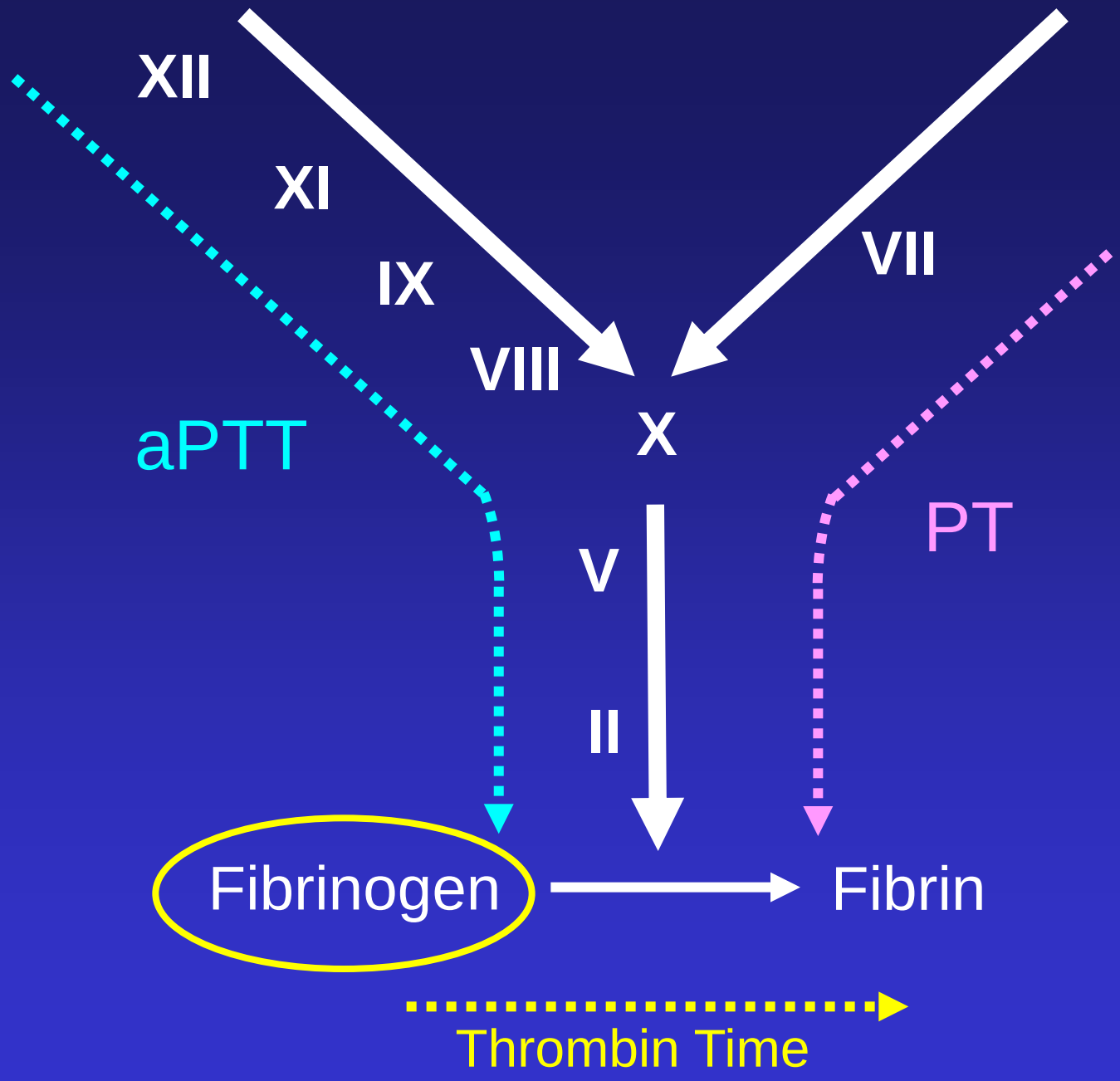
In Vitro Bleeding Time
Platelet function analyzer (PFA-100)

(Dade-Behring, Germany)

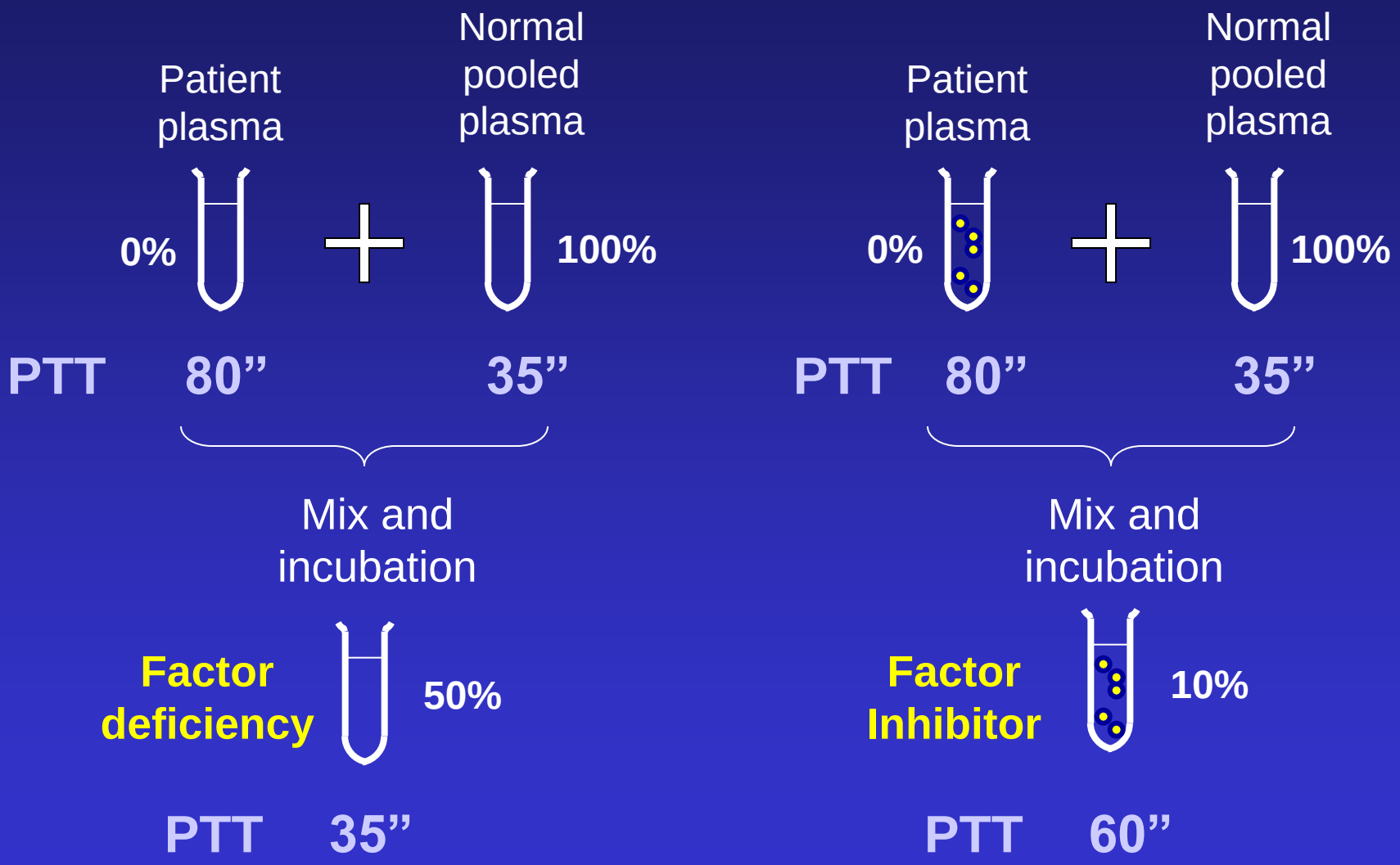
a point-of-care assay



- Citrated whole blood, stable for 4 hr at room temp.
- Measure high shear-dependent platelet function
- Closure Time
- Limitation: Platelet >80K, Hct >30%
- Cartridge membrane coated with
  1. Collagen-epinephrine (Col/EPI): primary screening
  2. Collagen-ADP (Col/ADP): differentiation


XII
XI
IX
VIII
VII
X
aPTT
PT
V
II
Fibrinogen
Fibrin
Thrombin Time

Mixing Test for Differentiation between Factor Deficiency and Factor Inhibitor



Laboratory Approach of Coagulation Inhibitors

Step 1 (general screening): compare P + N (1:1) vs. N

(1) correctable: pure factor(s) deficiency

(2) not correctable: inhibitors

Step 2 (identification of inhibitors): immediate vs. 1~2 hr incubation

(1) **immediate** prolongation:

a) lupus anticoagulant

b) factor IX inhibitor

c) high titer of factor VIII inhibitor

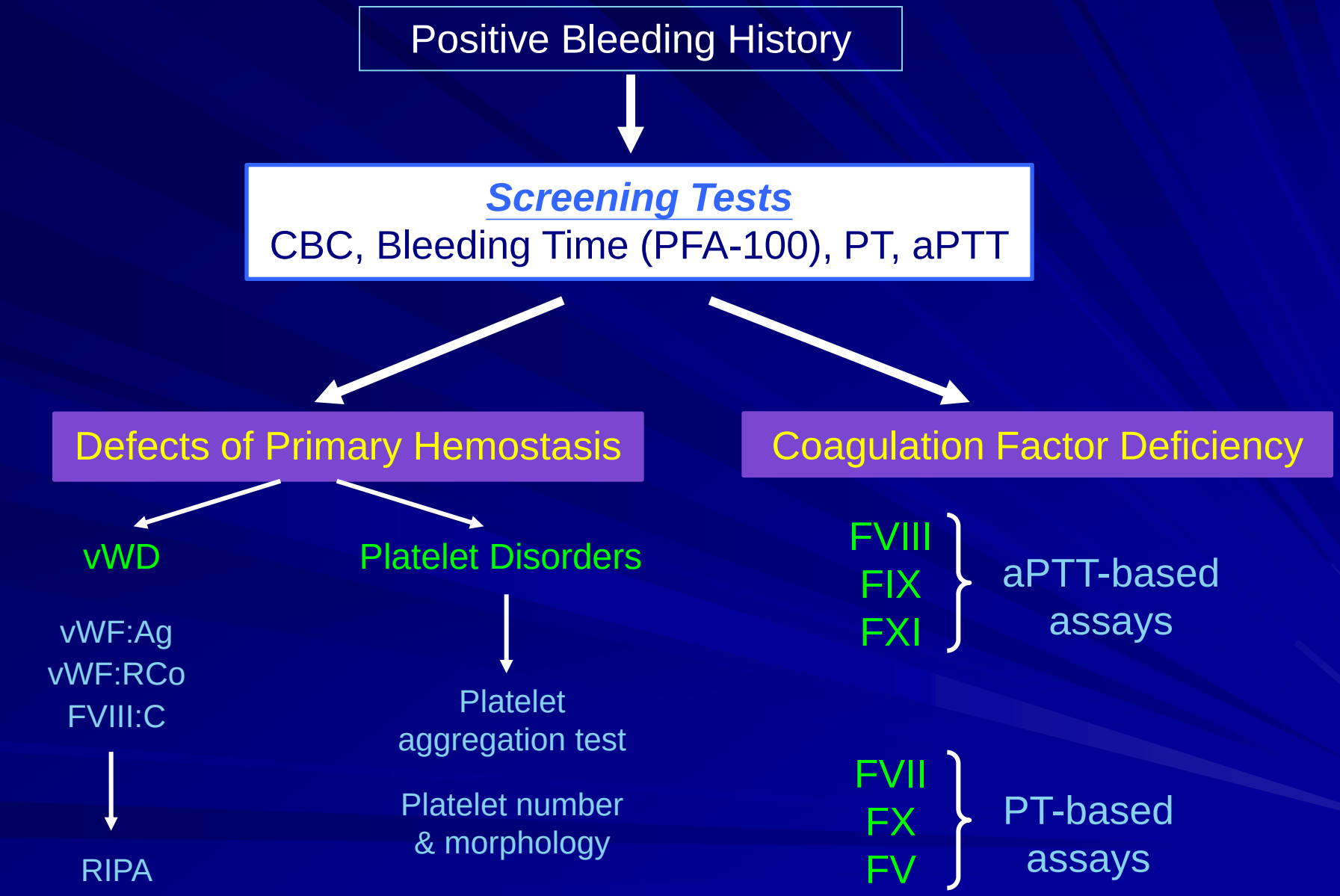
further screen: P (dilute 50X or 100X) + N (1:1)

(2) **time-dependent** prolongation:

factor VIII inhibitor

Step 3 (screening for low-titer inhibitors):

compare P + N(4:1) vs. N , incubation for 2 hr


Positive Bleeding History
Screening Tests
CBC, Bleeding Time (PFA-100), PT, aPTT
Defects of Primary Hemostasis
Coagulation Factor Deficiency
vWD
Platelet Disorders
vWF:Ag
vWF:RCo
FVIII:C
RIPA
vWF multimers
Platelet aggregation test
Platelet number & morphology
FVIII
FIX
FXI
aPTT-based assays
FVII
FX
FV
PT-based assays