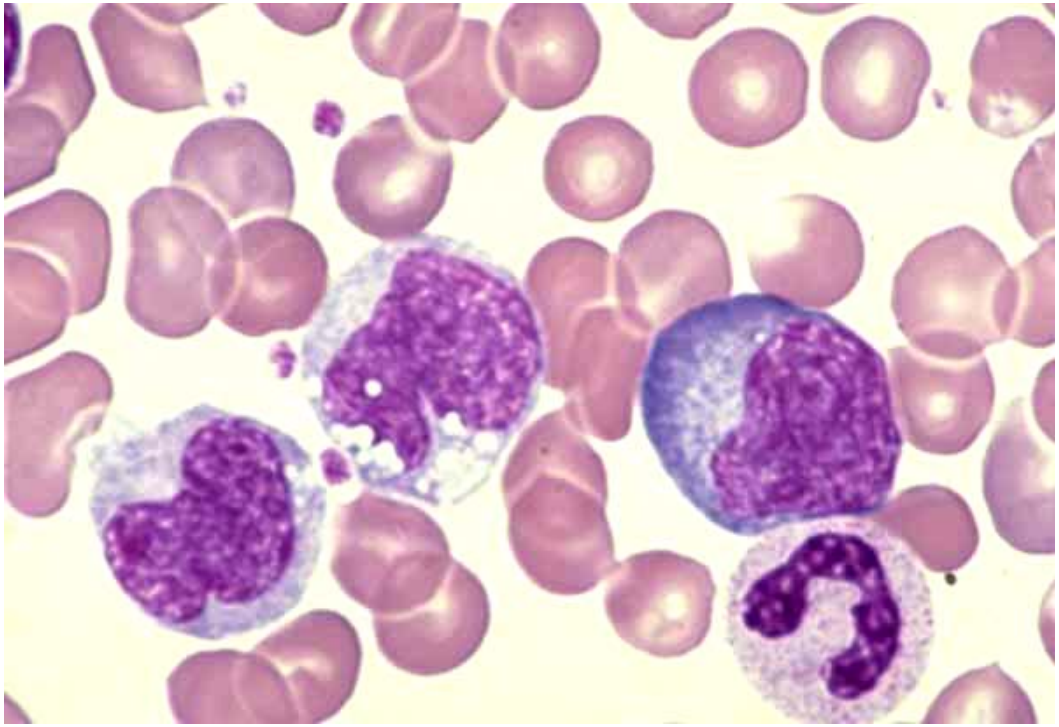
BASIC PHYSIOPATHOLOGY OF GENERAL HEMATOLOGY

A SYNOPSIS OF HEMATOLOGY



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Version 12.0, 2010

SERVICE OF HEMATOLOGY CHUV - Lausanne

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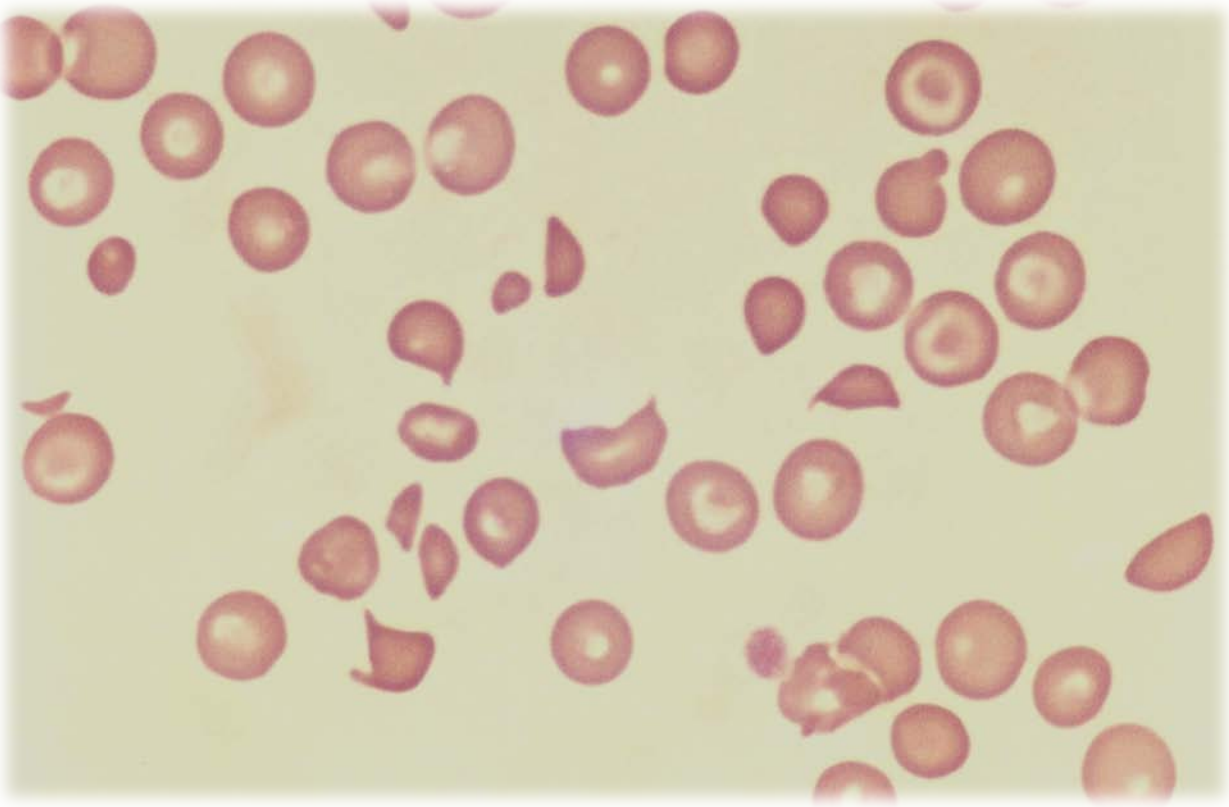
Part 1

RED BLOOD CELL PATHOLOGY



DIFFERENTIATION OF BLOOD CELLS

Early-acting hematopoietic growth factors

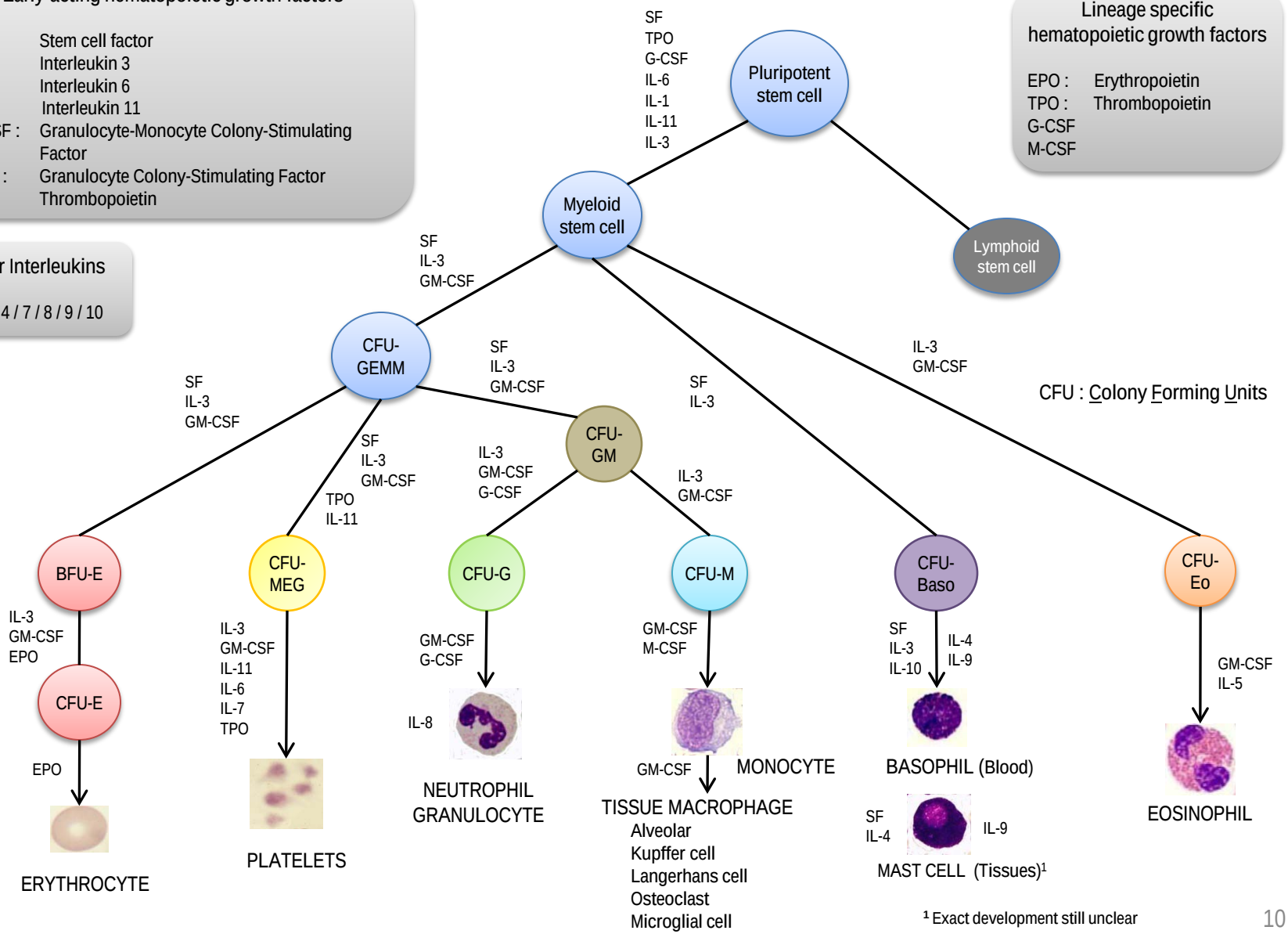
SF :	Stem cell factor
IL-3 :	Interleukin 3
IL-6 :	Interleukin 6
IL-11 :	Interleukin 11
GM-CSF :	Granulocyte-Monocyte Colony-Stimulating Factor
G-CSF :	Granulocyte Colony-Stimulating Factor
TPO :	Thrombopoietin

Other Interleukins

IL-1 / 4 / 7 / 8 / 9 / 10

Lineage specific hematopoietic growth factors

EPO :	Erythropoietin
TPO :	Thrombopoietin
G-CSF	
M-CSF	



[1] Exact development still unclear

NORMAL RANGES IN HEMATOLOGY

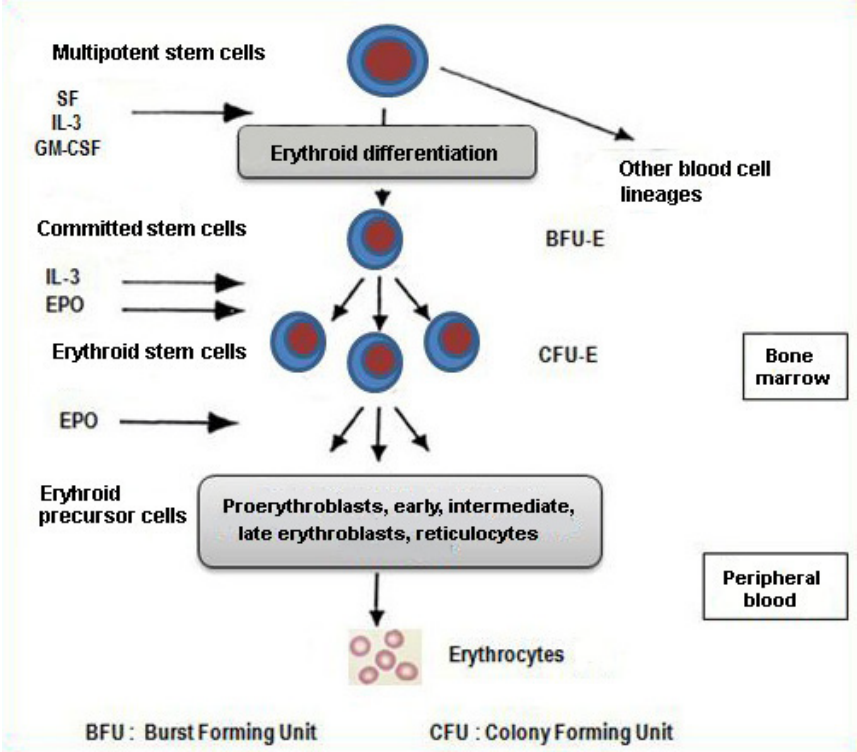
	UNIT	MAN	WOMAN
HEMOGLOBIN	g / L	133 – 177	117 – 157
HEMATOCRIT	%	40 – 52	35 – 47
RED BLOOD CELLS	T / L	4.4 – 5.8	3.8 – 5.2
MCV	fL	81 – 99	
MCH	pg	27 – 34	
MCHC	g / L	310 – 360	
RDW[1] (anisocytosis index)		< 15	
RETICULOCYTES (Relative count)	‰	5 – 15	
RETICULOCYTES (Absolute count)	G / L	20 – 120	
WHITE BLOOD CELLS	G / L	4 – 10	
PLATELETS	G / L	150 – 350	

T / L : Tera / L = 10^{12} / L
G / L : Giga / L = 10^{9} / L
fL : Femtoliter = L^{-15}
pg : Picogram = g^{-12}

[1] RDW : Red cell distribution width

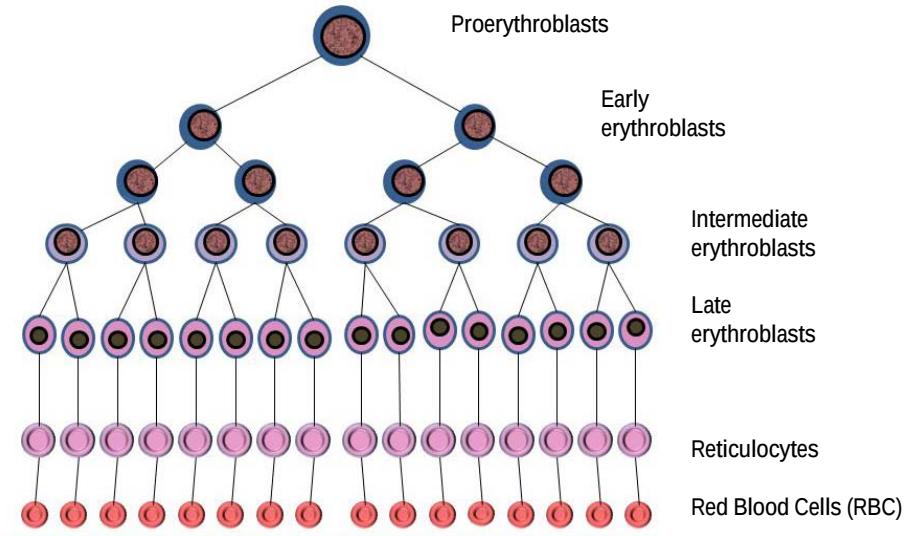
LCH-CHUV, 2009

ERYTHROPOIESIS



Classical schedule of erythropoiesis. Cytokines like Interleukin 3 (IL-3) act on stem cells and primitive BFU-E; Erythropoietin (Epo) acts on more mature BFU-E but principally on CFU-E and on the erythroblastic compartment

Modified from *Wajcman H., Lantz B., Girot R. : Les maladies du globule rouge 1992; Médecine-Sciences Flammarion : page 60.*



Amplification and maturation of the erythroid cell line from proerythroblasts to RBC

Hoffbrand A.V., Pettit J.E. : Essential Haematology, 3th edition; Blackwell Science : p.14.

EVALUATION OF ANEMIA (1)

3 PARAMETERS

3 INDICES

RETICULOCYTE COUNT

EVALUATION OF ANEMIA (2)

PARAMETERS

HEMOGLOBIN (g / L)

RED BLOOD CELL COUNT (T / L = 10^{12} / L)

HEMATOCRIT (%)

ANEMIA = DIMINUTION OF HEMOGLOBIN

(At sea level, WHO 1968)

Child (6 months-6 years)	< 110 g / L
Child (6 years-14 years)	< 120 g / L
Adult man	< 130 g / L
Adult woman	< 120 g / L
Pregnant woman	< 110 g / L

Influence of altitude : + 4% / 1'000 m

EVALUATION OF ANEMIA (3)

RED BLOOD CELL INDICES

MCV : Mean Corpuscular Volume *(Hct / RBC) x 10 (fL)*

MCH : Mean Corpuscular Hemoglobin *Hb / RBC (pg)*

MCHC : Mean Corpuscular Hemoglobin Concentration :
(Hb / Hct) x 100 or (MCH / MCV) x 1'000 (g / L)

MORPHOLOGICAL CLASSIFICATION OF ANEMIAS

	MCV	MCH	MCHC
Normocytic normochromic anemia	no	no	no
Microcytic hypochromic anemia	⇩	⇩	⇩
Macrocytic normochromic anemia	⇗	⇗	no

EVALUATION OF ANEMIA (4)
RETICULOCYTES

Absolute reticulocyte count :

< 120 G / L : Hyporegenerative anemia

> 120 G / L : Regenerative anemia

Reticulocyte production index (RPI)

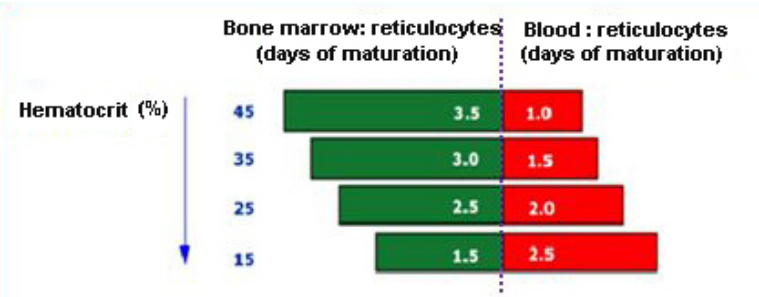
RPI = Reticulocytes (‰) / 10 x reticulocyte maturation time in blood (days)[1] x Hematocrit / 45

Normal : 1.0 - 2.0

Hyporegenerative anemia : < 2.0

Regenerative anemia : > 2.0

Reticulocyte maturation related to anemia severity[1]



[1] Reticulocyte have a total maturation time of 4.5 days :
- Normally 3.5 days in bone marrow and 1 day in peripheral blood
- In case of hematocrit / hemoglobin reduction reticulocytes leave the bone marrow earlier at a less mature stage, ➔ maturation > 1,0 day in peripheral blood *(where the reticulocyte count is performed)*

Reticulocytes distribution related to RNA[2] content :

HFR (High-Fluorescence Reticulocytes) : high — Immature reticulocytes *(IRF : Immature Reticulocyte Fraction[3])*

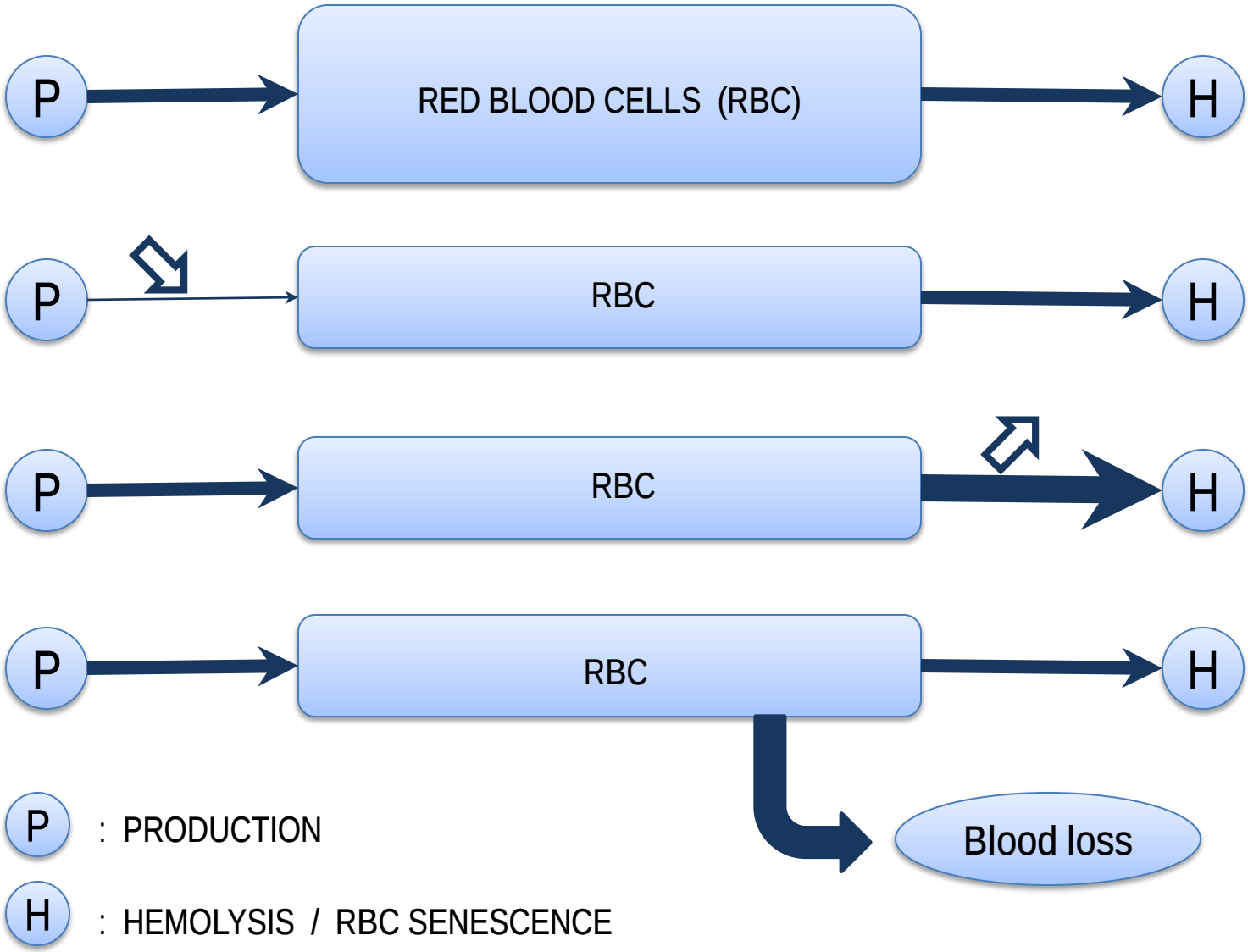
MFR (Medium-Fluorescence Reticulocytes : medium

LFR (Low-Fluorescence Reticulocytes : low — Mature reticulocytes

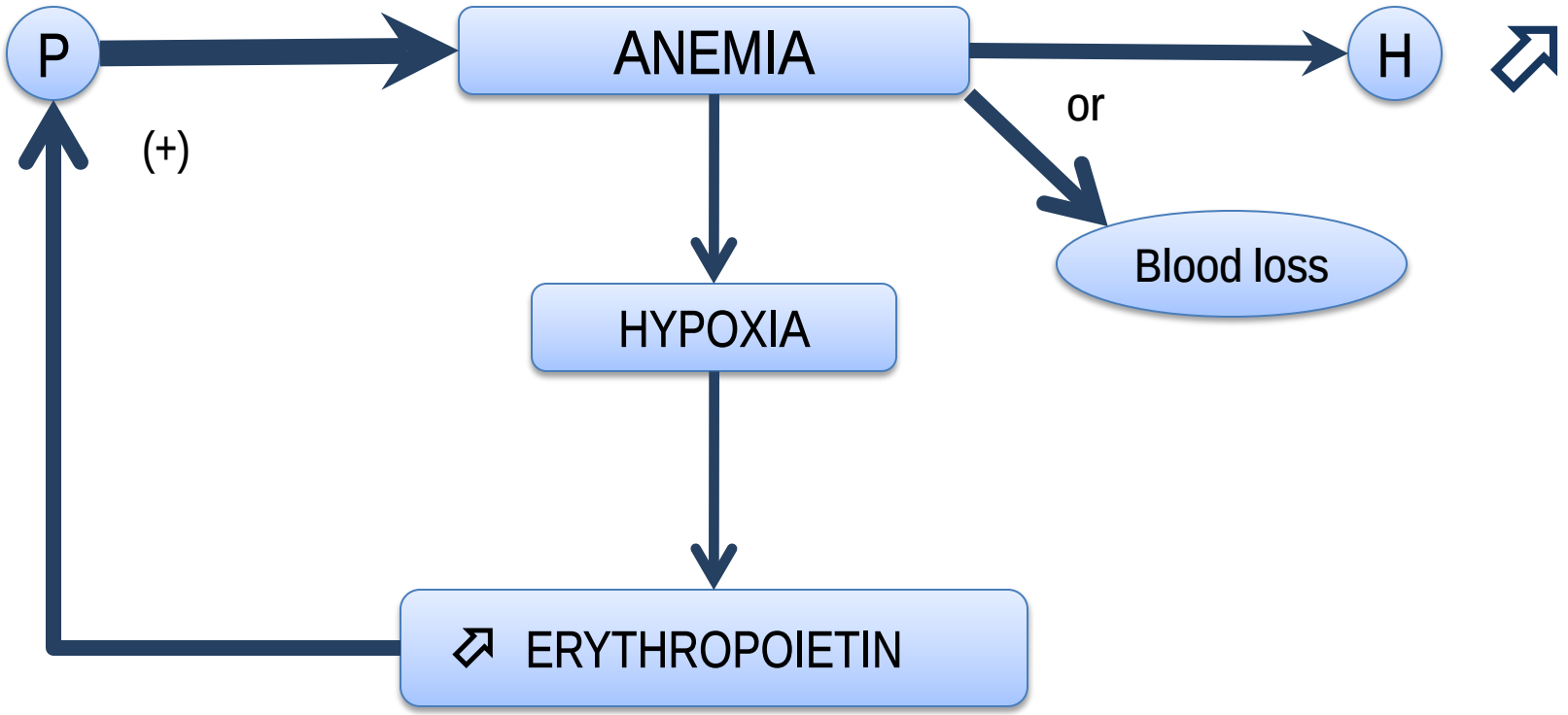
[2] By flow cytometry

[3] Increase of this fraction may precede the reticulocyte increase in peripheral blood. Therefore it can be an early sign of recovery or stimulation of erythropoiesis. e.g. : a) after bone marrow / stem cell transplantation; b) monitoring of EPO treatment

MECHANISMS OF ANEMIA (1)



MECHANISMS OF ANEMIA (2)

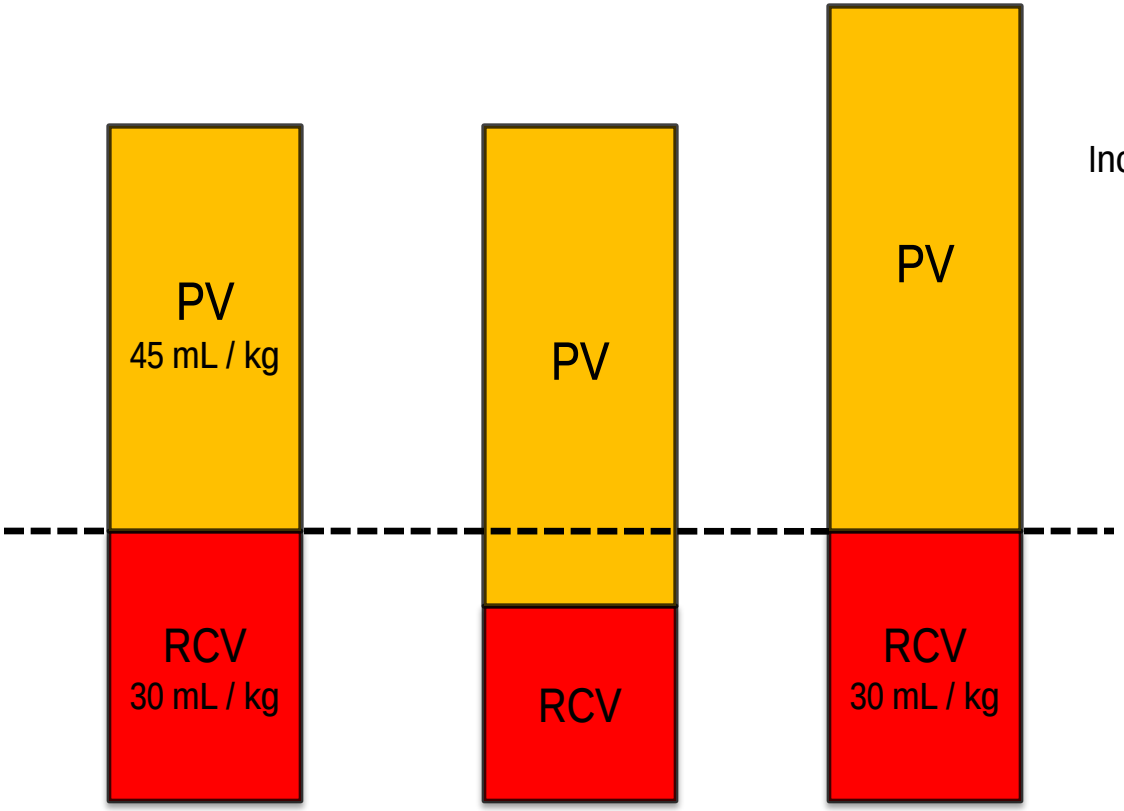


P : PRODUCTION

H : HEMOLYSIS / RBC SENESCENCE

MECHANISMS OF ANEMIA (3)

WHOLE BLOOD, RED CELL, PLASMA VOLUME



Increased plasma volume

Pregnancy
Paraprotein
Splenomegaly
Liver cirrhosis

RCV : Red Cell Volume

PV : Plasma Volume

ANEMIA
PATHOPHYSIOLOGICAL CLASSIFICATION

HYPOREGENERATIVE ANEMIA
(Reticulocyte count < 120 G / L / RPI < 2.0)

NORMOCYTIC NORMOCHROMIC
- Renal failure
- Pure red cell aplasia
- Bone marrow aplasia
- Bone marrow infiltration
- Anemia of chronic disease / Inflammatory anemia
- Hypothyroidism

MICROCYTIC HYPOCHROMIC
- Iron deficiency
- Anemia of chronic disease / Inflammatory anemia
- Iron utilization disorder (sideroblastic anemia, thalassemia)

MACROCYTIC NORMOCHROMIC
- Vitamin B_{12} and / or folate deficiency
- Cytotoxic drugs
- Alcoholism, liver diseases hypothyroidism
- Myelodysplastic syndrome
- Bone marrow aplasia

REGENERATIVE ANEMIA
(Reticulocyte count > 20 G / L / RPI > 2.0 / IRF ⇗)

NORMOCYTIC NORMOCHROMIC
- Acute blood loss
- Hemolytic anemia

HYPOREGENERATIVE NORMOCYTIC NORMOCHROMIC ANEMIA

MCV :	normal	81 – 99 fL
MCH :	normal	27 – 34 pg
MCHC :	normal	310 – 360 g / L
Reticulocyte count :		< 120 G / L

CLASSIFICATION

SOLITARY ANEMIA

RENAL FAILURE

PURE RED CELL APLASIA

HYPOTHYROIDISM[1]

PANCYTOPENIA *("CENTRAL" ORIGIN)*

BONE MARROW APLASIA[1]

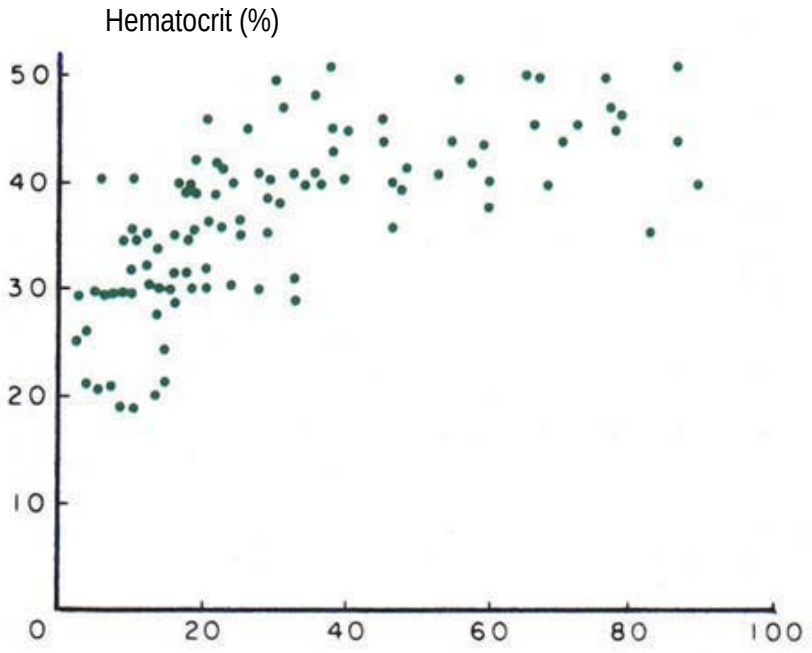
BONE MARROW INFILTRATION *(Acute leukemia, lymphoid neoplasm, metastatic cancer)*

MYELOFIBROSIS

HEMOPHAGOCYTOSIS

[1] Normocytic or slightly macrocytic anemia

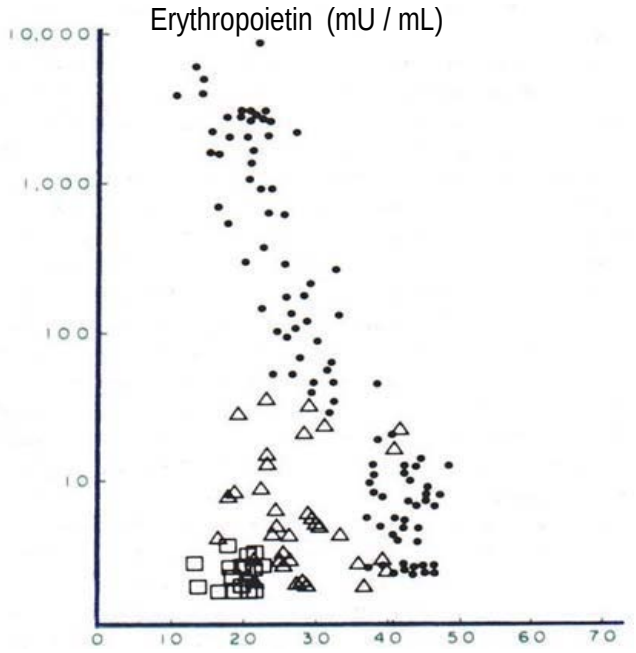
ANEMIA OF RENAL FAILURE



Creatinin clearance (mL / min / 1.73 m^2)

Relation between hematocrit and creatinin clearance

Radtke H.W., 1979.



Hematocrit (%)

Relation between hematocrit and endogenous erythropoietin

Renal anemia : □ Absence of kidney △ Presence of kidneys

- Non renal anemia

Caro J., 1979.

Treatment : rHuEpo 100-300 U / kg / week IV or SC

In Beutler E., Lichtman M.A., Coller B.S., Kipps T.J. : Williams Hematology, 5th edition 1995; McGraw-Hill : p. 456 & 458.

ERYTHROBLASTOPENIA - PURE RED CELL APLASIA

HEREDITARY

BLACKFAN-DIAMOND ANEMIA

ACQUIRED

PRIMARY

SECONDARY

THYMOMA (~ 5% of patients with thymoma have pure red cell aplasia)
LYMPHOID NEOPLASM
CANCER *(lung, breast, stomach, thyroid, biliary tract, skin)*
COLLAGEN VASCULAR DISEASE
PARVOVIRUS B19 INFECTION
PREGNANCY
DRUG INDUCED : Anticonvulsants
Azathioprine
Chloramphenicol
Sulfonamides
Isoniazid
Procainamide

BONE MARROW APLASIA
ETIOLOGY

HEREDITARY BONE MARROW APLASIA

- FANCONI ANEMIA

ACQUIRED BONE MARROW APLASIA

- IDIOPATHIC
- SECONDARY
  - Irradiation
  - Chemicals (benzene…)
  - Drugs
    - Obligatory bone marrow aplasia
      - Cytotoxic drugs (alkylating agents)
    - Occasional or uncommon bone marrow aplasia
      - Choramphenicol
      - Phenylbutazone
      - Gold salts
    - Viral infection (EBV, Hepatitis, Parvovirus B19, CMV, HIV)
    - Immune disorder (thymoma)
    - Paroxysmal Nocturnal Hemoglobinuria (PNH)
    - Hypoplastic myelodysplastic syndrome
    - Pregnancy

APLASTIC ANEMIA (1)

DRUG INDUCED BONE MARROW TOXICITY

OBLIGATORY :	dosis related	*Alkylating agents*
OPTIONAL :	dosis related	*Chloramphenicol*
	dosis unrelated	*Chloramphenicol*

CHLORAMPHENICOL INDUCED APLASTIC ANEMIA

TOXICITY	DOSE RELATED	DOSE UNRELATED
INCIDENCE	FREQUENT	UNCOMMON
BEGIN	IMMEDIATE	DELAYED (months)
SYMPTOMS	LIGHT	SEVERE (infection, bleeding)
COURSE	SPONTANEOUSLY FAVORABLE	FREQUENTLY FATAL

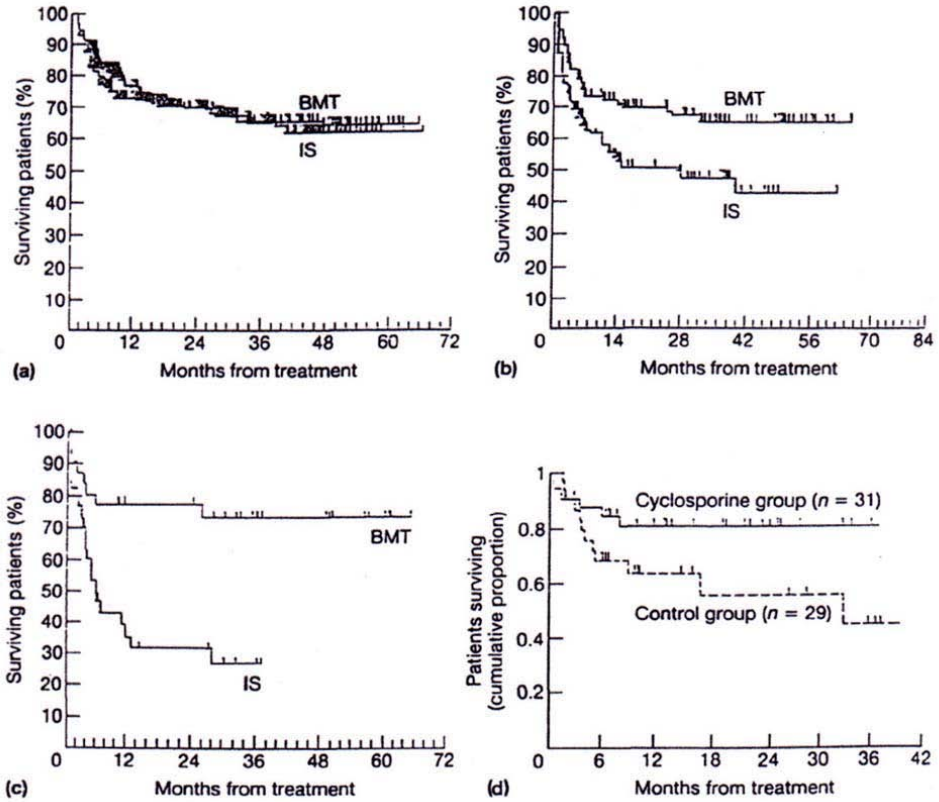
APLASTIC ANEMIA (2)
IDIOSYNCRASY[1] OVER 4 DECADES[2]

	1950 - 1959	1960 - 1969	1970 - 1979	1980 - 1989
Drugs[3]	427 (56%)	203 (60%)	523 (40%)	163 (20%)
Benzene and other solvants[4]	24 (3%)	14 (4%)	37 (3%)	21 (3%)
Insecticides	9 (1%)	29 (9%)	15 (1%)	11 (1%)
Idiopathic[5] / others[6]	296 (40%)	93 (27%)	717 (56%)	616 (76%)
Total	756	339	1292	811

[1] Idiosyncrasy : occasional or uncommon bone marrow depression
[2] Patients collective recruited in USA, Europe and Asia
[3] Chloramphenicol, Phenylbutazone, anticonvulsants, gold salts, others
[4] Benzene : obligatory toxicity or idiosyncrasy
[5] On the basis of some studies, 40-70% of idiosyncratic bone marrow aplasia are considered idiopathic
[6] Viral infection (EBV, hepatitis non-A, non-B, non-C, non-G, parvovirus, HIV), immune disease (eosinophilic fasciitis, thymoma, hypogammaglobulinemia, GvH : graft versus host disease in the context of immunodeficiency, pregnancy), PNH *(Paroxysmal Nocturnal Hemoglobinuria)*

Modified from data quoted by Young N.S. in Handin R.I., Lux S.E., Stossel T.P. : Blood, Principles & Practice of Hematology 1995; J.B. Lippincott : p. 303.

APLASTIC ANEMIA (3)
TREATMENT



(IS = anti-thymocyte globulin)

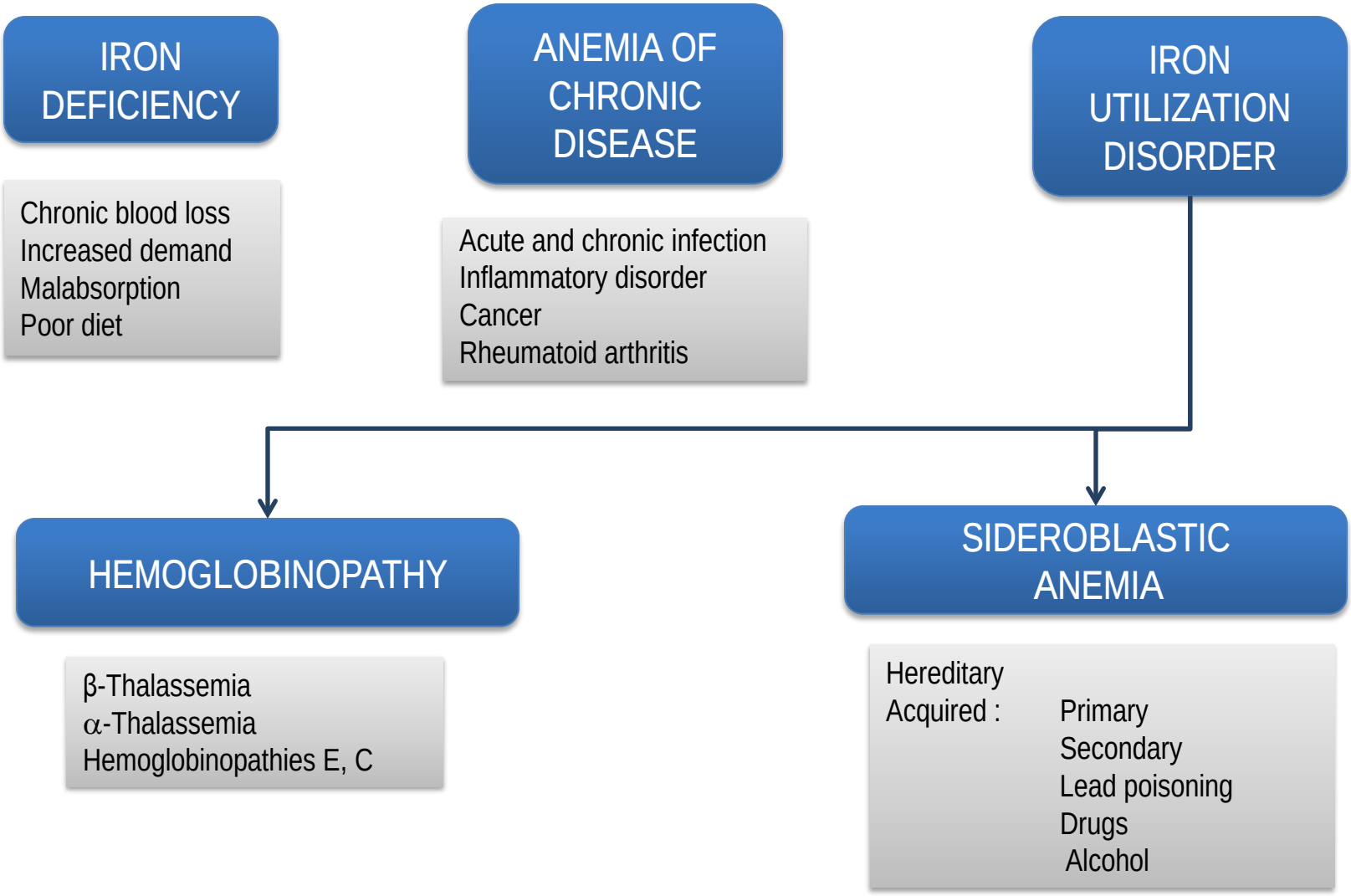
a) Comparison between allogeneic BMT and Immunosuppressive Treatment (IS). b) Neutrophils < 0.2 G / L, ($p < 0.01$).
c) Neutrophils < 0.2 G / L + infections (EBMT 1987). d) IS + high dose steroids ± cyclosporine *(Frickhofen et al.,1992).*

Probability to find an HLA-compatible sibling as bone marrow / hematopoietic stem cells donor : 20-30%

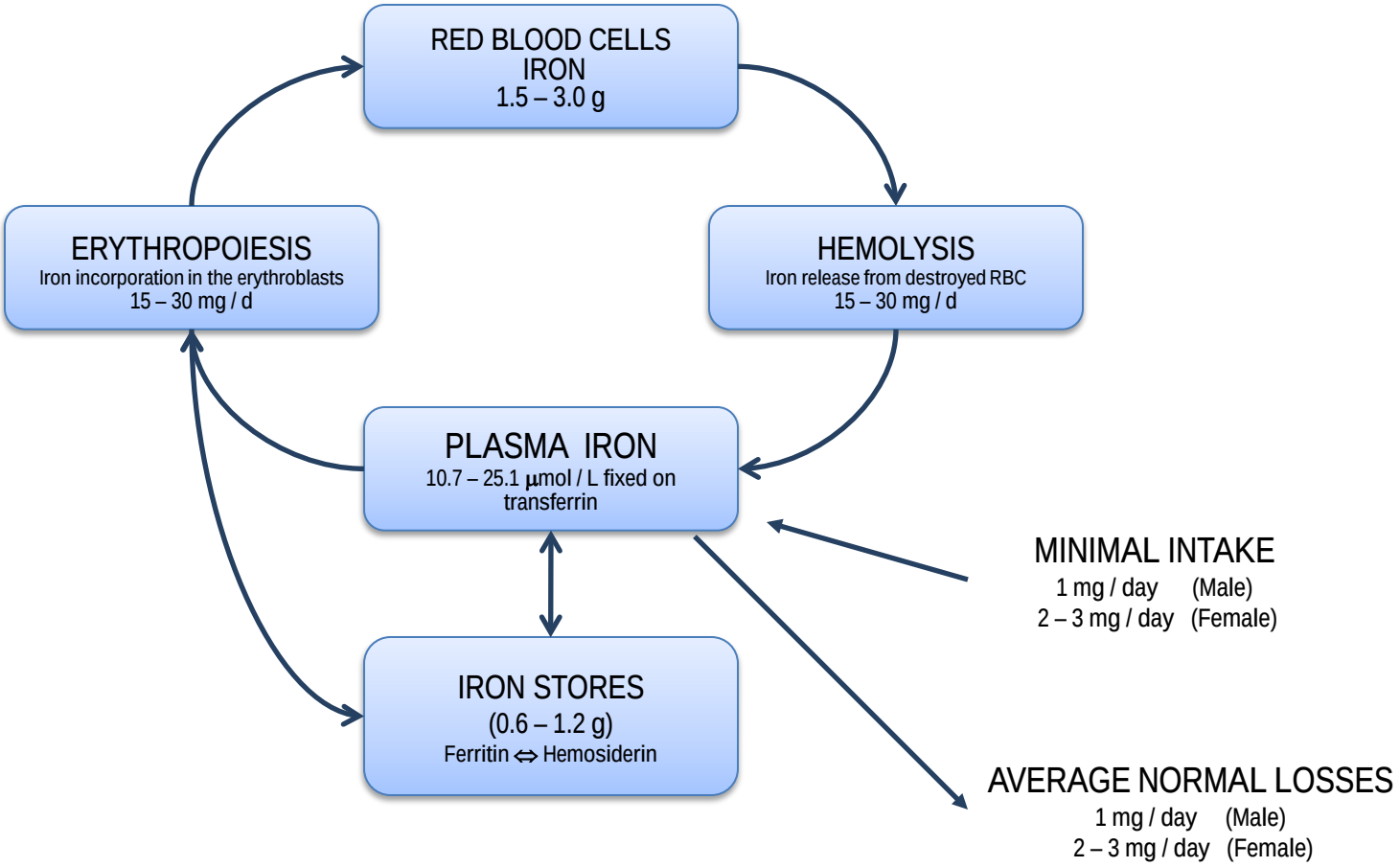
Adapted from Hoffbrand A.V., Pettit J.E. : Essential Haematology, 3th edition 1993; Blackwell Science p. 127.

MICROCYTIC HYPOCHROMIC ANEMIA

DECREASED MCV, MCH AND MCHC



IRON CYCLE



Normal range[1] :	Iron (serum)	12.5 – 25.1 µmol / L (M)	10.7 – 21.4 µmol / L (F)
	Transferrin	24.7 – 44.4 µmol / L	
	Ferritin (serum)	10 – 300 µg / L	

[1] *LCC-CHUV, 2009*

PHYSIOLOGICAL IRON LOSSES

MAN : 14 μg / kg / day *(Green, 1968)*
(0.9 – 1.0 mg / day)

WOMAN : 0.8 mg / day

+ menstruations : 1.4 – 2.2 mg / day
- – 50% if oral contraception
- \+ 100% if intrauterine device

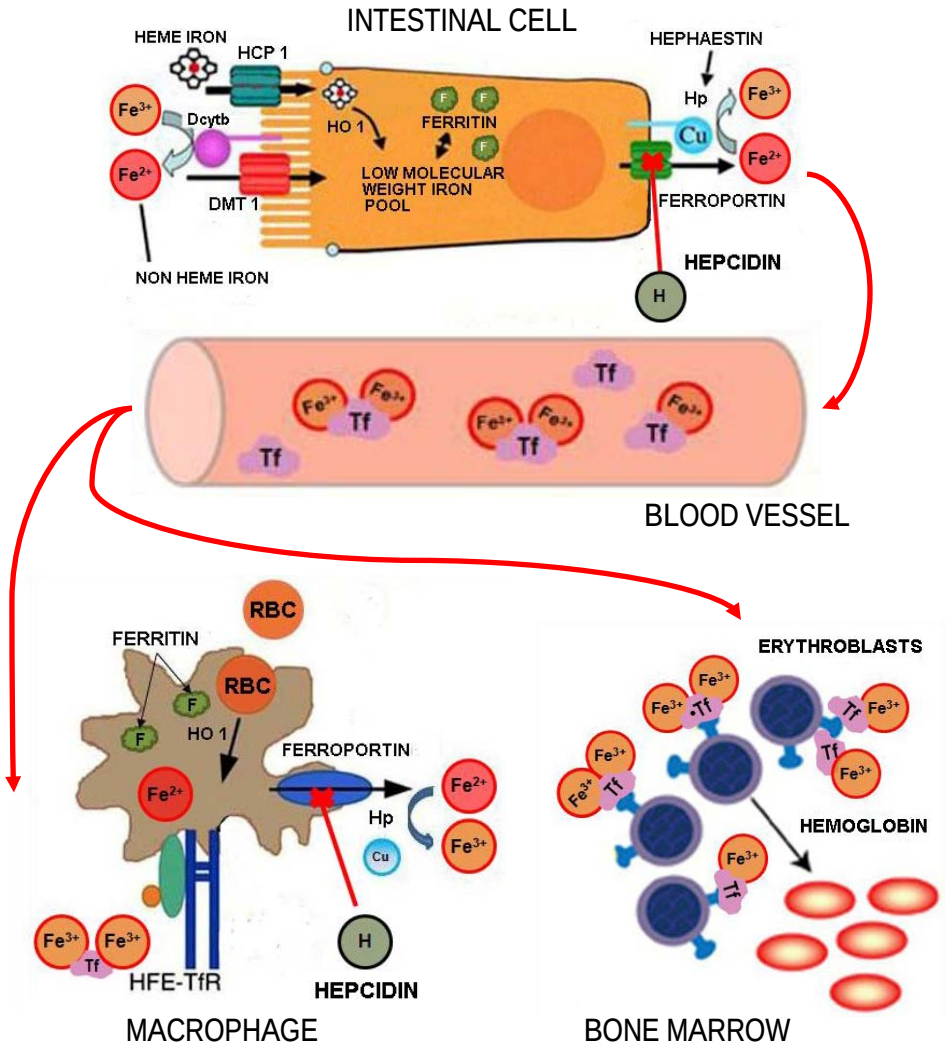
IRON BIOAVAILABILITY

ABSORPTION :

Heme iron 25 – 30%
Non heme iron 1 – 7%

- ⇗ Ascorbates, citrates, tartrates, lactates
- ⇘ Tannates, wheat, calcium, phosphates, oxalates, soya proteins

IRON METABOLISM



IRON ABSORPTION :

- **Heme iron** : by a special pathway, probably HCP 1[1], followed by heme degradation through **Heme-Oxygenase** (HO 1[6]) with iron recycling
- **Non-heme iron** : reduction of Fe^{+++} to Fe^{++} by **Dcytb**[2] with following absorption by DMT 1[3] to the intracellular labile iron pool then to ferritin

IRON CIRCULATION

Fe^{++} leaves the intestinal cell through the **Ferroportin** pathway, negatively regulated by **Hepcidin**
Iron is reoxidated to Fe^{+++} through **Hephaestin** (**Hp**[5]) in presence of Cu^{++}

Iron then binds to **Transferrin** (**Tf**) a specific bivalent transporter protein. By binding of **Tf** to the**Transferrin Receptors** (**TfR**[4]) iron can be delivered to the cells, in particular to the erythroblasts for heme synthesis

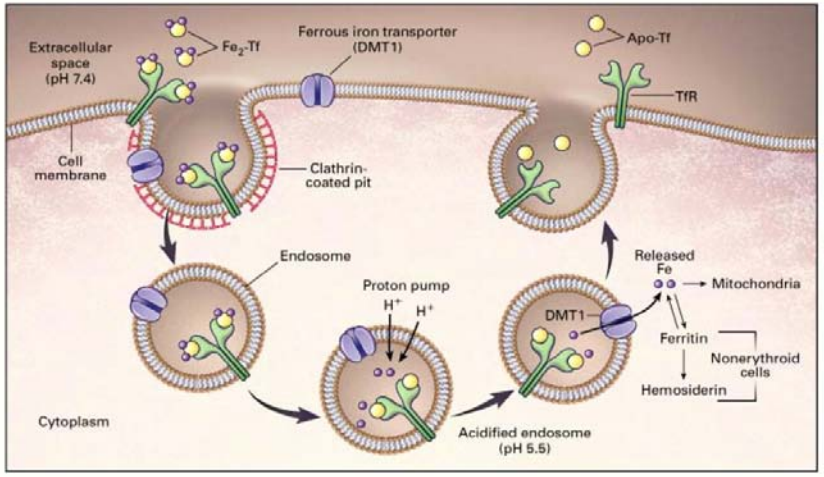
Iron is also stored in the macrophages.They also "recycle" the senescent RBC with recuperation and storage of their Heme iron
Release of iron from the stores proceeds by the **Ferroportin** pathway, also negatively regulated by **Hepcidin**

↗ **Hepcidin** : blocks Ferroportin by cellular internalization of the formed complex, stopping the process of iron release. This may lead to iron oveload in the cells with functional iron deficiency (*e.g. anemia of chronic disorders / inflammatory anemia*)

↘ **Hepcidin** : favours iron transfer and supply to the cells (*e.g. iron deficiency*)

[1] HCP 1 : Heme Carrier Protein 1
[2] Dcytb : Duodenal cytochrome b reductase
[3] DMT 1 : Divalent Metal Transporter 1
[4] TfR : Transferrin Receptor
[5] Hp : Hephaestine
[6] HO 1 : Heme Oxygenase 1
HFE : Human hemochromatosis protein

TRANSFERRIN CYCLE



TfR : Transferrin Receptor. Binds 2 molecules of bivalent transferrin
DMT 1 : Divalent Metal Transporter 1. Transport in the cell of non-heme iron
APO-Tf : Apotransferrin

Andrews N.C. : Disorders of Iron Metabolism. NEJM 1999; 341 : 1986-1995.

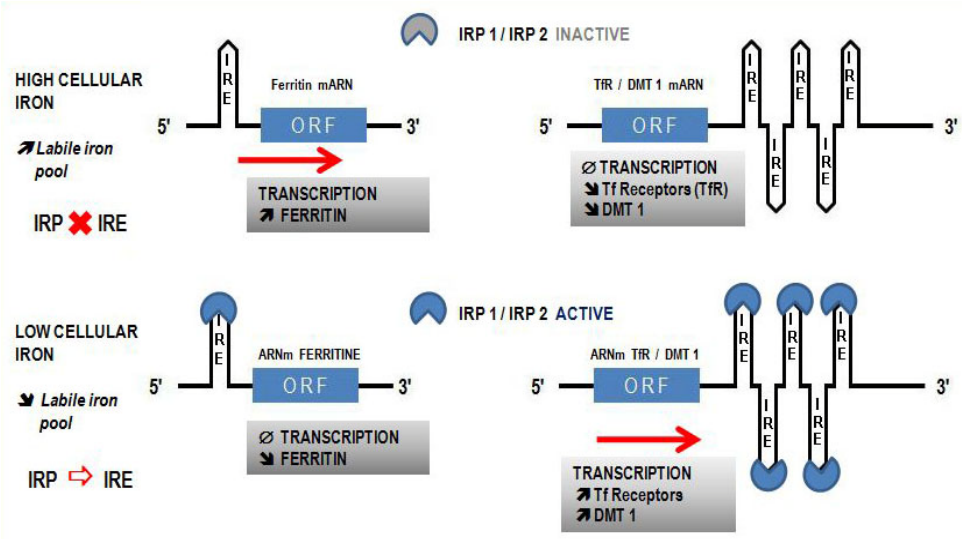
SYNTHESIS OF FERRITIN, TRANSFERRIN RECEPTOR AND DMT-1

IRP 1 / IRP 2 : Iron Regulatory Proteins *(sensors of intracellular labile iron)*
IRE and IREs(5) : Iron Responsive Elements (*ARNm motives*)

Interactions between IRE(s) and IRP lead to regulation of ferritin, DMT 1 and transferrin receptor synthesis related to the iron load of the labile intracellular pool

By **high** intracellular **iron pool**, IRP 1 and IRP 2 have low or absent activity leading to facilitated Ferritin mARN transcription with ⇗ **ferritin synthesis**. Transcription of TfR and DMT-1 mARN cannot proceed, leading to ⇘ **of TfR and DMT-1**, with reduction of iron absorption and transport capacity

By **low** intracellular **iron pool**, IRP-IRE binding leads to inhibition of initiation complex of Ferritin mARN transcription in 5' : ⇘ **of ferritin synthesis**
Stabilization of mARN in 3' by absence of endonuclease cleavage leads to ⇗ **of TfR and DMT-1 synthesis**



ORF : Open Reading Frame

STAGES OF IRON DEFICIENCY DEVELOPMENT

SERUM IRON - TRANSFERRIN - FERRITIN

	STAGE 1	STAGE 2	STAGE 3
FERRITIN	↘	↘	↘
IRON (Bone marrow)	↘	Absent	Absent
TRANSFERRIN (Serum)	Normal	↗	↗
IRON (Serum)	Normal	↘	↘
HEMOGLOBIN	Normal	Normal	↘
MCV	Normal	Normal	↘
MCHC	Normal	Normal	↘

	SERUM IRON	TRANSFERRIN	FERRITIN
IRON DEFICIENCY	↘	↗	↘
INFLAMMATORY ANEMIA	↘	↘	↗
IRON UTILIZATION DISORDER	↗	no / ↘	↗

SOLUBLE TRANSFERRIN RECEPTORS :

Increased in isolated iron deficiency and in this associated with inflammatory processes
Normal in isolated inflammatory anemia

RING SIDEROBLASTS :

Increased in sideroblastic anemia (indication to bone marrow examination), *cf. page 43*

ETIOLOGY OF IRON DEFICIENCY

Chronic blood loss
Increased iron demand
Malabsorption
Poor diet

CAUSES OF CHRONIC IRON LOSS

Uterine *(menorrhagia, metrorrhagia),* digestive bleeding *(hematemesis, melaena)*, parasites *(hookworm)*, hematuria
Chronic intravascular hemolysis *(Paroxysmal Nocturnal Hemoglobinuria)*
Frequent blood donations, phlebotomies, provoked bleedings *(Lasthénie de Ferjol syndrome)*

Chronic bleeding (microcytic hypochromic hyporegenerative anemia) must imperatively be distinguished from acute blood loss (normocytic normochromic regenerative anemia). Remember that 1 L of blood = 500 mg of iron

INCREASED IRON DEMAND

Pregnancy
Breast feeding *(maternal milk = 0.3 – 0.5 mg / L)*
Growth

IRON DEMAND IN PREGNANCY

Increased maternal total red cell volume	500 mg
Fetal needs	290 mg
Placenta	25 mg
Basal iron loss *(0.8 mg / d for 9 months)*	220 mg
TOTAL :	1'035 mg

FUNCTIONAL IRON DEFICIENCY

Absence of adequate erythropoietin response in case of anemia secondary to renal failure or to an inflammatory process with ferritin level in normal or high range *(cf. following page)*

TREATMENT OF IRON DEFICIENCY ANEMIA

CAUSAL TREATMENT

IRON SUBSTITUTION (anemia correction and iron stores reconstitution)

Oral substitution :

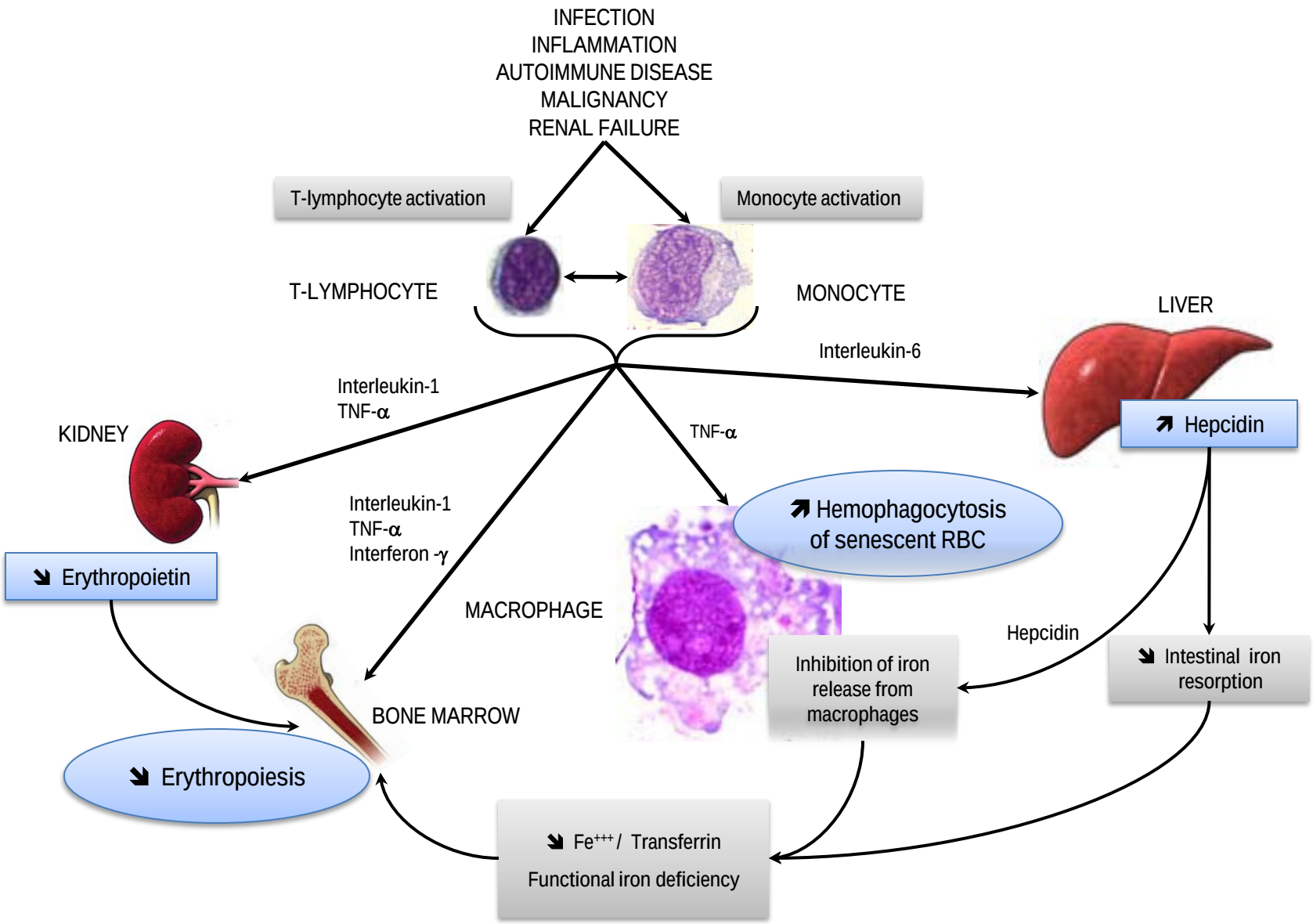
Basic data :1 L of blood = 500 mg of iron and 160 g of hemoglobin.1 g of hemoglobin : 500 / 160 = ± 3 mg of iron
Blood volume : 75 mL / kg. Iron reserves : 1'000 mg

Example : Woman, 56 years old, BW 50 kg, hemoglobin 80 g / L
Iron needs for anemia correction and iron stores reconstitution :
[Blood volume (L) x (160 - Hb patient) x 3] + 1'000 mg → [3.75 x (160 – 80) x 3] + 1'000 mg = 1'900 mg of iron
Patient receives 100 mg elementary iron q.d. with a mean resorption of 15 mg q.d.
Duration of substitution : 1'900 / 15 = 126 days (± 4 months)
Anemia correction within ± 1 month. Iron deficiency corrected when serum ferritin in normal range

Parenteral substitution : 100-200 mg IV 1-3 x weekly or perfusion of 1'000 mg (15 mg / kg) of ferric carboxymaltose once or twice

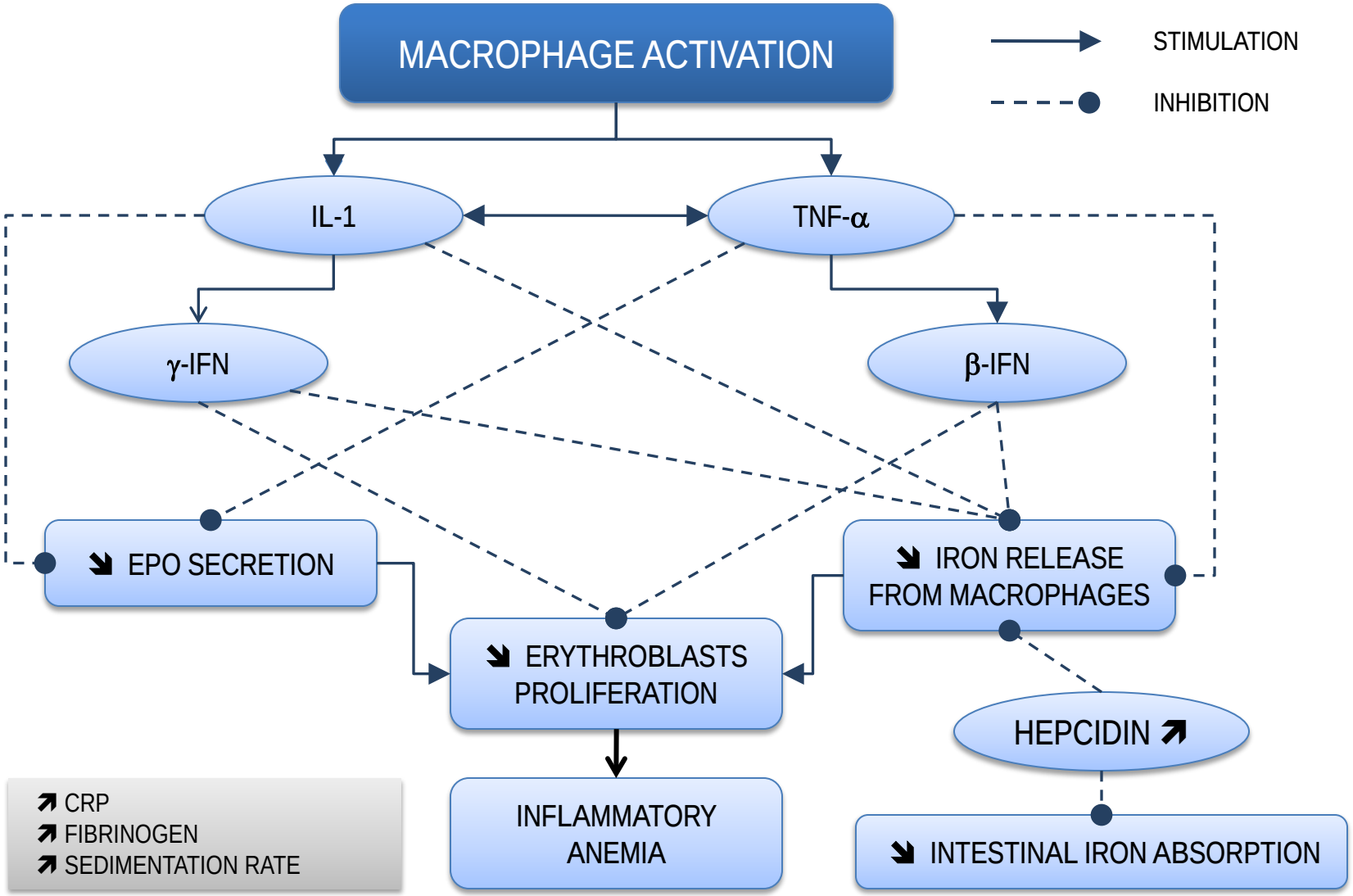
Indications :
Functional iron deficiency (Hb content in reticulocytes (CHr) < 28 pg ; percentage of hypochromic RBC (HYPO) : > 5%)
Malabsorption syndrome
Digestive oral iron intolerance
Poor patient compliance
Important chronic, persisting hemorrhage

ANEMIA OF CHRONIC DISORDERS / INFLAMMATORY ANEMIA (1)



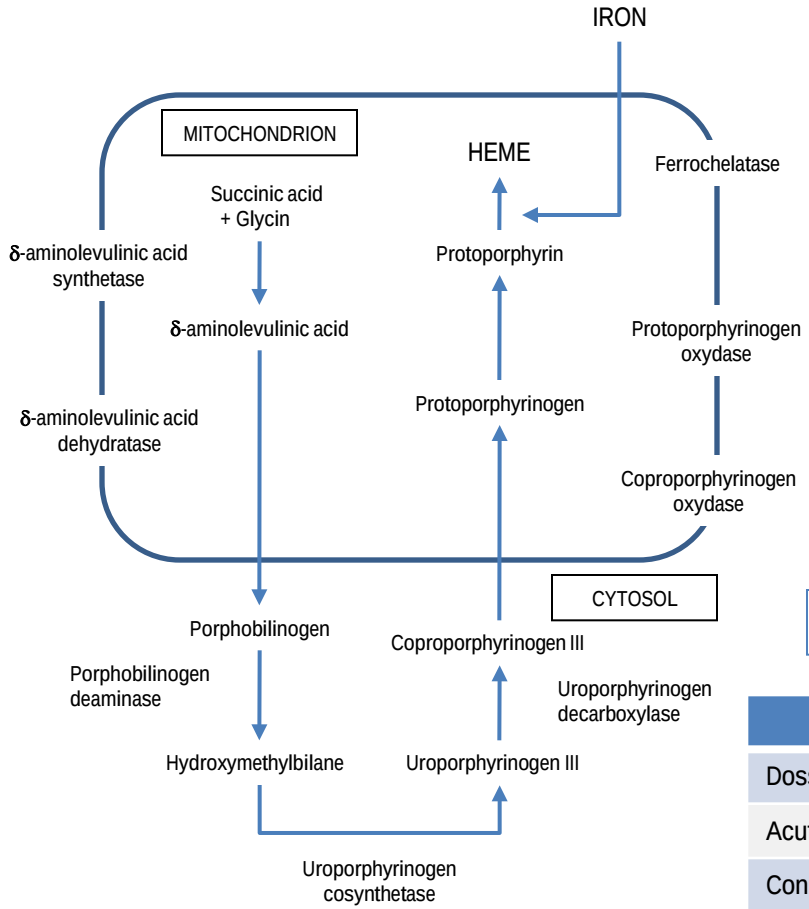
ANEMIA OF CHRONIC DISEASE / INFLAMMATORY ANEMIA (2)

ROLE OF MACROPHAGE RELEASED CYTOKINES IN ITS PATHOPHYSIOLOGY



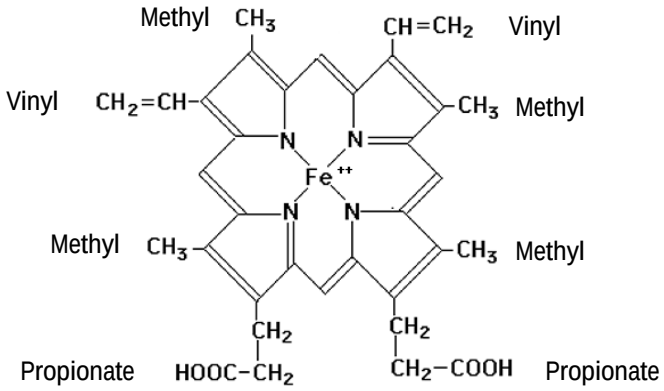
Modified from Means R.T.& Krantz S.B., Blood 1992; 80 :1639-1647.

HEME SYNTHESIS



Wajcman H., Lantz B., Girot R. : Les maladies du globule rouge 1992; Médecine-Sciences. Flammarion : p. 418 & 420.

Porphyric nucleus + iron

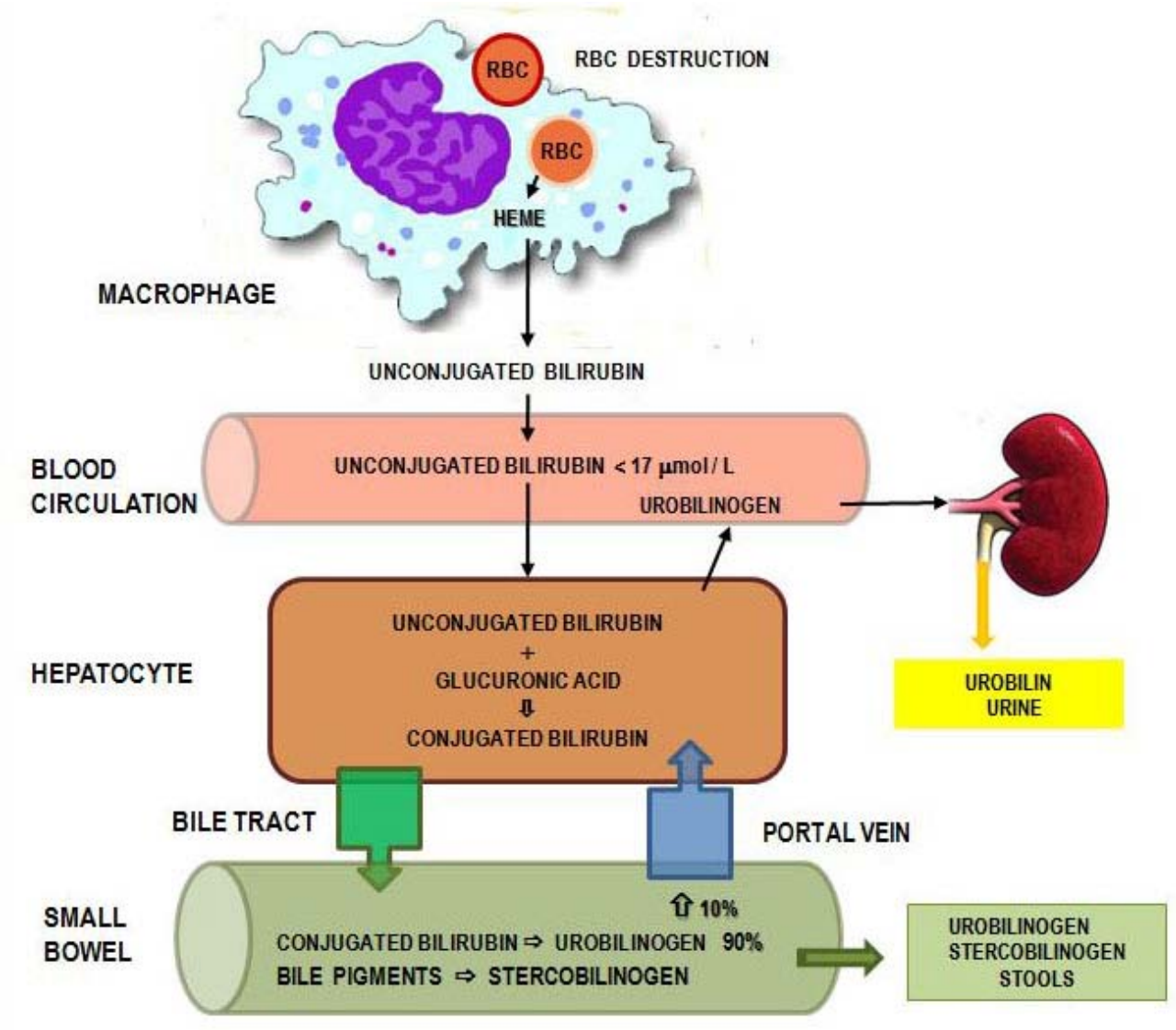


The heme molecule

HEPATIC (H) AND ERYTHROPOIETIC (E) PORPHYRIAS

DISEASE	TYPE	ENZYME DEFICIENCY
Doss porphyria	H	ALA dehydratase
Acute intermittent porphyria	H	Porphobilinogen deaminase
Congenital erythropoietic porphyria	E	Uroporphyrinogen cosynthetase
Cutaneous porphyria	H	Uroporphyrinogen decarboxylase
Hereditary coproporphyria	H	Coproporphyrinogen oxydase
Porphyria variegata	H	Protoporphyrinogen oxydase
Protoporphyria	E	Ferrochelatase

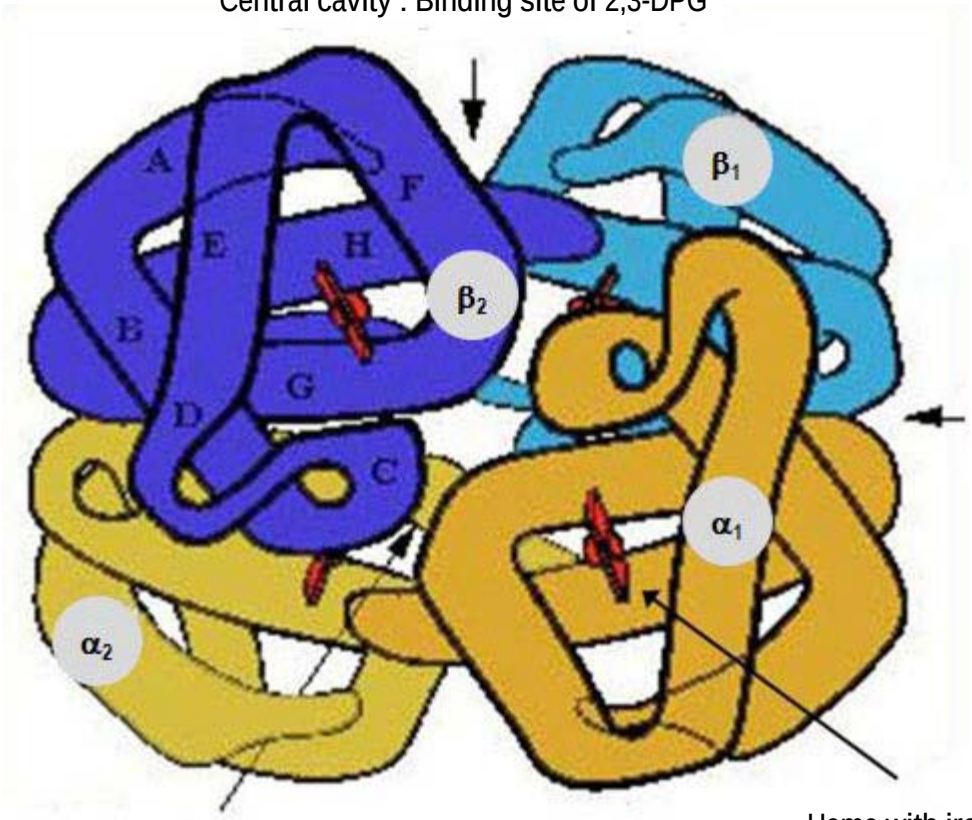
HEMOGLOBIN DEGRADATION



HEMOGLOBIN STRUCTURE

Central cavity : Binding site of 2,3-DPG

$\alpha_2\beta_2$ dimer



$\alpha_1\beta_1$ dimer

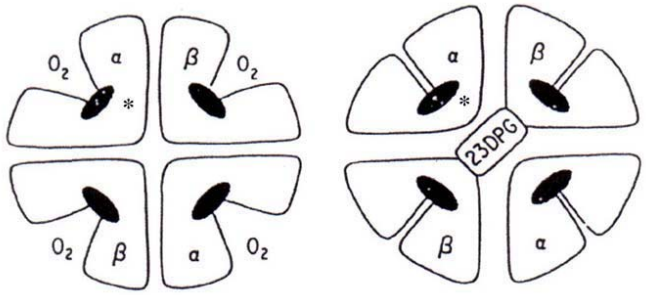
$\alpha_1\beta_1$ contact area

Heme with iron atom

$\alpha_1\beta_2$ contact area

Hemoglobine tetramer with contact areas

HEMOGLOBIN / INTERACTION O_2 AND 2,3-DPG



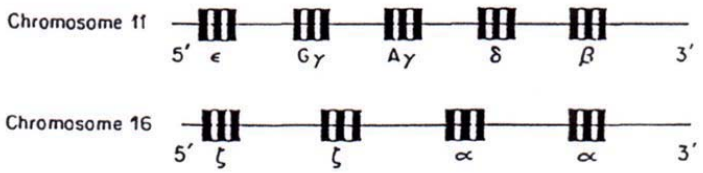
OXYHEMOGLOBIN DEOXYHEMOGLOBIN

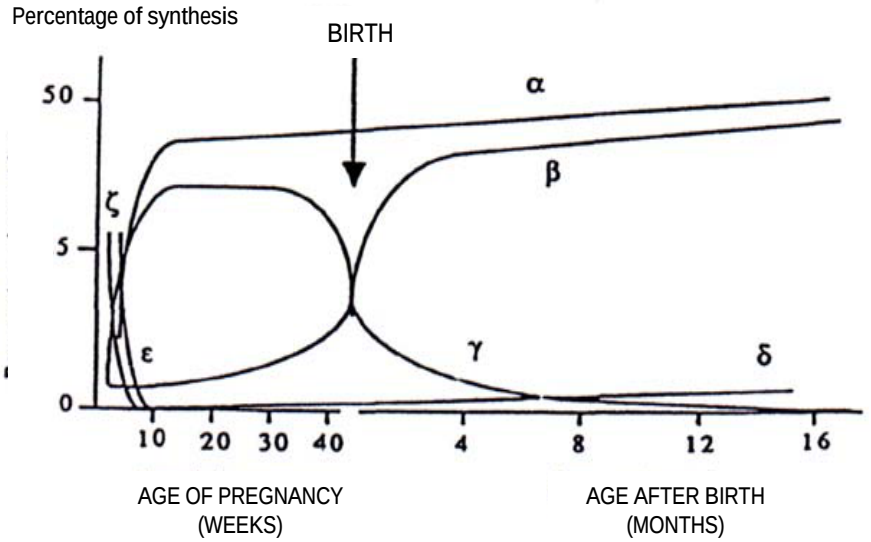
* Heme

Competition between oxygen and 2,3-diphosphoglycerate *(2,3-DPG)*

	GLOBIN STRUCTURE	HEMOGLOBIN
Embryonic hemoglobins	$\xi_2 \varepsilon_2$	Gower 1
	$\xi_2 \gamma_2$	Portland
	$\alpha_2 \varepsilon_2$	Gower 2
Adult hemoglobins	$\alpha_2 \beta_2$	A
	$\alpha_2 \delta_2$	A_2 (1.5 – 3.0%)
	$\alpha_2 \gamma_2$	F (< 1%)

GENES CODING FOR THE DIFFERENT GLOBIN CHAINS

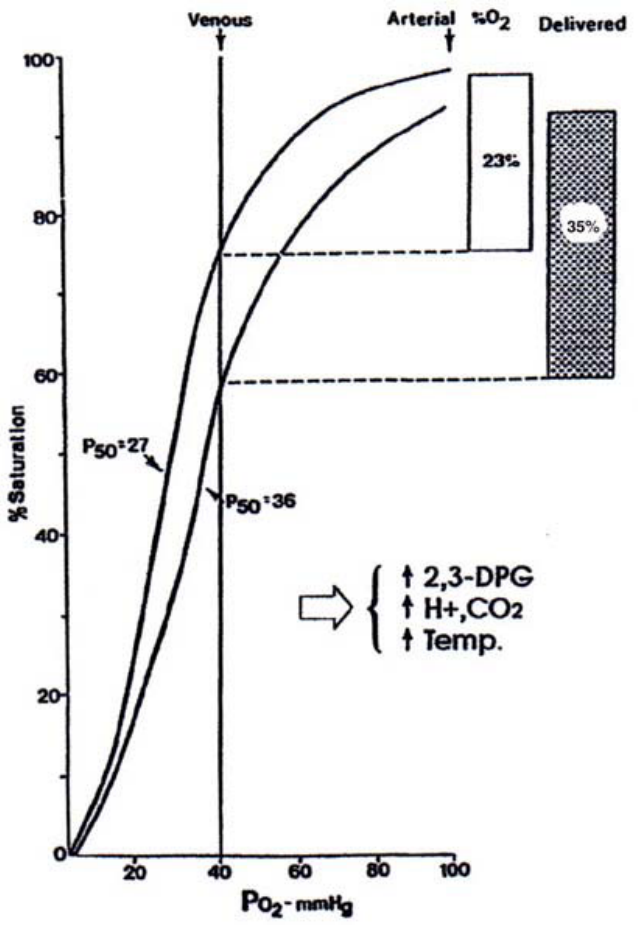




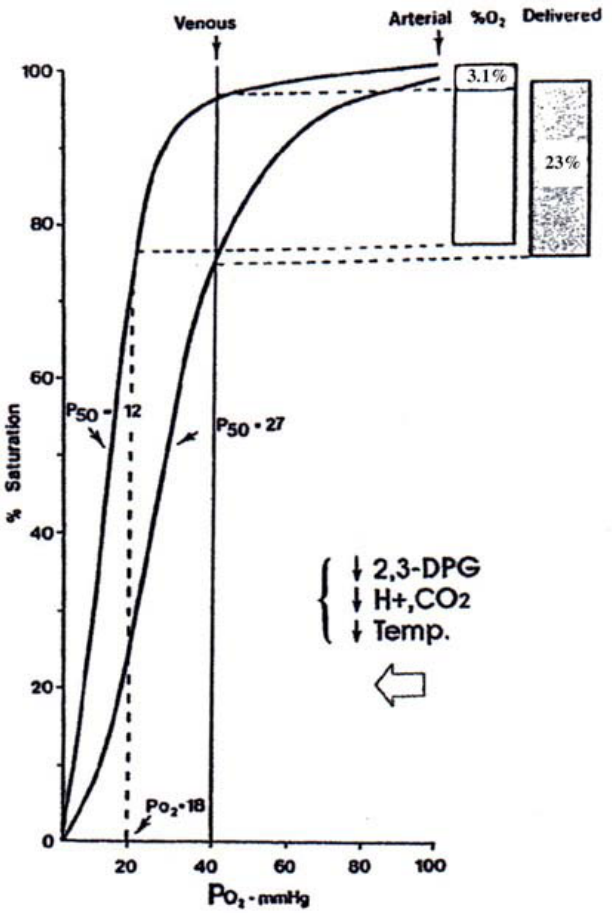
Synthesis of the different globin chains during ontogenesis

Wajcman H., Lantz B., Girot R. : les maladies du globule rouge 1992; Médecine-Sciences Flammarion : p. 12.

HEMOGLOBIN DISSOCIATION CURVE



Right shift of the hemoglobin dissociation curve through
⇗ of 2,3-DPG : ⇘ of oxygen affinity of hemoglobin
In this situation : 12% increase of O_2 tissues delivery



Left shift of the hemoglobin dissociation curve through
⇘ of 2,3-DPG : ⇗ of oxygen affinity of hemoglobin
in this situation : 20% diminution of O_2 tissues delivery

ANEMIA WITH IRON UTILIZATION DISORDER (1)
SIDEROBLASTIC ANEMIA

PATHOPHYSIOLOGY

Anomaly of porphyric nucleus synthesis
Presence of ring sideroblasts *(bone marrow)*
Role of vitamin B_6 *(Pyridoxin)*

CLASSIFICATION

Acquired sideroblastic anemia :
- *Primary*
- *Secondary*
  - *Lead*
  - *Isoniazid*
  - *Chloramphenicol*
  - *Pyrazinamide*
  - *Alcohol*

Hereditary sideroblastic anemia :
- *X - linked*
- *Autosomal*
- *Mitochondrial*

ANEMIA WITH IRON UTILIZATION DISORDER (2)
THALASSEMIA

PATHOPHYSIOLOGY

GLOBIN SYNTHESIS DEFECT

Great genetic heterogeneity at molecular level (DNA lesions, i.e. more or less important deletions, point mutations)

α-Thalassemia : ↘ or absence of α-chain synthesis of globin

β-Thalassemia : ↘ or absence of β-chain synthesis of globin

CENTRAL *(BONE MARROW) AND PERIPHERAL HEMOLYSIS THROUGH TETRAMERS INSTABILITY*

α_4 for β-Thalassemia

β_4 for α-Thalassemia *(Hemoglobin H)*

α-THALASSEMIA

CHROMOSOME 16

CLINICAL VARIETIES

Normal	α α / α α
Asymptomatic carrier	– α / α α
α-Thalassemia minor	– – / α α *or* – α / – α
Hemoglobin H disease	– – / – α
Moderate, sometimes severe chronic anemia *Splenomegaly* *Inclusion bodies*	
Hemoglobin Bart	– – / – –
Hydrops fetalis *Hb Bart = γ_4*	

DIAGNOSIS

Search for inclusion bodies

Electrophoresis of a fresh[1] hemolysate at alkaline or neutral pH. Isoelectric focusing (Hb H)

DNA analysis

[1] Hb H is unstable !

β-THALASSEMIA

β-THALASSEMIA MINOR

β / β^{+}-thal (heterozygocity)

"Micropolyglobulia" : e.g. *RBC : 6.2 T / L*
Hb : 105 g / L
MCV : 62 fL

Target cells, coarse basophilic stippling. Hb electrophoresis : ⇗ Hb A_2 and F
Genetic counseling

β-THALASSEMIA MAJOR

β^{0}-thal / β^{0}-thal (homozygocity) or
β^{0}-thal / β^{+}-thal (double heterozygocity)

Severe anemia, hemolytic icterus, erythroblasts on blood smear
Splenomegaly, hepatomegaly
Growth retardation
Hb electrophoresis : ⇩ or absence of Hb A
Hb F 20-80 %
***Treatment** : Transfusions, iron chelation, allogeneic stem cell / bone marrow transplantation*

MACROCYTIC NORMOCHROMIC HYPOREGENERATIVE ANEMIA

MCV :	⇗	> 99 fL
MCH :	⇗	> 34 pg
MCHC :	normal	310 – 360 g / L
Reticulocyte count :		< 120 G / L

CLASSIFICATION

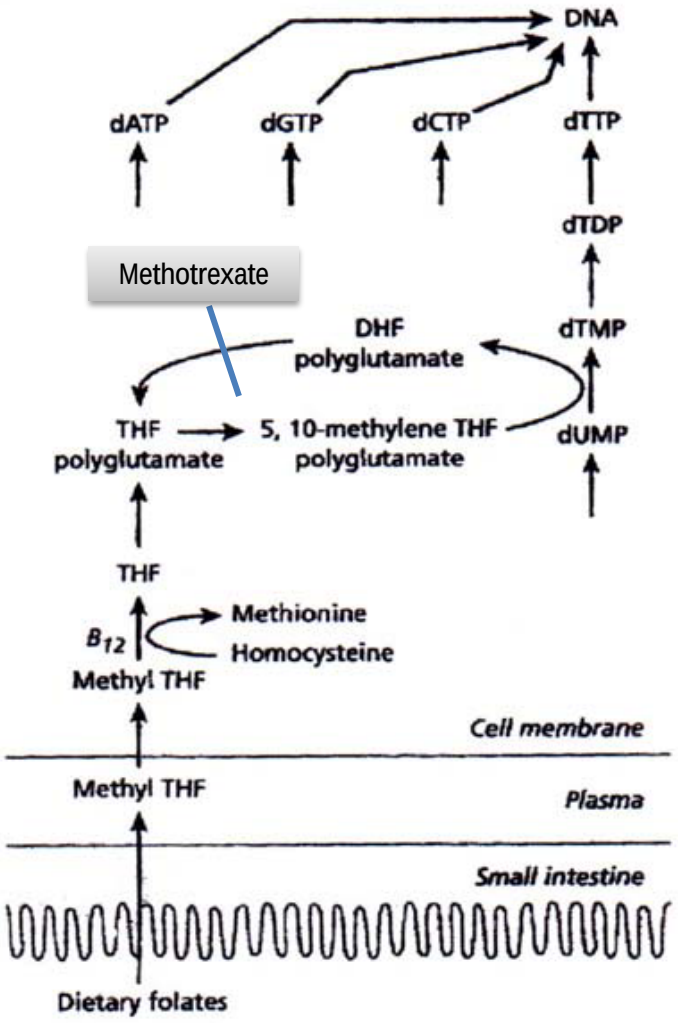
MEGALOBLASTIC MACROCYTIC ANEMIA

Vitamin B_{12} deficiency
Folate deficiency
Cytotoxic drugs
- *6-mercaptopurin*
- *5-fluorouracil*
- *Cytosine arabinoside*
- *Hydroxyurea*
- *Methotrexate*
- *Zidovudin (AZT)*

NON MEGALOBLASTIC MACROCYTIC ANEMIA

Alcoholism
Liver disease
Myxedema
Myelodysplastic syndrome

MEGALOBLASTIC MACROCYTIC ANEMIA
PATHOPHYSIOLOGY



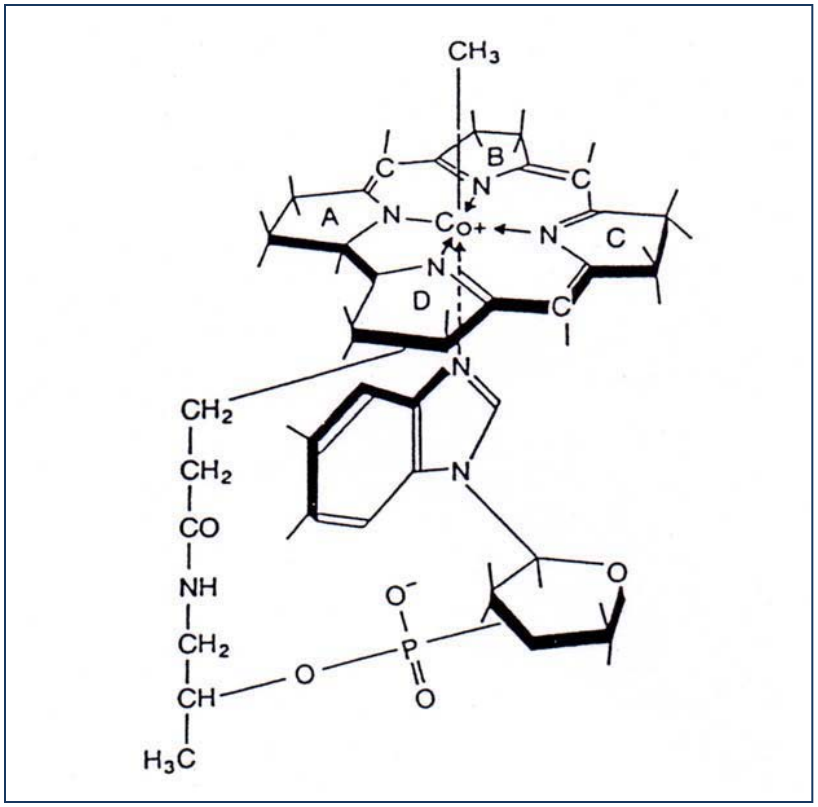
Role of vitamin B_{12} (cobalamin) and folates in DNA metabolism

Methyl THF :	*methyltetrahydrofolate*	*A :*	*adenine*
THF :	*tetrahydrofolate*	*G :*	*guanine*
DHF :	*dihydrofolate*	*C :*	*cytosine*
MP :	*monophosphate*	*T :*	*thymidine*
DP :	*diphosphate*	*U :*	*uridine*
TP :	*triphosphate*	*d :*	*deoxyribose*

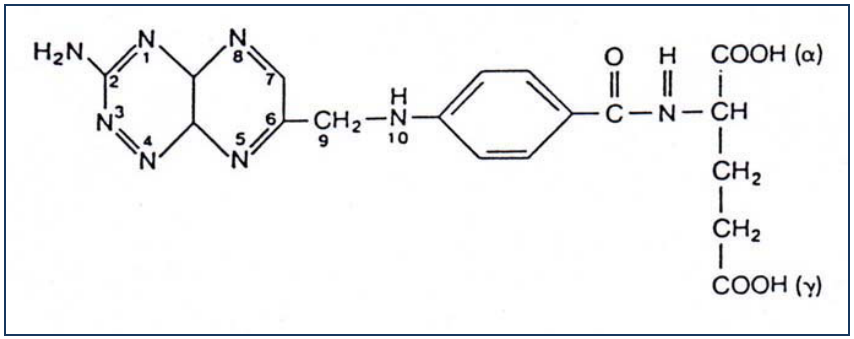
Methionine deficiency might be the cause of myelin synthesis anomaly, leading to the neurological signs and symptoms found in vitamin B_{12} deficiency

Hoffbrand A.V., Moss P.A.H., Pettit J.E. : Essential Haematology, 5th edition 2006; Blackwell Publishing : p. 47.

VITAMIN B_{12} AND FOLATES
CHEMICAL STRUCTURE



Structure of methylcobalamin *(plasma)*
Other compounds : deoxyadenosylcobalamin (tissues), hydroxocobalamin and cyanocobalamin (used in treatment of vitamin B_{12} deficiency)



Structure of folic acid (pteroylglutamic acid) *:* pteridine nucleus + para-aminobenzoic acid + glutamate(s)

Hoffbrand A.V., Pettit J.E. : Essential Haematology, 3th edition 1993; Blackwell Science : p. 54 & 57.

VITAMIN B_{12} AND FOLATES
GENERAL DATA

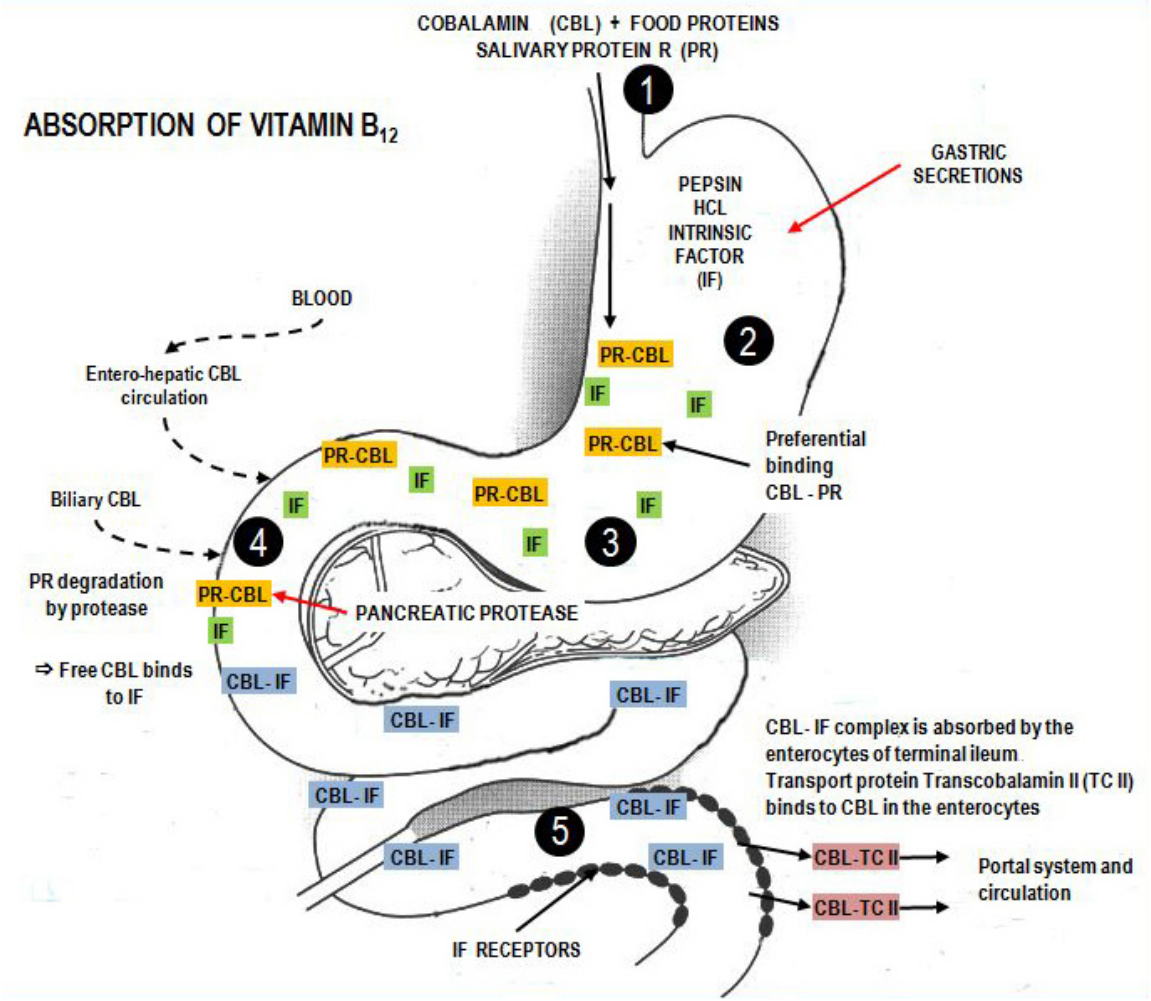
	VITAMIN B_{12}	FOLATES
Balanced diet (/ day)	7 – 30 µg	200 – 250 µg
Daily needs	1 – 2 µg	100 – 150 µg
Origin	Animal	Vegetables, liver, yeast
Cooking (heat)	Few effect	Thermolabile
Reserves	2 – 3 mg	10 – 12 mg
Exhaustion of stores	2 – 4 years	3 – 4 months
Absorption		
Site	Ileum	Jejunum
Mechanism	Intrinsic factor[1]	Methyltetrahydrofolate conversion
Plasmatic transport	Transcobalamins (TC) TC II : transport and intracellular transfer of cobalamins TC I [2] : transports the major part of circulating cobalamins TC III : isoprotein of TC I	Albumin
Active physiological forms	Methyl- and deoxyadenosylcobalamins	Polyglutamates
Compounds used for therapeutic substitution	Hydroxocobalamin Cyanocobalamin	Folic acid (pteroylglutamic acid)
Serum levels (physiological)	133 – 675 pmol / L[3]	> 5.3 nmol / L[3]

[1] Cobalamins of dietary origin are unspecifically bound to proteins. In the stomach, peptic digestion at low (acid) pH separates dietary proteins from cobalamins which then bind to Protein R *(or haptocorrin)* of salivary origin. In the duodenum, degradation of Protein R by pancreatic proteases allows binding of the cobalamins to intrinsic factor of gastric origin

[2] TC I and TC III are abundant in secondary granules of neutrophils

[3] *LCC-CHUV, 2009*

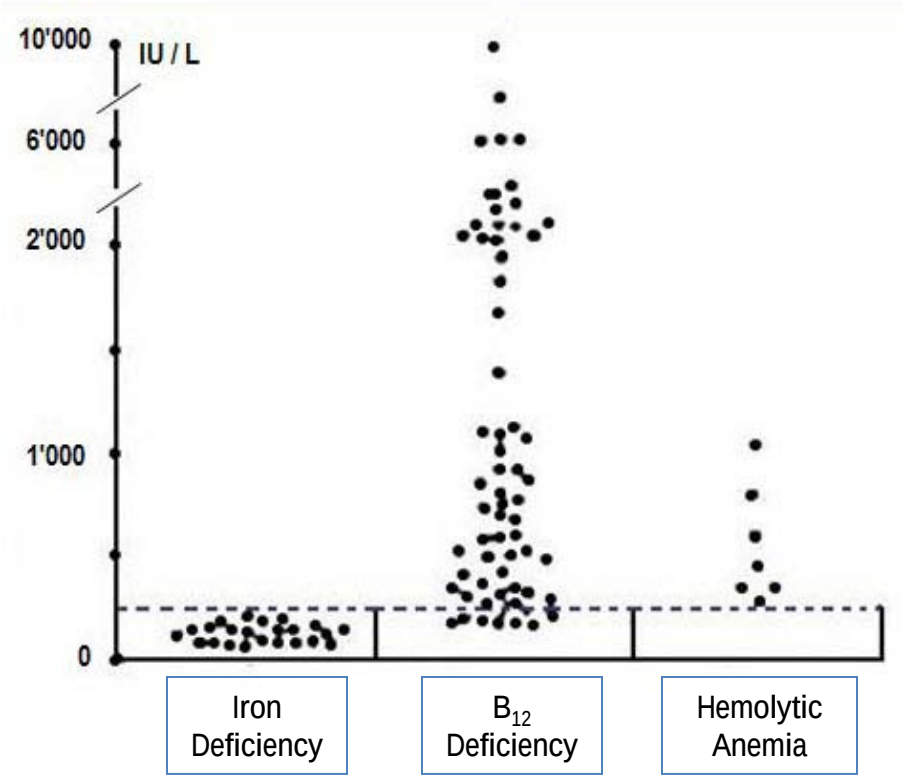
ABSORPTION OF VITAMIN B_{12}



PHYSIOPATHOLOGICAL MECHANISMS OF VITAMIN B_{12} (COBALAMINE) DEFICIENCY

1. Cobalamin dietary deficiency
2. Anomaly of cobalamine - food dissociation
3. Quantitative or qualitative defect of Intrinsic Factor (IF)
4. Deficiency of pancreatic protease
   Abnormal utilization of vitamin B_{12} by bacterias (blind loop syndrome), fish worm (diphyllobothrium latum)
5. Anomaly of ileal mucosa and / or of the IF receptors and / or transfer in the enterocyte

LDH AND ANEMIA



LDH activity in iron deficiency, megaloblastic and hemolytic anemias
Dotted line : upper limit of the reference interval

Modified from Emerson P.M., Wilkinson J.H., Br J Haematol 1966; 12 : 678-688.

MEGALOBLASTIC ANEMIA WITH DNA SYNTHESIS ANOMALY

Nuclear maturation slowdown
Optimal hemoglobin concentration reached before the usual 4 mitosis
Reduction of the number of mitosis
Increased size of the cells
Bone marrow : *megaloblasts*
Peripheral blood : *megalocytes ("macroovalocytes")*
Intramedullary and peripheral hemolysis
Bone marrow with megaloblastic hyperplasia by erythroid stem cell recruitment through erythropoietin

SCHILLING TEST

Saturation of transcobalamins by IM injection of 1 mg vitamin B_{12}
Oral administration of 0.5 -1 μg radiolabeled vitamin B_{12}
48 hours urine collection and measure of excreted radioactivity
In case of pathological result repeat the test with concomitant oral intrinsic factor administration (IF)

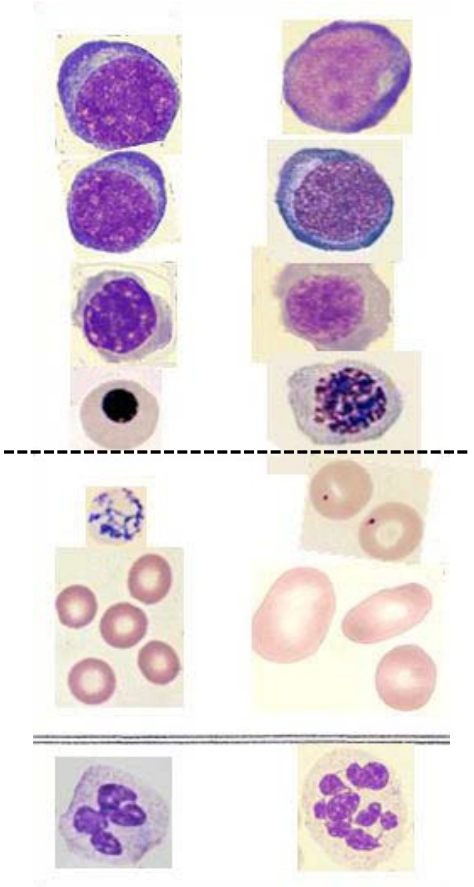
	Urinary excretion of radiolabeled vitamin B_{12} (%)	
	B_{12} alone	B_{12} + IF
Normal subject	18 (9 – 36)	–
Pernicious anemia	0.5 (0 – 1.2)	13 (6 – 31)
Malabsorption (Gluten enteropathy)	3.6 (0 – 19)	3.3 (0 – 10)

Results obtained with 0.5 μg of radiolabeled oral vitamin B_{12}

Modified from Lee G.R., Wintrobe's Clinical Hematology, 9th edition 1993; Lea & Febiger : p. 776.

NORMAL AND MEGALOBLASTIC ERYTHROPOIESIS

BONE MARROW	NORMAL ERYTHROPOIESIS	MEGALOBLASTIC ERYTHROPOIESIS
CELLULARITY	NORMAL	INCREASED



BLOOD	
	PROERYTHROBLASTS
	EARLY ERYTHROBLASTS
	INTERMEDIATE ERYTHROBLASTS
	LATE ERYTHROBLASTS
	RETICULOCYTES
	RED BLOOD CELLS
WHITE BLOOD CELLS	
	NEUTROPHILS

MEGALOBLASTS (Asynchronism of nucleocytoplasmic maturation)
NORMAL HEMOGLOBIN SYNTHESIS
HOWELL-JOLLY BODIES
LOW OR ABSENT RETICULOCYTE COUNT
MACROCYTES MEGALOCYTES
HYPERSEGMENTED NEUTROPHILS

Modified from Chandrasoma P., Taylor C.R. : Concise Pathology, 3th edition 1998; Appleton & Lange.

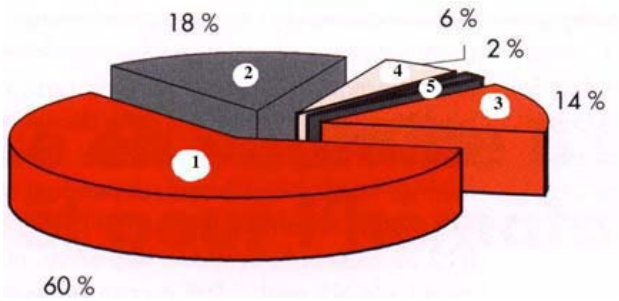
CAUSES OF VITAMIN B_{12} DEFICIENCY

MALABSORPTION

Gastric origin : *Achlorhydria*
Pernicious anemia
Partial or total gastrectomy
Congenital intrinsic factor deficiency

Intestinal origin : *Resection of terminal ileum*
Crohn's disease
Gluten induced enteropathy
Fish tapeworm (Diphyllobothrium latum) infestation

Dietary deficiency



1. Non dissociation of Vitamin B_{12} from the transport proteins or insufficient digestion of dietary vitamins B_{12}
2. Pernicious anemia
3. Undefined
4. Malabsorption
5. Poor diet

Distribution of causes of vitamin B_{12} deficiency in adults

Andrès E. et al. : Hématologie 2007; 13 : 186-192.

PERNICIOUS ANEMIA (1)

PATHOPHYSIOLOGY

Atrophic gastritis of immune origin with lack of intrinsic factor

HEMATOLOGY

Macrocytic megaloblastic anemia
Neutropenia with hypersegmented neutrophils
Thrombocytopenia

CLINICAL ASPECTS

Atrophic glossitis (Hunter's glossitis), dyspepsia
Combined degeneration of the dorsal *(posterior)* and lateral spinal columns *(paresthesias, pain, gait disturbance, pallesthesia diminution, pyramidal syndrome)*
→ *Methionine synthesis defect ?*
Psychiatric symptoms *(irritability, depression)*
Melanic skin hyperpigmentation *(uncommon !)*
Sterility, asthenospermia

PERNICIOUS ANEMIA (2)
LABORATORY

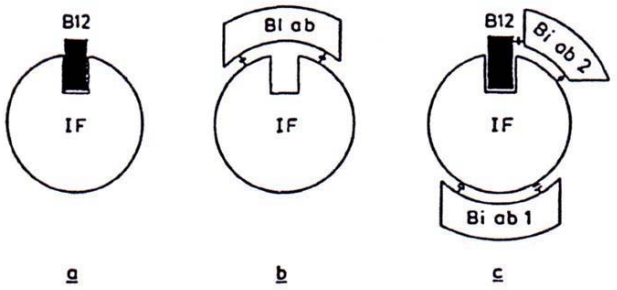
SCHILLING TEST

Pathological but normalized after simultaneous administration of vitamin B_{12} + intrinsic factor

ANTIBODY SCREENING

	Antiparietal cells (± 90%) [1]	Anti-intrinsic factor (± 50%)
Specificity	–	+
Sensitivity	+	–

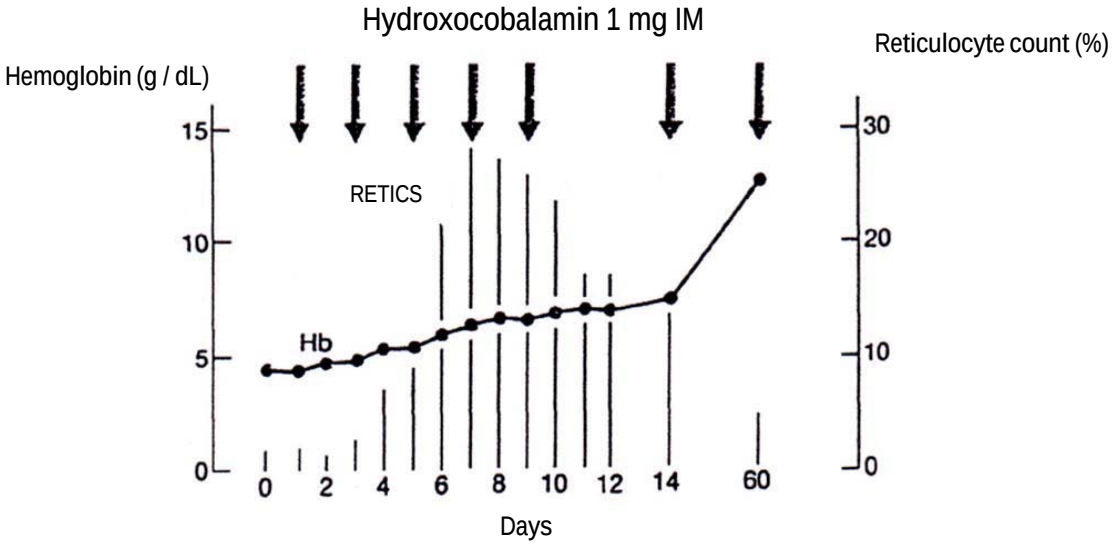
[1] Antiparietal cells antibodies can be found in normal individuals (5-20%) and in myxedema (~ 30%)

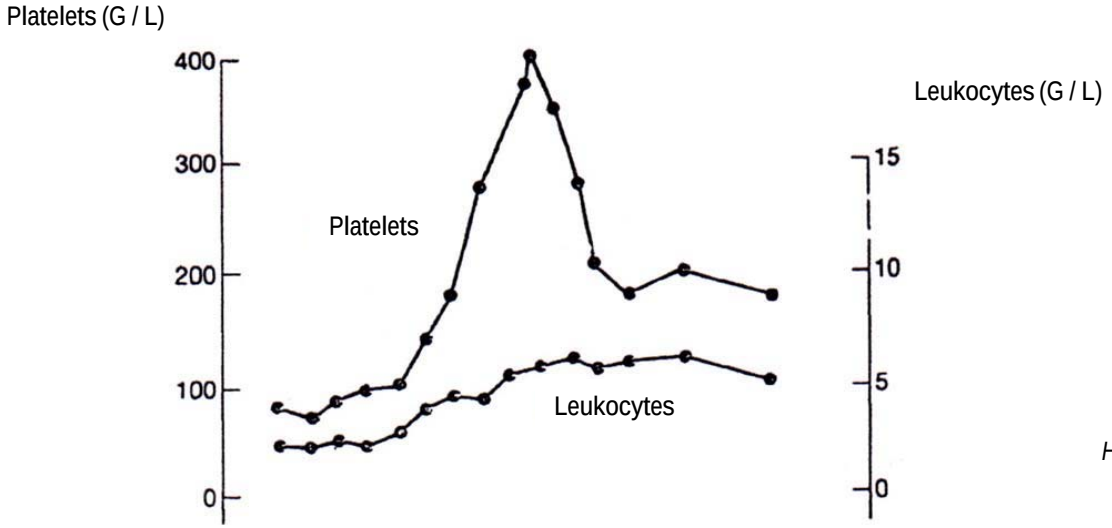


Schematic presentation of intrinsic factor (IF), vitamin B_{12} and of antibody directed against intrinsic factor :
a) Normal binding between IF and vitamin B_{12}
b) Blocking antibody
c) Coupling antibody

Modified from Lee G.R. : Wintrobe's Clinical Hematology, 9th edition 1993; Lea & Febinger : p. 753.

PERNICIOUS ANEMIA (3)
RESPONSE TO HYDROXOCOBALAMIN SUBSTITUTION





Hoffbrand A.V., Pettit J.E. : Essential Haematology, 3th edition; Blackwell Science : p. 70.

CAUSES OF FOLATE DEFICIENCY

DIETARY DEFICIENCY

MALABSORPTION

Gluten induced enteropathy
Wide jejunal resection
Crohn's disease

INCREASED DEMAND

Physiological : *Pregnancy*
Lactation
Prematurity
Growth

Pathological : *Hemolytic anemia*
Cancer, myeloid or lymphoid neoplasm
Inflammatory process

DRUGS

Anticonvulsants (e.g. : Diphenylhydantoin)
Barbiturates
Salazopyrin

ALCOHOLISM

WORKUP OF MACROCYTIC ANEMIA
WITH OR WITHOUT NEUTROPENIA AND / OR THROMBOCYTOPENIA

1. RETICULOCYTE COUNT

   Regenerative anemia ?

2. FOLATES AND VITAMIN B_{12} SERUM LEVELS

   DNA synthesis disorder ?

3. TESTS OF THYROID FUNCTION

   Hypothyroidism ?

4. ALCOHOLISM INVESTIGATION

5. IF 1-4 NEGATIVE $\rightarrow$ BONE MARROW CYTOLOGY AND HISTOLOGY

   Myelodysplastic syndrome ?
   Bone marrow aplasia ?

NORMOCYTIC NORMOCHROMIC REGENERATIVE ANEMIA

MCV :	normal	81 – 99 fL
MCH :	normal	27 – 34 pg
MCHC :	normal	310 – 360 g / L
Reticulocyte count :		> 120 G / L

ACUTE BLOOD LOSS (1)

BLOOD LOSS	% BLOOD VOLUME	SYMPTOMS
0.5 – 1.0 L	10 – 20	Possible vaso-vagal signs
1.0 – 1.5 L	20 – 30	Tachycardia / hypotension
1.5 – 2.0 L	30 – 40	Reversible hypovolemic shock
> 2.0 L	> 40	Irreversible hypovolemic shock

ACUTE BLOOD LOSS (2)

Evolution in 2 phases :

1. Hypovolemia (1-3 days)
2. Volemia normalization

Anemia is only found during phase of volemia correction

Anemia normocytic normochromic as far as iron stores not exhausted

To be remembered : 1 L of blood = 500 mg of iron

Increase of the reticulocyte count from the 4th day, possibly neutrophilic leukocytosis with left shift, myelocytosis *(presence of some peripheral blood myelocytes and metamyelocytes)*, thrombocytosis

Treatment :

Phase 1 : *packed red cells and plasma*
Phase 2 : *packed red cells*

HEMOLYTIC ANEMIA
BASIC DATA (1)

HISTORY

Ethnic origin, family history
Stay in a foreign country
Drug treatment
Prior transfusion(s), pregnancy(-ies)

CLINICAL FEATURES

Jaundice
Splenomegaly

HEMOGRAM

Normocytic normochromic anemia

Particular situations :

Absence of anemia in case of compensated hemolysis
Microcytic anemia : thalassemia, hemoglobinopathies E, C, PNH[1]
Macrocytic anemia : high reticulocyte count, associated folate deficiency

Regeneration signs

Polychromasia
Increased reticulocyte count
Presence of peripheral blood erythroblasts

Red blood cell morphology

Spherocytes, schistocytes, sickle cells, target cells

[1] PNH : Paroxysmal Nocturnal Hemoglobinuria (iron deficiency due to chronic hemoglobinuria)

HEMOLYTIC ANEMIA
BASIC DATA (2)

BLOOD CHEMISTRY

- ⇗ unconjugated bilirubin
- ⇗ L D H
- ⇘ haptoglobin
- ⇗ fecal stercobilinogen
- Urobilinuria

ISOTOPIC TESTS (^{51}Cr) : cf. following page

EXTRAVASCULAR HEMOLYSIS

"Sensitization" of circulating RBC and destruction by the monocyte / macrophage system (*spleen, lymph nodes, bone marrow)*

INTRAVASCULAR HEMOLYSIS

- ⇗ plasmatic Hb (> 50 mg / L)
- Hemoglobinuria
- Hemosiderinuria

HEMOLYSIS DUE TO CORPUSCULAR ANOMALY

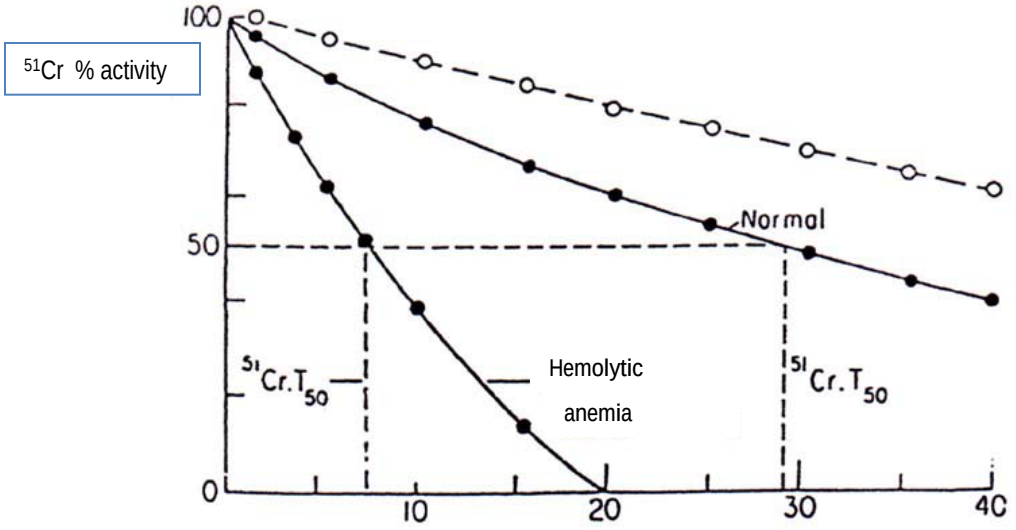
- Hereditary *(except PNH[1])*
- Homozygous or heterozygous

HEMOLYSIS DUE TO EXTRACORPUSCULAR ANOMALY

- Acquired

[1] PNH : Paroxysmal Nocturnal Hemoglobinuria

MEASURE OF RED BLOOD CELLS HALF LIFE
^{51}Cr LABELLING

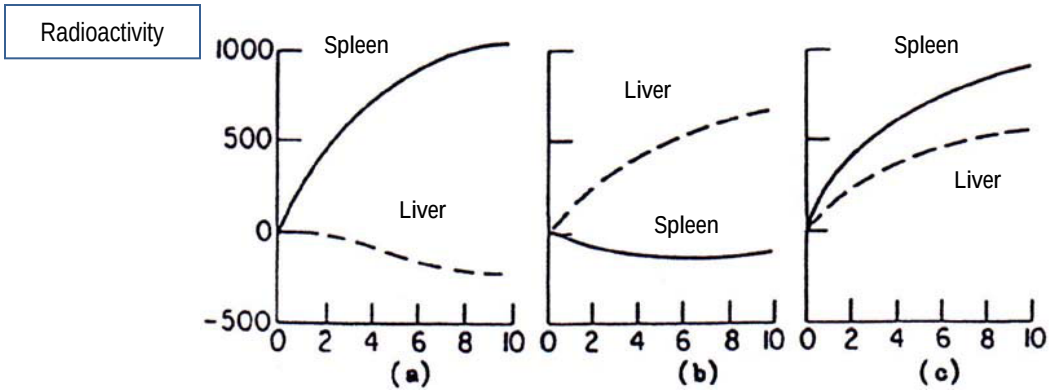


Measure of RBC half life with ^{51}Cr labeling ($^{51}CrT_{50}$)

o- -o- -o : Theoretical curve

•—•—• : Normal curve with half life of 30 ± 2 days

Pathological curve with half life < 10 days



External counts during ^{51}Cr test :

a) Predominant splenic sequestration *(hereditary spherocytosis)*
b) Predominant hepatic sequestration *(sickle cell disease)*
c) Mixed sequestration *(splenic and hepatic)* *(some forms of immune hemolytic anemia)*

Modified from Hoffbrand A.V., Pettit J.E. : Essential Haematology, 3th edition 1993; Blackwell Science : p. 76.

HEMOLYTIC ANEMIA DUE TO CORPUSCULAR DEFECT

ENZYMOPATHY

RBC MEMBRANE ANOMALY

HEMOGLOBINOPATHY

Diminution (or absence) of globin chains synthesis

THALASSEMIAS (cf. pages 44-46)

Substitution (or deletion) of a residue on a globin chain

SICKLE CELL DISEASE

HEMOGLOBINS E, C

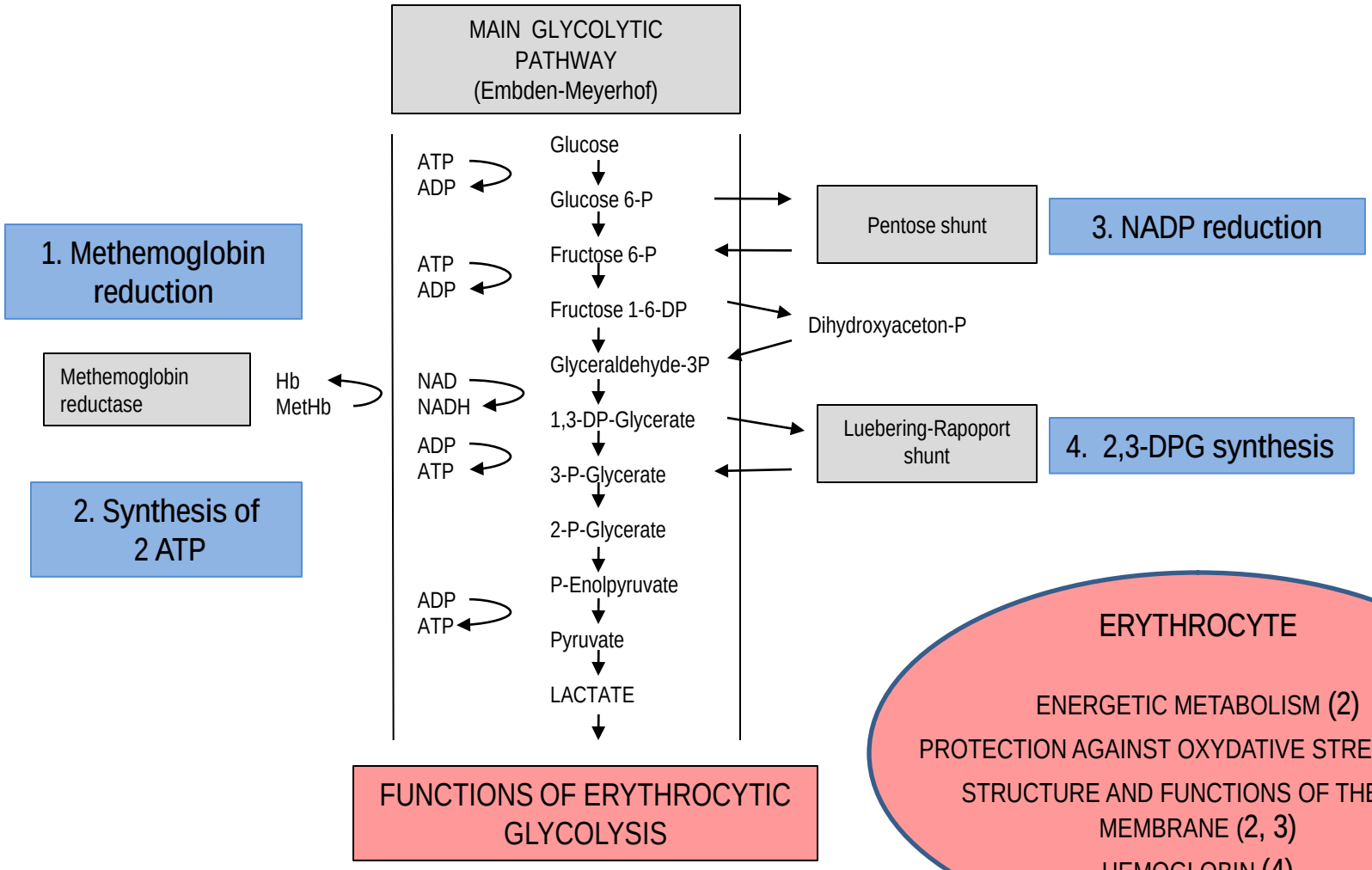
UNSTABLE HEMOGLOBINS

HEMOGLOBINS M[1]

HEMOGLOBINS WITH INCREASED OR REDUCED OXYGEN AFFINITY

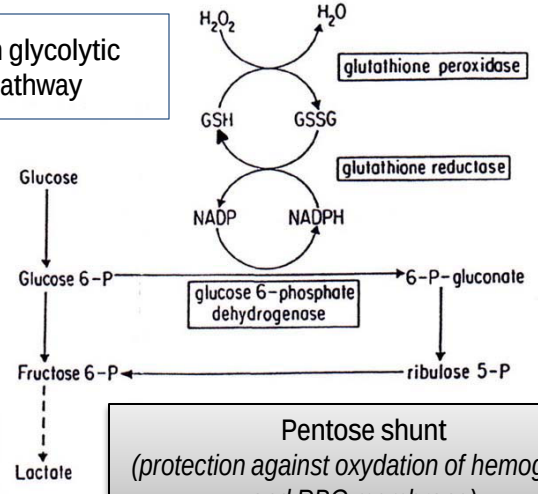
[1] M : Methemoglobin

ERYTHROCYTIC GLYCOLYSIS (1)

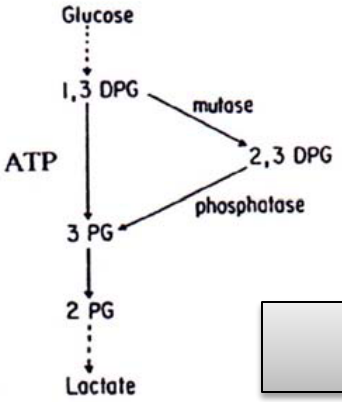


GLYCOLYSIS (2) / STRUCTURE OF THE RBC MEMBRANE

Main glycolytic pathway



Pentose shunt
(protection against oxydation of hemoglobin and RBC membrane)

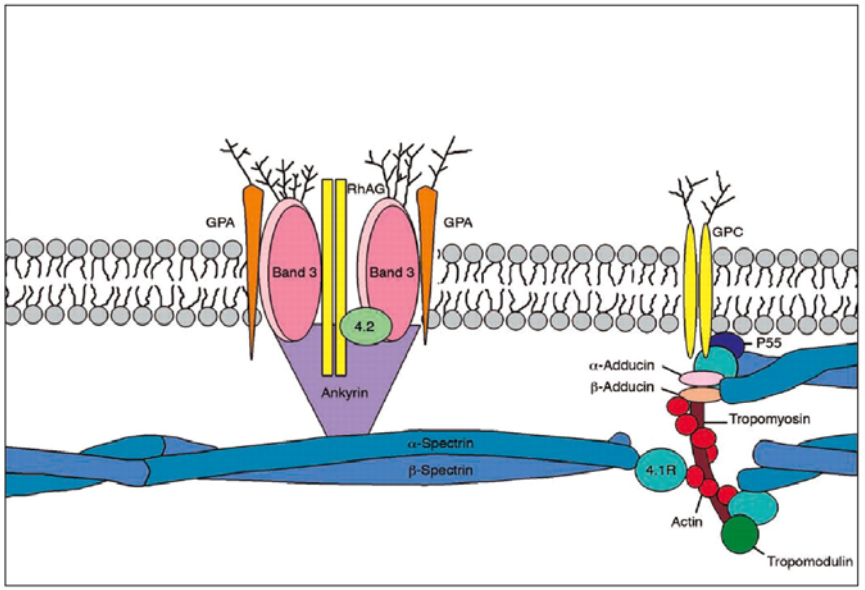


Luebering-Rapoport shunt
(synthesis of 2,3-DPG)

STRUCTURE OF RBC MEMBRANE

Composite structure with double layer lipidic membrane anchored to a two-dimensional elastic network (**cytoskeleton**) with tethering sites (transmembrane proteins)
Vertical fixation involves cytoplasmic part of **Band 3** protein, **Ankyrin**, **Protein** 4,2 and **Spectrin**. The horizontal interaction involves **Spectrin** (α- and β-chains), **Protein** 4.1.R, **Actin**, **Tropomodulin**, **tropomyosin** and **Adducin**. Protein 4.1R interacts with **Glycophorin C** (GPC) and P55

RhAG : Rhesus Antigens GPA : Glyocophorin A



Iolascon, A. et al. Haematologica 2008;93:1283-1288

RED BLOOD CELL ENZYMOPATHY

FREQUENT

PENTOSE SHUNT

Glucose-6-phosphate dehydrogenase (G-6-PD) deficiency
(> 400 .10^6 cases, > 300 variants)

EMBDEN-MEYERHOF PATHWAY

Pyruvate kinase deficiency *(< 1'000 cases)*
Glucose phosphate isomerase deficiency *(< 200 cases)*

UNCOMMON

EMBDEN-MEYERHOF PATHWAY

Deficiency in : Hexokinase, phosphofructokinase, aldolase, triose phosphate isomerase, diphosphoglycerate mutase, phosphoglycerate kinase (< 20 cases)

GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENCY (G-6-PD) (1)

Amino acid substitution in some variants of G-6-PD

Variants	Position of residue				
	68	126	188	227	323
B (+)	Valine	Asparagine	Serine	Arginine	Leucine
A (+)		Aspartic acid			
A (-)	Methionine				
A (-)				Leucine	
A (-)					Proline
Mediterranean			Phenylalanine		

B (+) : *Usual form : predominant*

A (+) : *30% African colored : normal activity*

A (-) : *11% African American : activity 5-15% of normal*

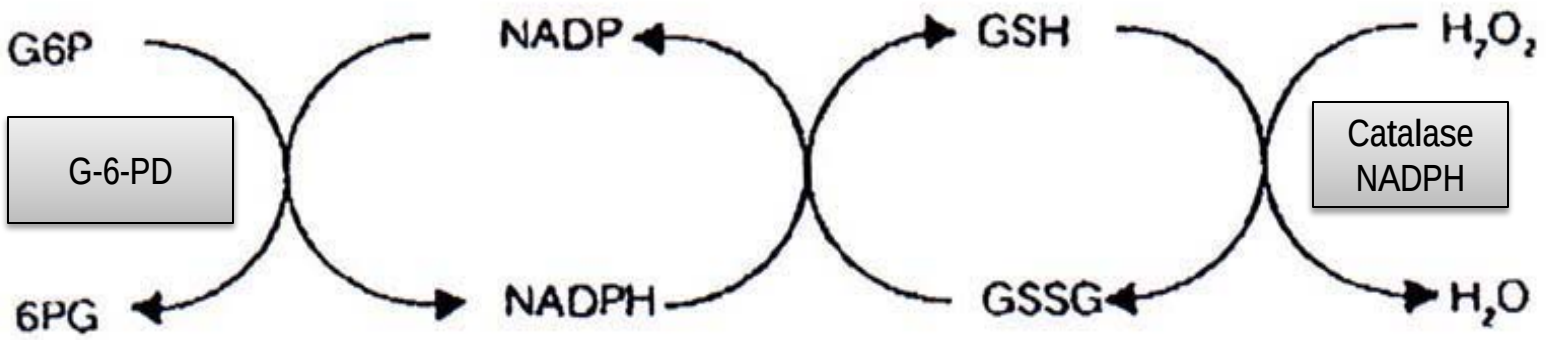
Mediterranean [formerly B (-)] : *Activity < 1%*

X-linked recessive deficiency

Hemolysis : *Chronic (uncommon)*

Usually induced by : drugs, fever, fava beans (Favism)

GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENCY (G-6-PD) (2)
PATHOPHYSIOLOGY



Reduced glutathione (GSH) protects the -SH groups of the RBC membrane and hemoglobin

During hemolytic crisis, presence of *Heinz bodies* in the RBC after staining with brilliant cresyl blue : denatured hemoglobin *(oxidized)*

Decrease in hemolysis during reticulocyte response *(young RBC are relatively enzyme rich)*

GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENCY (G-6-PD) (3)

Main substances able to induce hemolytic crisis in G-6-PD deficiency[1]

ANTIMALARIAL DRUGS

Primaquine, pamaquine, pentaquine, quinine

SULFONAMIDES

Sulfacetamide, sulfamethoxazole, sulfanilamide, sulfapyrine, sulfoxone, thiazosulfone

ANTIBIOTICS AND BACTERIOSTATIC AGENTS

Para-aminosalicylic acid, nalidixic acid, nitrofurantoin, chloramphenicol, methylene blue, niridazole

ANALGESICS

Acetanilide, amidopyrine, paracetamol

OTHERS

Toluidin blue, naphtalene, phenylhydrazine, probenecid, trinitrotoluen

FOOD

Beans *(fava beans…)*

[1] Because of disease polymorphism, these substances are not necessarily dangerous for all G-6-PD deficient subjects. Nevertheless they should be avoided because of the unpredictable tolerance of each subject

Modified from Wajcman H., Lantz B., Girot R. : Les maladies du globule rouge 1992; Médecine-Sciences Flammarion : p. 262.

ANOMALY OF RED BLOOD CELL MEMBRANE

HEREDITARY SPHEROCYTOSIS

- AUTOSOMAL DOMINANT *(cf. following pages)*
- AUTOSOMAL RECESSIVE *(frequent in Japan; protein 4.2 mutations)*
- AUTOSOMAL DOMINANT WITH ACANTHOCYTOSIS

HEREDITARY ELLIPTOCYTOSIS

- Anomaly of spectrin, protein 4.1

HEREDITARY STOMATOCYTOSIS

ABETALIPOPROTEINEMIA WITH ACANTHOCYTOSIS[1]

[1] Not to be mistaken for acanthocytosis secondary to severe liver disorder

HEREDITARY SPHEROCYTOSIS
AUTOSOMAL DOMINANT (1)

PATHOPHYSIOLOGY

Anomalies of spectrin, ankyrin, band 3, which may be combined
Spherocytes with loss of plasticity and splenic trapping *(sequestration)*

Volume generally normal
Diameter ⇩
Surface ⇩

Increase of membrane permeability for Na^+ (glycolytic activity ⇗)

CLINICAL FEATURES

Chronic hemolytic anemia

⇗ if : pregnancy
exercise
intercurrent viral infection (EBV, etc)

Splenomegaly
Negative Coombs test
⇘ osmotic resistance
⇗ autohemolysis, corrected by glucose
Pure splenic RBC destruction
Aplastic crises *(Parvovirus B19)*
Frequent cholelithiasis

TREATMENT

Splenectomy *(severe forms only)*

AUTOSOMAL DOMINANT HEREDITARY SPHEROCYTOSIS (2)

Clinical classification of hereditary spherocytosis (HS)

	Trait	Light HS	Moderate HS	Moderate to severe HS[1]	Severe HS[1]
Hb (g / L)	Normal	110 – 150	80 – 120	60 – 80	< 60
Reticulocyte count (‰)	1 – 30	30 – 80	**≥ 80**	**≥ 100**	**≥ 100**
Spectrin content[2] (% of normal)	100	80 – 100	50 – 80	40 – 80	20 – 50
Spherocytes	-	+	+	+	+ with poikilocytosis
Osmotic resistance	normal	normal / ↘	↘↘	↘↘	↘↘
Autohemolysis	slightly ↗	↗↗	↗↗	↗↗	↗↗↗

[1] Values in absence of transfusion. Patients with severe HS are transfusion dependent

[2] Reference values (± SD) : 245 ± 27 x 10^5 spectrin dimers / RBC
In most patients ankyrin content is reduced in parallel. A low number of patients present with absence of band 3 or protein 4.2; in this case HS is light to moderate with normal amounts of spectrin and ankyrin

Modified from Eber S.W., Armbrust R., Schroter W., J Pediatr 1990; 117 : 409-416, & Pekrun A., Eber S.W., Kuhlmey A., Schroter W., Ann Hematol 1993; 67 : 89-93.

PAROXYSMAL NOCTURNAL HEMOGLOBINURIA (PNH) (1)
PATHOPHYSIOLOGY

Mutation of a gene on chromosome X coding for the glycosyl phosphatidyl inositols *(membrane anchoring proteins)* named PIGA *(= Phosphatidyl Inositol Glycan complementation class A) with deficiency of membrane anchor proteins*

3 types of RBC :

PNH I :	normal
PNH II :	intermediate
PNH III :	abnormal

RBC lysis by complement due to membrane protein anomalies like :

CD55 :	Decay Accelerating Factor (DAF)
CD59 :	Membrane Inhibitor of Reactive Lysis (MIRL) or Homologous Restriction Factor (HRF)

Clonal anomaly of hematopoietic stem cell

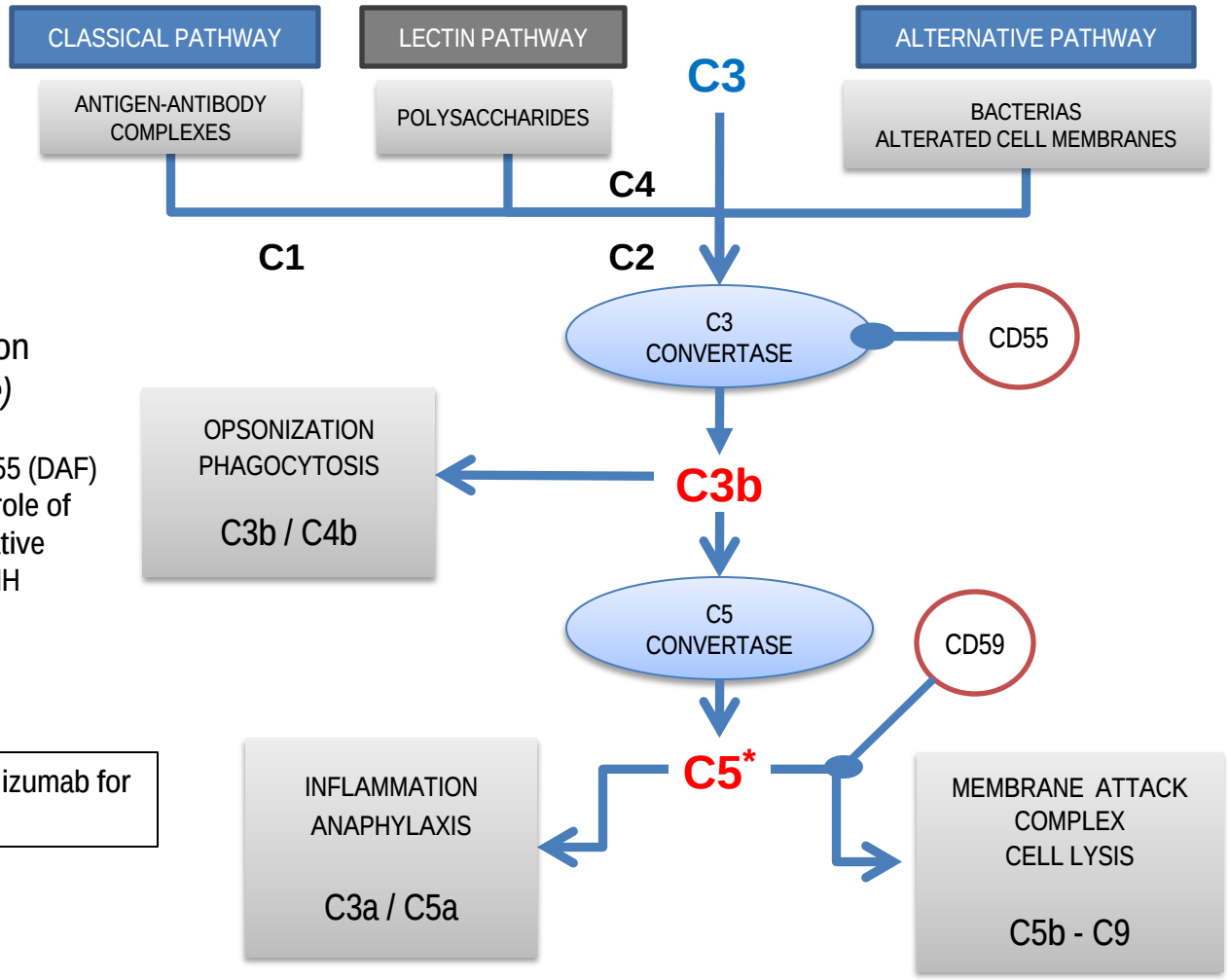
Lysis affects also neutrophils and platelets which also present functional anomalies

Relation with *aplastic anemia*

PAROXYSMAL NOCTURNAL HEMOGLOBINURIA (PNH) (2)

Outline of the complement activation pathways *(classical and alternative)*

The 2 membrane regulatory proteins CD55 (DAF) and CD59 (NIRL/HRF) play an inhibitory role of the complement activation by the alternative pathway. They are missing on RBC in PNH



* Target for monoclonal antibody Eculizumab for treatment of PNH. Cf. page 78

PAROXYSMAL NOCTURNAL HEMOGLOBINURIA (PNH) (3)

CLINICAL FEATURES

Hemolytic anemia with hemoglobinuria (nocturnal)

↘ of pH during sleep ? (controversial)

Depending on the size of the PNH III clone. Promoted by infections, surgery, violent exercise, alcohol, transfusions

Splenomegaly

Thromboembolic manifestations (*Budd-Chiari syndrome : thrombosis of hepatic veins)*

Median survival : 14.6 years *(Socié G. et al., Lancet 1996; 348 : 573-577.)*

Causes of death : Thromboses
Hemorrhage

Possible evolution : Aplastic anemia
Acute leukemia

DIAGNOSIS

Immunophenotyping : Deficiency(-ies) of CD55 (DAF), CD59 (MIRL / HRF), CD58 (LFA-3) on *RBC;*
CD55, CD59, CD58, CD16, CD24 and CD66b on *neutrophils :* markers anchored on the cellular membrane by the way of Glycosyl Phosphatidylinositols (GPI-linked)
FLAER test *(Sutherland D.R. et al., Cytometry Part B (Clinical Cytometry) 2007; 72B : 167-77 and Am J Clin Pathol 2009; 132 : 564-72)*

Ham-Dacie test *(acid test[1])*

Sucrose test[1]

TREATMENT

Transfusion

Eculizumab (monoclonal antibody anti-C5)

Iron substitution if deficiency (may increase hemolysis by stimulation of PNH III clone)

Allogeneic stem cell transplantation (ev. bone marrow) in severe cases

[1] These tests are obsolete and should be replaced by immunophenotyping

ANOMALY OF HEMOGLOBIN
HEMOGLOBINOPATHY

Approximately 1'000 mutants (*2008)*
Frequent mutants : S, E, C

SICKLE CELL DISEASE (Hb S) : *cf. following pages*

HEMOGLOBIN E

β26 Glu → Lys
South-East Asia
Microcytic anemia with target cells

HEMOGLOBIN C

β6 Glu → Lys
Africa
Microcytic anemia with target cells

UNSTABLE HEMOGLOBINS

Hb Zurich (β63 His → Arg)
Hemolysis with Heinz bodies after intake of oxidant drugs *(sulfonamides)*

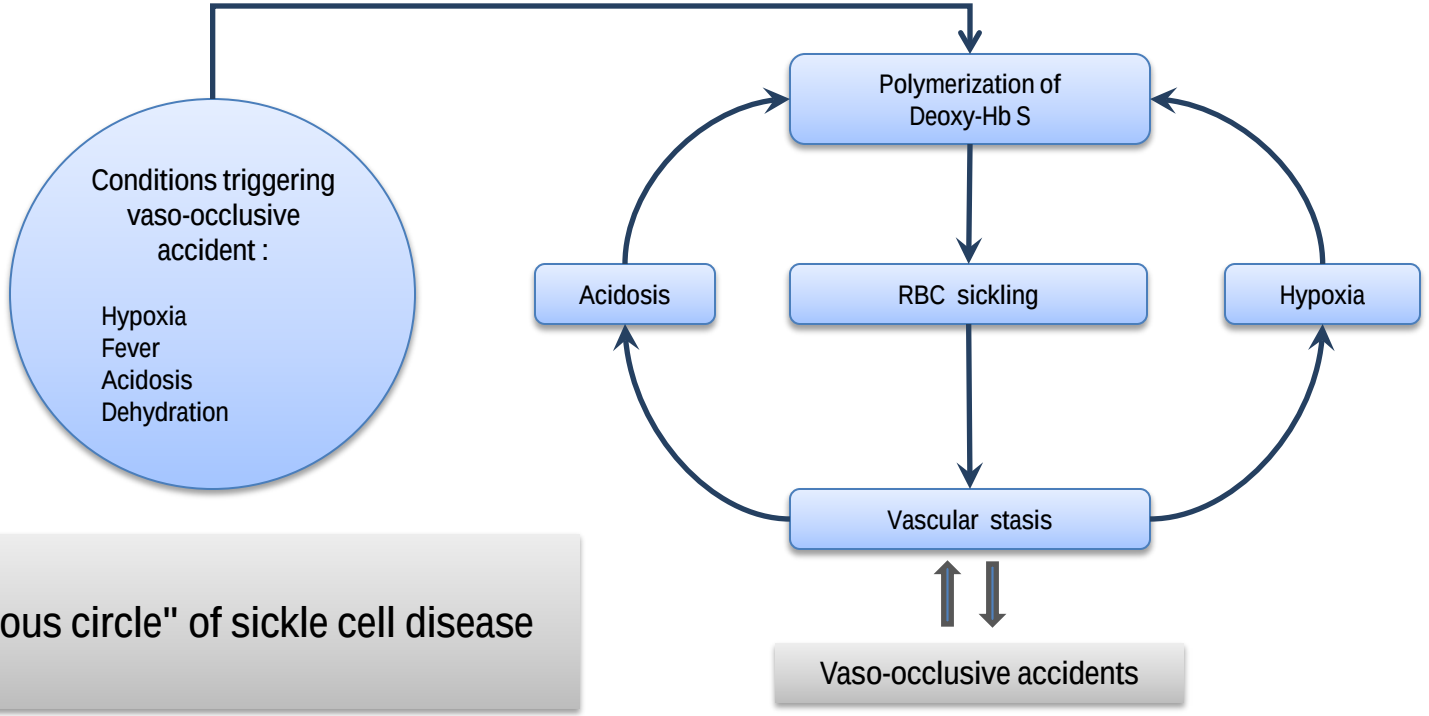
HEMOGLOBINS M

Cyanosis due to methemoglobinemia

HEMOGLOBINS WITH INCREASED OR REDUCED OXYGEN AFFINITY

SICKLE CELL DISEASE (1)
PATHOPHYSIOLOGY

Autosomal recessive transmission
Hemoglobin S : β6 Glu → Val
Polymerization in deoxygenated form : shape alteration of RBC to *drepanocytes ("sickling")* with loss of plasticity



"Vicious circle" of sickle cell disease

Modified from Wajcman H., Lantz B., Girot R. : Les maladies du globule rouge 1992; Médecine-Sciences Flammarion : p. 184.

SICKLE CELL DISEASE (2)

Africa, Arabia, India, Mediterranean region, African Americans

CLINICAL FEATURES

HETEROZYGOUS VARIETY (A - S)

Approximately 30% of Hemoglobin S
Asymptomatic, occasionally kidneys may be affected with hyposthenuria, hematuria *(microinfarctions of medullary zone)*
Avoid severe hypoxemia (apnea diving, general anesthesia)
Protection against malaria

HOMOZYGOUS VARIETY (S - S)

Symptomatic since the age of 6 months : Hb F $\rightarrow$ Hb S
5 typical clinical manifestations :

1. Vaso-occlusive crises
2. Splenic sequestration crises (children < 4 years)
3. Aplastic crises
4. Hemolytic crises
5. Infectious complications

DIAGNOSIS

Hemoglobin electrophoresis
Screening by Emmel test or *in vitro RBC sickling test (Sodium metabisulfite as reducing agent)*

TREATMENT

Rest / hydration / analgesia / exchange transfusion(s)
Hydroxyurea *(increased synthesis of Hb F)*

HEMOLYTIC ANEMIA DUE TO EXTRACORPUSCULAR DEFECT

IMMUNOLOGICAL

AUTOIMMUNE (AIHA)

Warm autoantibodies : IgG, IgA ± C3, C3 alone

- Idiopathic AIHA (20%)
- Secondary AIHA (80%)
  - Lymphoid neoplasm (50%)
  - Infectious disease (30%)
  - Lupus erythematosus, other systemic autoimmune disease (15%)
  - Cancer (ovary, stomach), drugs, others (5%)

Cold autoantibodies *(cold agglutinins)* : IgM + C3

- Polyclonal *(idiopathic, EBV, CMV, Mycoplasma pneumoniae)*
- Monoclonal *(lymphoid neoplasm, cold agglutinins disease)*

ALLOIMMUNE

Transfusion accident *(ABO or Rhesus incompatibility)*
Neonatal hemolytic anemia
Organ or bone marrow graft with ABO incompatibility

IMMUNOALLERGIC

Drugs *(penicillin and derivatives)*

TOXIC

INFECTIOUS

MECHANICAL

HYPERSPLENISM

All causes of splenomegaly, e.g. hepatic cirrhosis with portal hypertension. Presence of associated other cytopenias

HEMOPHAGOCYTOSIS

Viral, bacterial, mycotic and parasitic infections in immunodeficient patients

TOXIC HEMOLYTIC ANEMIA (1)
OXIDATIVE ORIGIN

PATHOPHYSIOLOGY

Hemoglobin oxidation to methemoglobin, then transformation to *hemichromes* which precipitate to form *Heinz bodies.* Oxidation of RBC membrane components

RESPONSIBLE SUBSTANCES

Industrial chemicals *(nitrites, chlorates, naphtalene, aniline derivatives)*
Drugs

MAIN DRUGS SUSCEPTIBLE TO INDUCE OXYDATIVE HEMOLYTIC CRISIS

ANTIMALARIALS :

Pamaquine, pentaquine, primaquine, quinine

SULFONAMIDES :

Sulfacetamide, sulfamethoxazole, sulfanilamide, sulfapyridine, sulfoxone, thiazosulfone, etc.

ANTIBIOTICS AND BACTERIOSTATIC AGENTS :

Para-aminosalicylic acid, nalidixic acid, nitrofurantoin, chloramphenicol, etc.

ANTIPARASITIC DRUGS :

Niridazole

ANALGESICS :

Acetanilide, amidopyrine, paracetamol, phenacetin, etc.

OTHERS :

Chloramine, formaldehyde, chlorates, nitrites, methylene blue, toluidine blue, naphtalene, phenylhydrazine, probenecid, trinitrotoluene

TOXIC HEMOLYTIC ANEMIA (2)
MULTIFACTORIAL ORIGIN

LEAD POISONING
- Pathophysiology
  - Heme synthesis defect *(inhibition of porphyrin metabolism enzymes)*
  - Inhibition of pyrimidine-5-nucleotidase
  - Inhibition of membrane pumps activity
- Clinical features
  - Acute abdominal pain
  - Neurological signs *(central and peripheral)*
  - Articular, renal, hepatic manifestations, arterial hypertension
- RBC morphology
  - Coarse basophilic stippling

COPPER POISONING
- Pathophysiology
  - Enzymatic inhibition *(G-6-PD in particular)*
- Clinical features
  - Vomiting, abdominal pain
  - Hepatic cytolysis, renal failure
- Etiology
  - Vine treatment
  - Wilson disease
  - Contamination of dialysis fluids

VENOMS *(spiders, snakes, scorpions)*

HEMOLYTIC ANEMIA OF INFECTIOUS ORIGIN

DIRECT ACTION ON RED BLOOD CELL

PARASITES

MALARIA

Plasmodium falciparum, vivax, malariae, ovale

Protection by : Enzymopathy
Hemoglobinopathy
Membrane anomaly
Blood group Duffy (-) : *Pl. vivax*

BABESIOSIS

BACTERIAS

CLOSTRIDIUM PERFRINGENS (septic abortion)

BARTONELLOSIS (Oroya fever)

OTHER PATHOPHYSIOLOGICAL MECHANISM

Immunological *(cold agglutinins due to Mycoplasma pneumoniae, EBV infection)*

Microangiopathic hemolysis *(HIV)*

HEMOLYTIC ANEMIA DUE TO MECHANIC RBC FRAGMENTATION (1)
SCHISTOCYTES

CARDIOVASCULAR DISORDERS

Valvular heart disease, operated or not
Anomalies of great blood vessels *(aortic coarctation)*
Extracorporeal circulation

MICROANGIOPATHY

THROMBOTIC THROMBOCYTOPENIC PURPURA (TTP[1]) *(Moschcowitz syndrome)*

ADAMTS 13 deficiency *(metalloproteinase cleaving high molecular weight von Willebrand factor multimers)*

Clinical features : *Fever*
Hemolytic anemia
Thrombocytopenia
Neurological symptoms
Renal failure

Treatment : *Plasma exchanges (3 – 4 L / 24 h)*

HEMOLYTIC UREMIC SYNDROME (HUS[2])

Sporadic form *(D^{*-}HUS) : ± 10% pediatric cases*
Epidemic form *(D^{*+}HUS) : Verotoxin associated (Escherichia coli O157 : H7) : children ± 85%, adults ± 15%*

Clinical features : *Predominant renal failure*
Gastroenteritis with bloody diarrheas (D^+ HUS)

Treatment : *Dialysis*

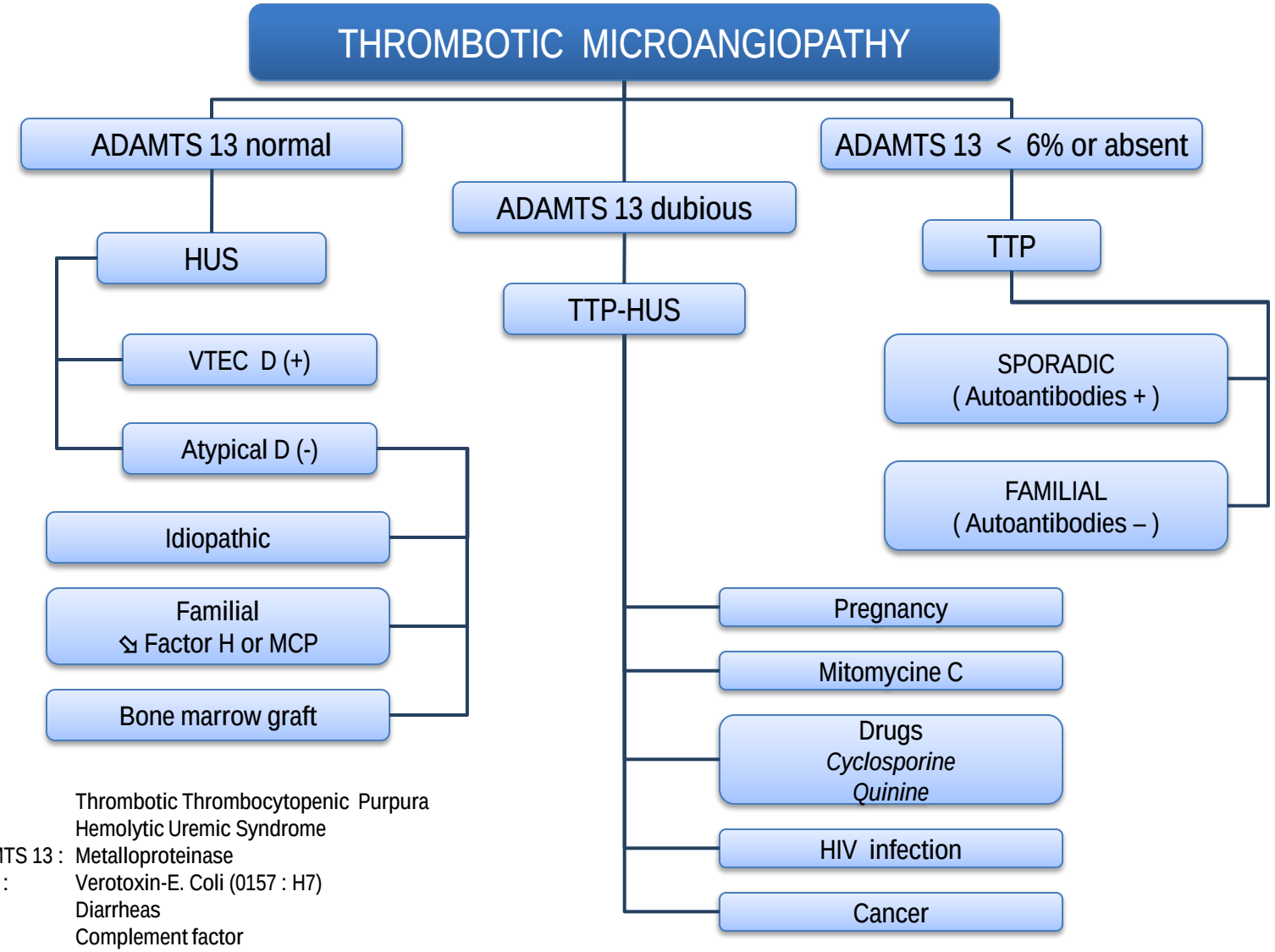
* *Diarrheas*

DISSEMINATED INTRAVASCULAR COAGULATION

TRAUMATIC ORIGIN *(march hemoglobinuria)*

[1]TTP : Thrombotic Thrombocytopenic Purpura
[2]HUS : Hemolytic Uremic Syndrome

HEMOLYTIC ANEMIA DUE TO MECHANIC RBC FRAGMENTATION (2)
SCHISTOCYTES

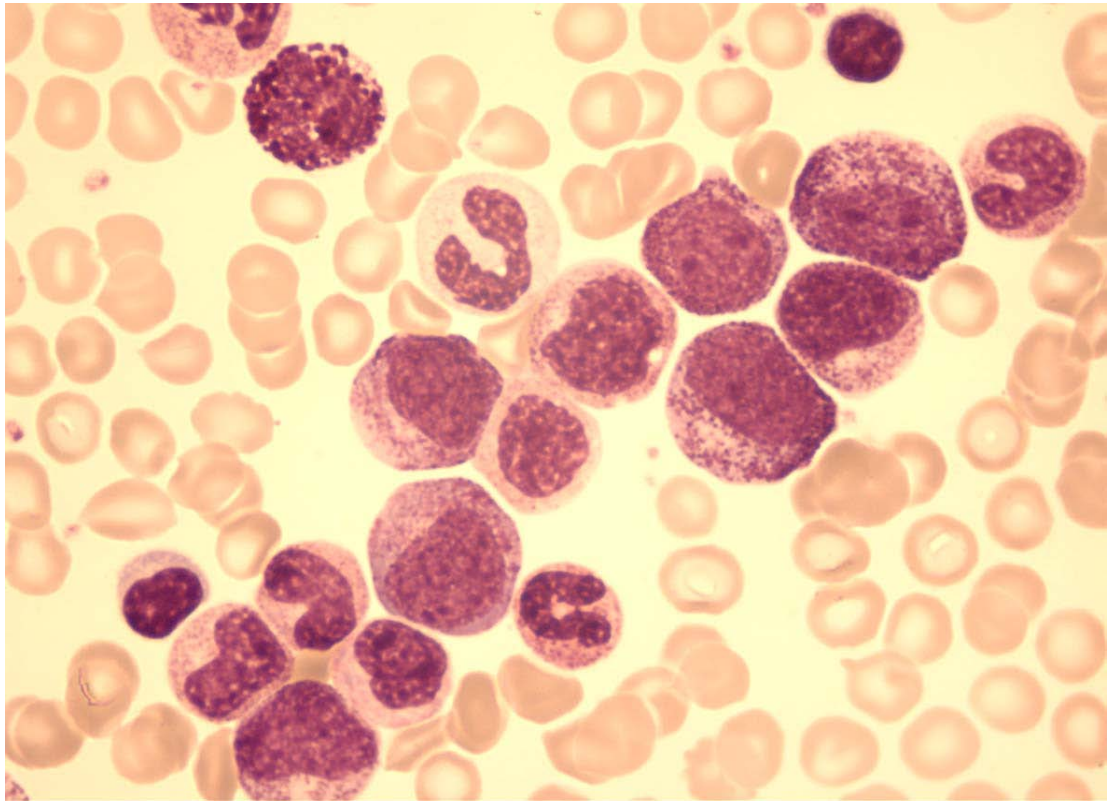


TTP : Thrombotic Thrombocytopenic Purpura
HUS : Hemolytic Uremic Syndrome
ADAMTS 13 : Metalloproteinase
VTEC : Verotoxin-E. Coli (0157 : H7)
D : Diarrheas
H : Complement factor
MCP : Membrane Cofactor Protein

Modified from Liu J., J Thromb Thrombolysis 2001; 11 : 261-272, quoted in Hoffman et al. : Hematology, Basic Principles and Practice 4th edition 2005; Elsevier : p. 2288.

Part 2

WHITE BLOOD CELL PATHOLOGY



DIFFERENTIAL LEUKOCYTE COUNT

	RELATIVE VALUES (%)	ABSOLUTE VALUES (G / L)
NEUTROPHILS	40 – 75	1.8 – 7.5
EOSINOPHILS	1 – 5	0.05 – 0.3
BASOPHILS	0 – 1	0.01 – 0.05
MONOCYTES	2 – 8	0.2 – 0.8
LYMPHOCYTES	25 – 40	1.5 – 4.0

LCH-CHUV, 2009

Left shift :

Band neutrophils (non segmented neutrophils)

> 1.0 G / L if leukocyte count > 4 G / L

*> 25% if leukocyte count ≤ **4** G / L*

Important to distinguish between relative and absolute values :

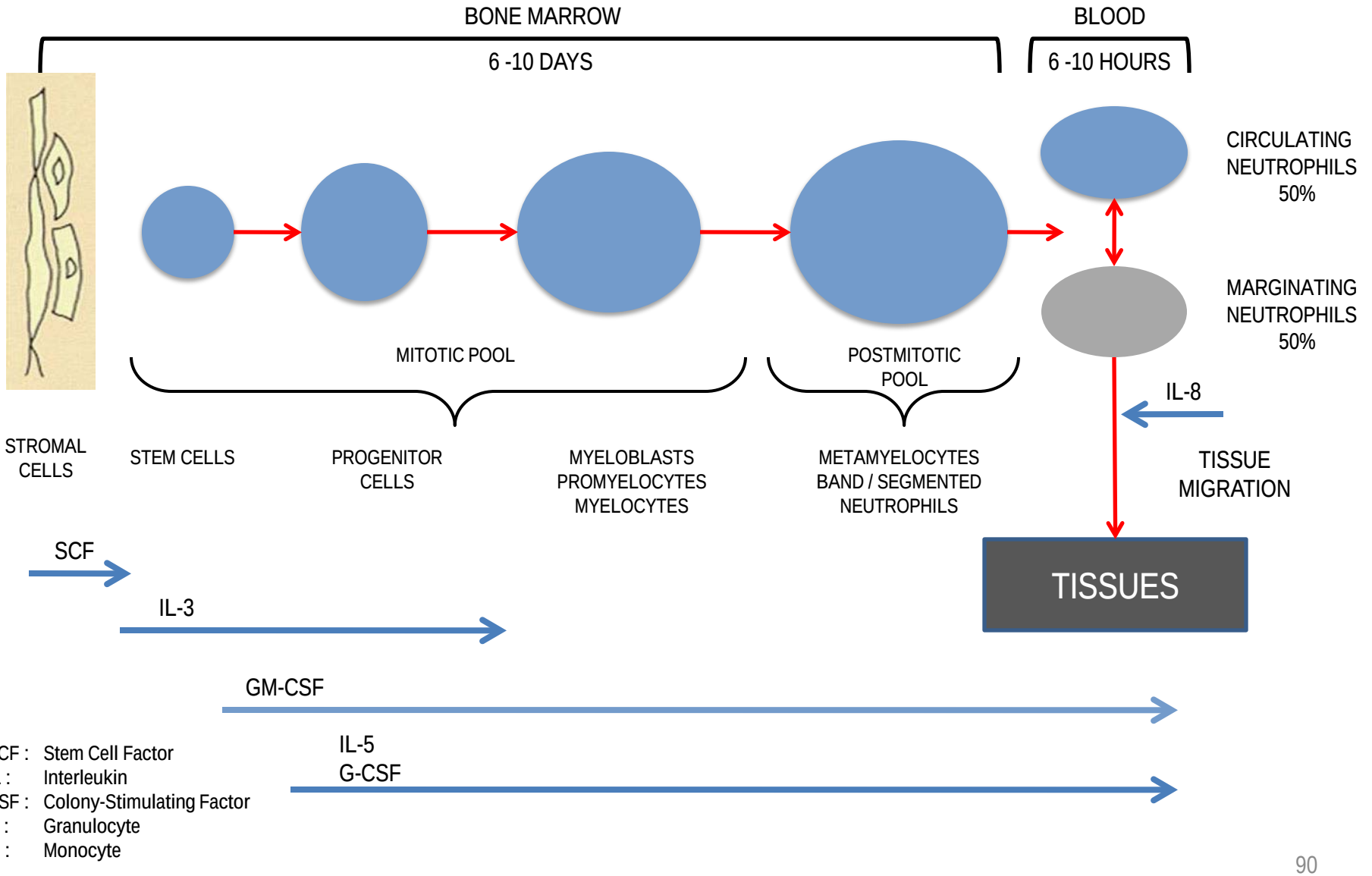
e.g. : chronic lymphocytic leukemia

Leukocyte count : 100 G / L
Neutrophils : 2%
Lymphocytes : 98%

→ *Neutropenia relative but non absolute*

→ *Lymphocytosis relative <u>and</u> absolute*

NEUTROPHIL GRANULOCYTES
KINETICS



SCF : Stem Cell Factor
IL : Interleukin
CSF : Colony-Stimulating Factor
G : Granulocyte
M : Monocyte

ETIOLOGY OF NEUTROPHILIC LEUKOCYTOSIS (*NEUTROPHILIA)*
(NEUTROPHIL COUNT > 7.5 G / L)

PHYSIOLOGICAL, USUALLY MODERATE

- Neonate
- Violent exercise
- Menstruation
- Pregnancy

PATHOLOGICAL

- Inflammatory process
  - Bacterial infection localized *(abscess)* or generalized *(septicemia)*
  - Cancer
  - Inflammatory arthritis
- Tissue necrosis *(myocardial infarction, pancreatitis, etc.)*
- Regenerative phase of acute blood loss or hemolytic anemia
- Tobacco smoking, stress
- Drugs *(Steroids, G-CSF, GM-CSF, lithium)*
- Myeloproliferative neoplasms

TOXIC CHANGES OF NEUTROPHILS

Leukocytosis *(leukocyte count > 10 G / L)*

Neutrophilia *(neutrophil count > 7.5 G / L)*

Neutrophil left shift : band neutrophil count > 1.0 G / L (or > 25% if leukocyte count ≤ **4.0** G / L)

Coarse granules or neutrophils, toxic granules

Doehle bodies *(basophilic cytoplasmic inclusions)*

Cytoplasmic vacuoles

Myelocytosis *(usually moderate)*

Toxic changes are seen in inflammatory process (acute or chronic bacterial infection, cancer, inflammatory arthritis and tissue necrosis)

Possible exceptions : neutropenia of salmonellosis, lymphocytosis of brucellosis and pertussis

MYELOCYTOSIS AND ERYTHROBLASTOSIS

DEFINITION

Presence in the peripheral blood of immature cells of neutrophilic lineage *(metamyelocytes, myelocytes, promyelocytes)* with or without erythroblasts

	Erythroblasts	Myelocytosis
Inflammatory process (bacterial infection, cancer, etc.[1])	–	+
Rupture of bone marrow-blood barrier (skeletal cancer metastasis with bone marrow infiltration)	+	+
Chronic myelogenous leukemia	– / +	+++
Primary myelofibrosis	+ (+)	+ (+)
Regeneration phase after acute blood loss or hemolysis	+ to +++	+
Recovery from agranulocytosis, G-CSF, GM-CSF	–	+ (+)

[1] An important leukocytosis associated with toxic changes of neutrophils and myelocytosis is called leukemoid reaction

NEUTROPENIA

DEFINITIONS

RELATIVE NEUTROPENIA : < 40%

ABSOLUTE NEUTROPENIA : < 1.8 G / L

AGRANULOCYTOSIS : < 0.5 G / L

(major risk of infection)

CLASSIFICATION OF ABSOLUTE NEUTROPENIAS

PSEUDONEUTROPENIA

Excess neutrophil margination *(fasting patient, correction after meal)*

Splenic sequestration ("pooling") - Hypersplenism

TRUE NEUTROPENIA

Reduced production and / or excessive destruction / demand

TRUE NEUTROPENIA (1)
REDUCED PRODUCTION

QUANTITATIVE

- Bone marrow aplasia
- Bone marrow infiltration
- Bone marrow fibrosis
- T-cell large granular lymphocytic leukemia
- Cyclic neutropenia
- Chronic ethnic or idiopathic neutropenia

QUALITITIVE

- Vitamin B_{12} and / or folate deficiency
- Myelodysplastic syndrome

TRUE NEUTROPENIA (2)
REDUCED PRODUCTION AND / OR EXCESSIVE DESTRUCTION

INFECTIOUS NEUTROPENIA[1]

Viral *(influenza, hepatitis, varicella, measles, rubeola, EBV, HIV)*

Bacterial *(salmonellosis, brucellosis, sepsis with Gram negative germs)*

Parasitic *(malaria)*

IMMUNE NEUTROPENIA

Alloimmune *(neonatal neutropenia)*

Autoimmune *(disseminated lupus erythematosus, rheumatoid arthritis, drugs)*

Immunoallergic

Drugs Mianserin *(antidepressant),* sulfasalazine, phenylbutazone *(antiinflammatory agents),* cotrimoxazole *(antiinfective)*, metamizole *(analgesic)*, carbamazepine *(anticonvulsant), carbimazole (antithyroid drug)*

[1] Immune pathogenic mechanism possible

HEREDITARY MORPHOLOGICAL NEUTROPHIL ANOMALIES

PELGER-HUET ANOMALY

Neutrophils with bilobate nucleus *(not to be mistaken for neutrophil left shift !)*
Autosomal dominant anomaly[1]

MAY-HEGGLIN ANOMALY

Basophilic cytoplasmic inclusions (RNA)[2]
Moderate thrombocytopenia with giant platelets
Autosomal dominant anomaly

ALDER-REILLY ANOMALY

Coarse purple granules in neutrophils, monocytes and lymphocytes
Autosomal recessive anomaly

CHEDIAK-HIGASHI SYNDROME

Giant granules in neutrophils, eosinophils, monocytes and lymphocytes
Neutropenia *(infection)*
Thrombocytopenia *(hemorrhage)*
Hepatosplenomegaly
Autosomal recessive anomaly

[1] Acquired variety in myelodysplastic syndrome : "pelgeroid" nuclei = pseudo-Pelger
[2] Döhle bodies

EOSINOPHILS

FUNCTIONS

Positive chemotaxis for histamine *(secreted by mastocytes)*

Immune complex phagocytosis

Destruction of certain parasite larvae after prior antibody sensitization

EOSINOPHILIA (> 0.3 – 0.5 G / L)

Parasitosis *(helminths)*

Allergy *(allergic rhinitis, bronchial asthma)*

Drug *(penicillins, cephalosporins, analgesics, phenothiazines, anticonvulsants…)*

Systemic inflammatory disease *(polyarteritis nodosa)*

Cancer

Adrenal insufficiency

Hypereosinophilic syndrome

Myeloid and lymphoid neoplasms

Acute myeloid leukemia with inv(16) or t(16;16)
Myeloid and lymphoid neoplasms with eosinophilia and anomalies of PDGFRA, PDGFRB or FGFR1
Chronic eosinophilic leukemia, NOS[1]

[1] Not Otherwise Specified

BASOPHILS / MASTOCYTES

DEFINITION

Blood : basophilic granulocytes

Tissues : tissue basophils or mastocytes

FUNCTIONS

Surface receptors for IgE Fc fragment
"Bridging" effect of several IgE molecules by the specific allergen with degranulation and release of histamine *(bronchospasm in asthma bronchiale),* heparin and a chemotactic factor for eosinophils

BASOPHILIA *(> 0.05 – 0.1 G / L)*

Myeloproliferative neoplasm
Allergy
Hypothyroidism

MASTOCYTOSIS (cf. Myeloproliferative neoplasms / WHO classification 2008 on page 118)

MONOCYTES / MACROPHAGES (1)
FUNCTIONS

Chemotaxis, phagocytosis, killing

Antigen presentation to lymphocytes with help of HLA class I (T CD8 +) or class II (T CD4 +, B) molecules

Secretion

- Hydrolases *(acid phosphatase)*
- Lysozyme
- Complement fractions
- Tumor Necrosis Factor *(TNF)*
- Interleukin-1 *(IL-1)*

Brain :	Fever
Liver :	CRP
Neutrophils :	Activation
T lymphocytes :	GM-CSF, G-CSF, M-CSF, IL-2-7
NK lymphocytes :	Activation
Endothelial cells :	Proliferation, GM-CSF, M-CSF, IL-1, IL-5-7

Activation by γ-Interferon, TNF and GM-CSF

CRP :	C-Reactive Protein
IL :	Interleukin
CSF :	Colony-Stimulating Factor
G :	Granulocyte
M :	Monocyte

MONOCYTES / MACROPHAGES (2)

ABSOLUTE MONOCYTOSIS (> 0.8 – 1.0 G / L)

REACTIVE

Infectious disease *(tuberculosis, bacterial endocarditis, salmonellosis, brucellosis, malaria)*

Recovery phase of bacterial infection

Recovery from agranulocytosis

Alcoholic hepatic disease

G-CSF or GM-CSF treatment

MALIGNANT

Chronic myelomonocytic leukemia

Acute myeloid leukemia with t(9;11), acute myelomonocytic leukemia, acute monocytic leukemia

MONOCYTOPENIA

Hairy cell leukemia

LYMPHOCYTES / LYMPHOID ORGANS

LYMPHOID ORGANS

Primary : Bone marrow *(lymphoid stem cells : CFU-L, B-cell differentiation and maturation)*

Thymus *(T-cell differentiation and maturation, thymic selection)*

Secondary : (B and T) Lymph node

Spleen

Digestive tract mucosa

Respiratory tract mucosa

PROPORTION OF B- AND T-LYMPHOCYTES IN BONE MARROW AND PERIPHERAL BLOOD

BONE MARROW	PERIPHERAL BLOOD
B ≥ T	T > B
CD8 > CD4	CD4 > CD8

B-LYMPHOCYTES

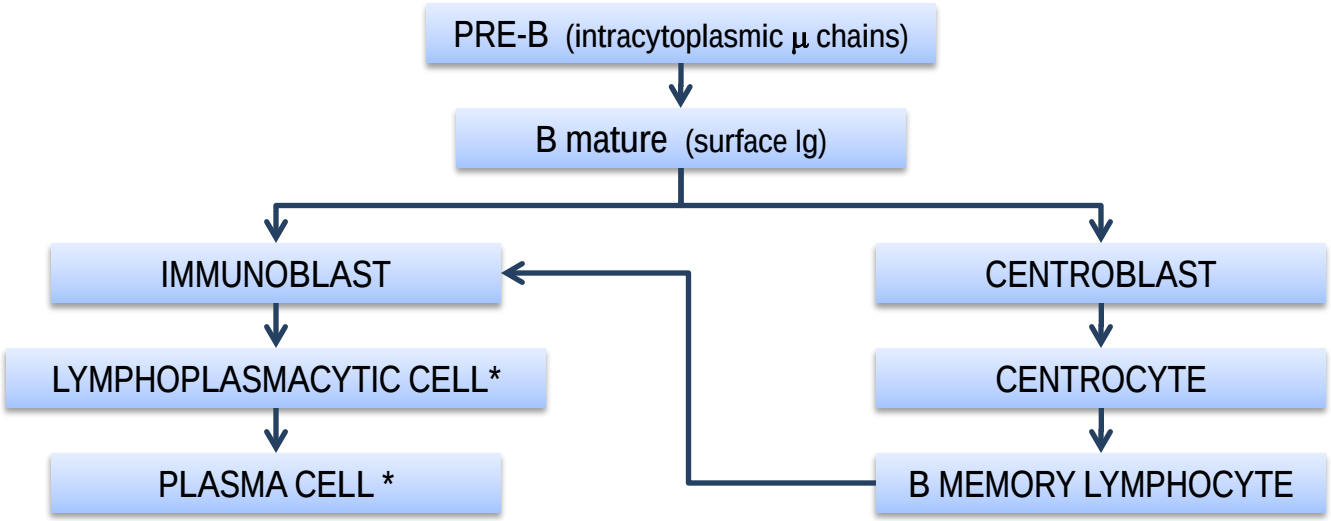
BONE MARROW

PRECURSORS :	CFU-L CD34 +
PRO-B :	CD34 +, TdT +, HLA-DR +
EARLY PRE-B :	Rearrangement of immunoglobulins genes (heavy chains then light chains) CD19 and CD20 expression
PRE-B :	Intracytoplasmic μ chains expression
IMMATURE B :	Surface IgM expression

MIGRATION TO BLOOD AND SECONDARY LYMPHOID ORGANS

$\rightarrow$ MATURE B CELLS (surface IgM and IgD expression)

STEPS OF B-LYMPHOCYTE MATURATION IN SECONDARY LYMPHOID ORGANS



* Plasmatic immunoglobulin (Ig) secretion

	IgG	IgA	IgM	IgD	IgE
Molecular weight (x 1'000)	140	160[1] (400[2])	900	170	190
Sedimentation constant	7 S	7 S[1] (11 S[2])	19 S	6.5 S	8 S
Placental transfer	Yes	No	No	No	No
Serum level (g / L)	8 – 12	1.4 – 4.0	0.5 – 1.9	0.03 – 0.4	0.0001
Half life (d)	21	7	5	2.8	2.3
Heavy chain	γ (1-4)	α (1-2)	μ	δ	ε
Light chain	κ or λ				

[1] Serum IgA
[2] Secretory IgA

Examples :
IgG $\gamma_2\kappa_2$ or $\gamma_2\lambda_2$
IgM $(\mu_2\kappa_2)_5$ or $(\mu_2\lambda_2)_5$ (pentamers)

T-LYMPHOCYTES / THYMIC SELECTION

MEDULLARY PRECURSORS (CFU-L) CD34 +

MIGRATION TO THYMUS

CORTICAL ZONE :

TCR expression (T-Cell Receptor), CD2, CD3

TCR gene rearrangement ($_{\gamma\delta}$ then $_{\alpha\beta}$)

Positive selection[1] *:* amplification of CD4 + CD8 + thymocytes with affinity for " self " class I and II molecules of the HLA system

MEDULLARY ZONE :

Negative selection[1] *:* elimination of thymocytes with affinity for class I and II HLA molecules in contact with " self " antigens (clonal deletion)

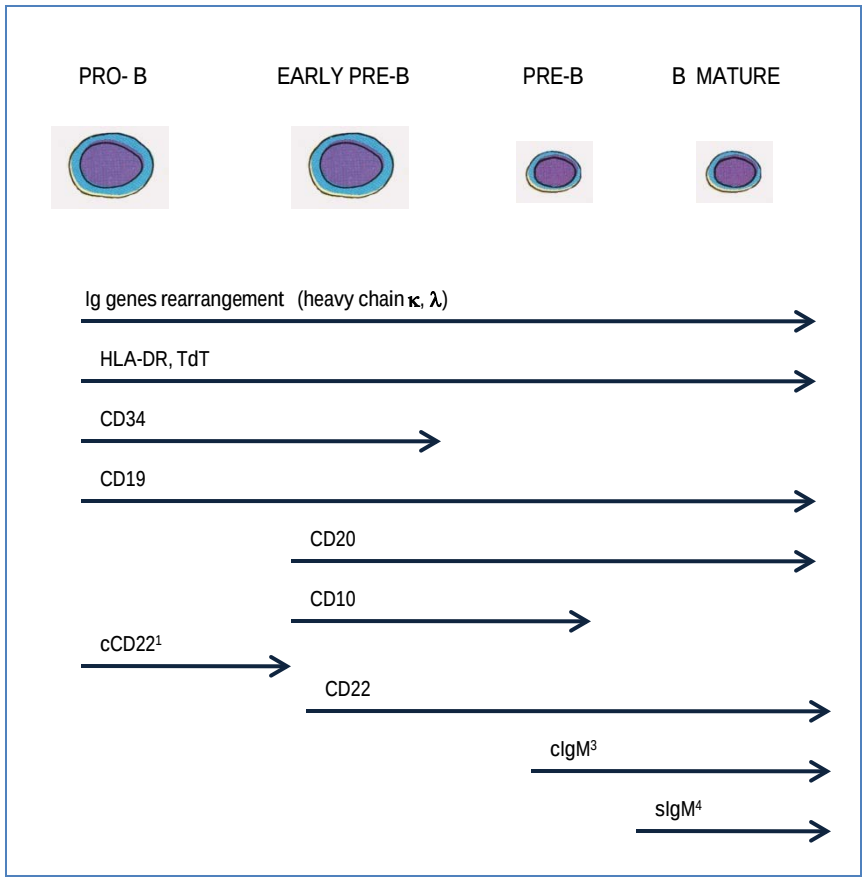
Expression of CD2, CD3, *CD4* + *CD8* - or CD4 - CD8 +

MIGRATION TO PERIPHERAL BLOOD AND SECONDARY LYMPHOID ORGANS

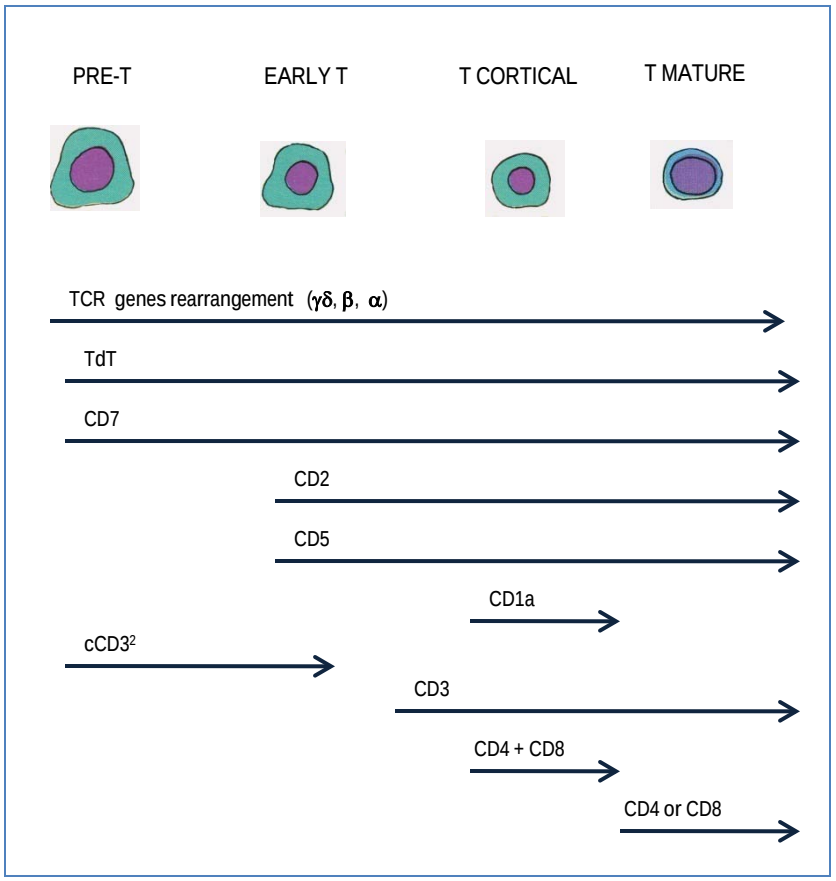
[1] During positive and negative selections approximately 90% of T-lymphocytes (thymocytes) are eliminated through apoptosis (cell death)

B- AND T-LYMPHOCYTE DIFFERENTIATION MARKERS

B-LYMPHOCYTE DIFFERENTIATION



T-LYMPHOCYTE DIFFERENTIATION



[1] **cCD22 : intracytoplasmic CD22**
[2] **cCD3 : intracytoplasmic CD3**
[3] **cIgM : intracytoplasmic IgM**
[4] **sIgM : surface IgM**

NK-LYMPHOCYTES (NATURAL KILLER LYMPHOCYTES)

Large granular lymphocytes (LGL variety)

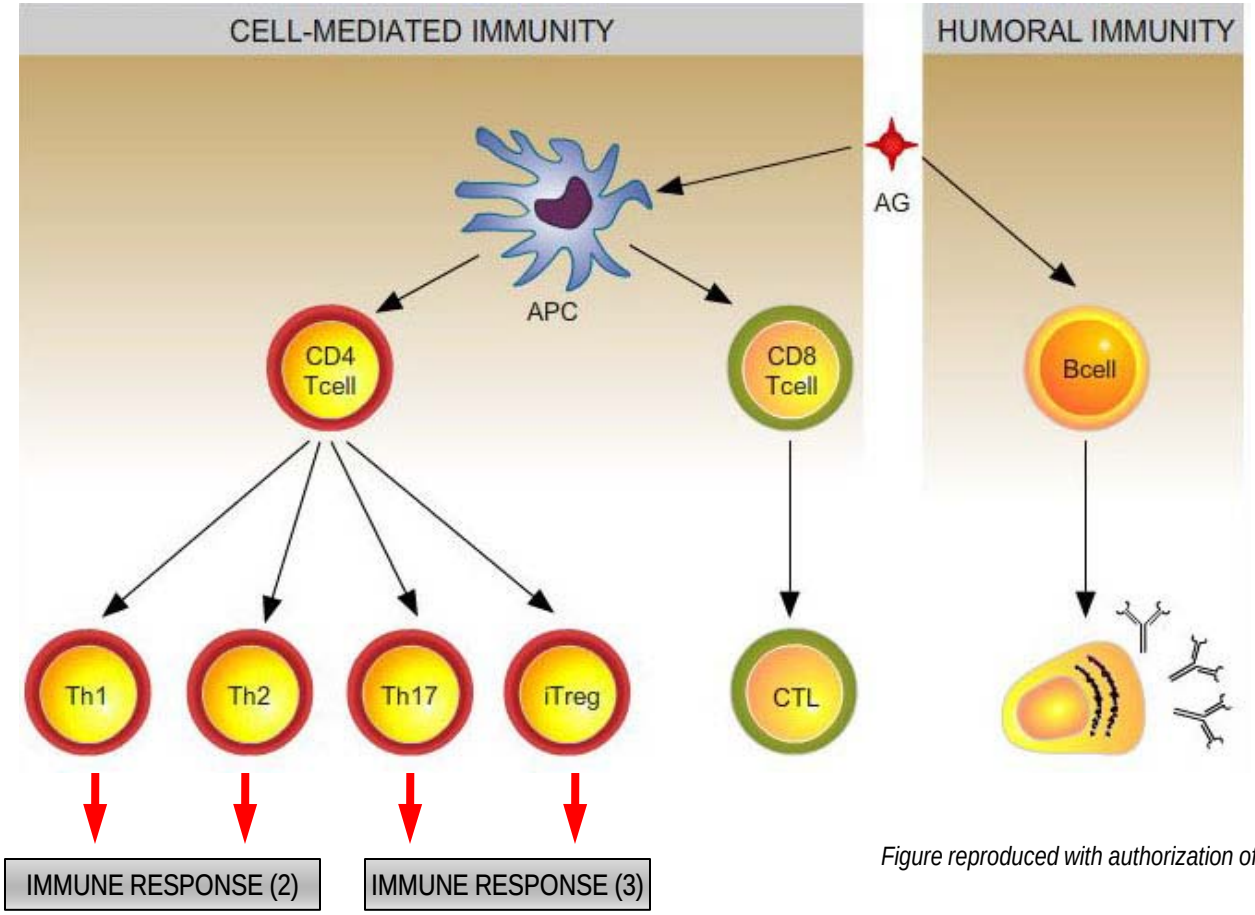
CD3 - , CD8 + / - , CD16 +, CD56 + , CD57 + / - , absence of TCR

Cytotoxicity

1. Inhibited by the presence of surface receptors for HLA class I molecules expressed by "self " cells
   Stimulated by reduced synthesis (or transport) of HLA class I molecules
   (virus infected cells, tumor cells)

2. CD16 + (Fc receptor) : binding of antibody to surface antigen $\rightarrow$ binding of a NK lymphocyte by the Fc, leading to activation

LYMPHOCYTES / IMMUNE RESPONSE (1)



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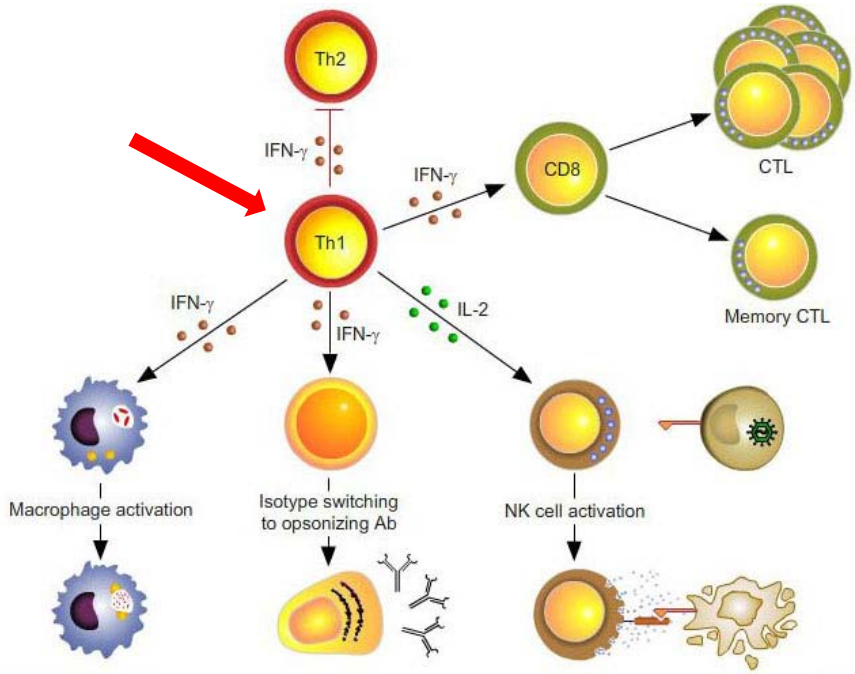
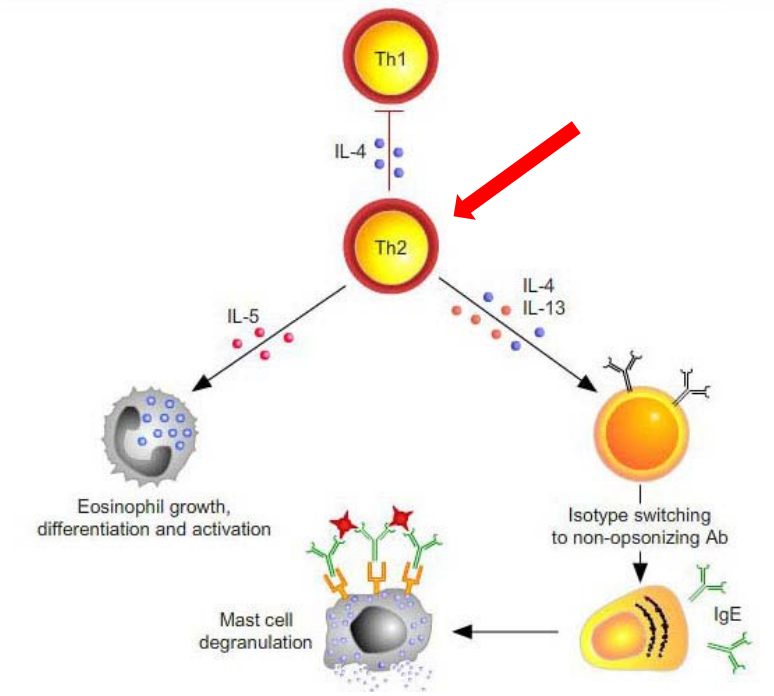
Functionally, the adaptive immune system can be divided into two arms : **cell-mediated and humoral** immunity. B cells are responsible for the humoral response. B cells interact directly with antigen (Ag) and then differentiate into antibody-secreting cells. T cells are responsible for the cell-mediated immunity. They recognize antigens as short antigen fragments presented on the surface of antigen-presenting cells (APC)

T cells exist as two main functional groups : the **Helper T cells** (Th), which respond to antigen by producing cytokines and the **cytotoxic T cells** (CTL) which respond to antigen by releasing cytotoxins. Depending on signals they receive from APC, the helper T cells can differentiate into four main subsets, with distinct profile of cytokines *(Th1, Th2, Th17 and iTreg)*

LYMPHOCYTES / IMMUNE RESPONSE (2)



Th1 cells are required for defense against intracellular pathogens. They are characterized by the production of **IFN-γ** and **IL-2**. IFN-γ activates the microbicidal activity of macrophages, stimulates B cells to produce antibodies that are involved in the opsonization and phagocytosis of particulate microbes, and enhances the development of long-term memory **CD8** T cells. IL-2 increases the cytolytic activity of natural killer cells *(CTL NK)*



Th2 cells are required for defense against extracellular pathogens. They are characterized by the production of **IL-4**, **IL-5** and **IL-13**. IL-4 stimulates B cell proliferation and induces isotype class switch to **IgG1** and **IgE** and so plays a role in IgE-dependent mast cell-mediated reactions. IL-5 acts largely on eosinophils. IL-13 is homologous to IL-4 and induces many of the same functions, including inducing IgE isotype switching

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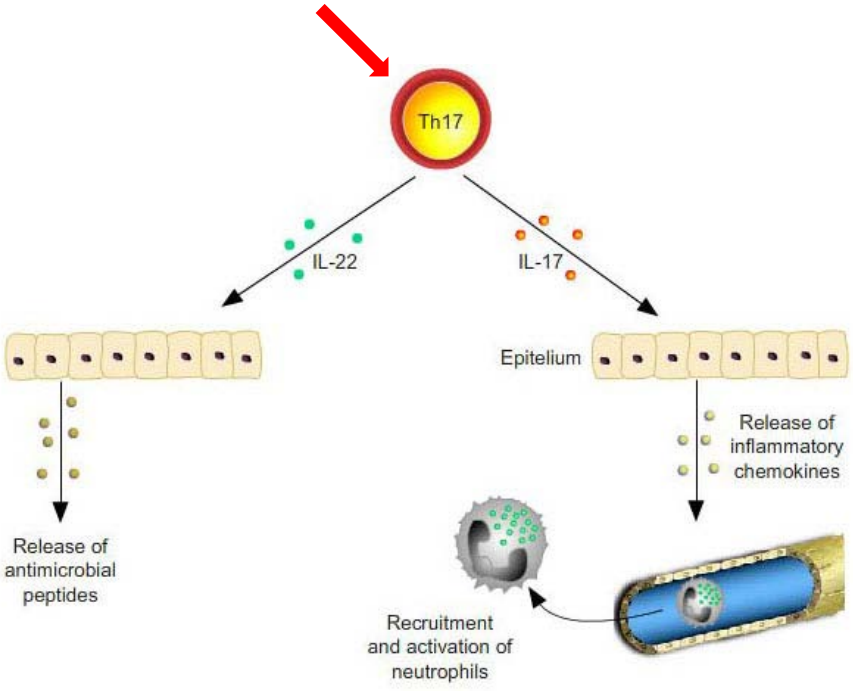
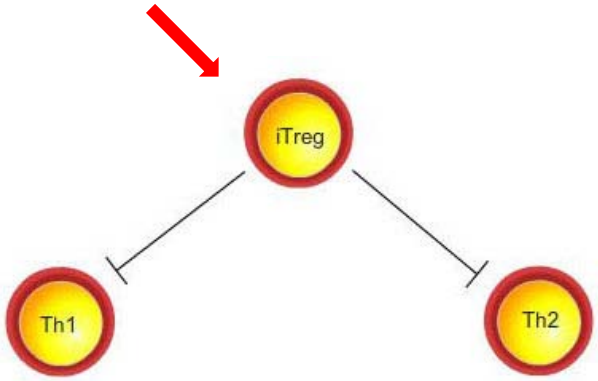
LYMPHOCYTES / IMMUNE RESPONSE (3)

LYMPHOCYTES Th 17



Th17 cells are the most recently discovered subset of Th cells and are thought to be important effector cells in host defense against extracellular bacteria and fungi. They are characterized by the production of IL-17 and IL-22. IL-17 triggers the release of pro-inflammatory chemokines by epithelial cells, and various other tissues and cell types, helping thus the recruitment of neutrophils. IL-22 increases acute-phase reactants in hepatocytes and induces the expression of β-defensins in epithelial cells of the gastrointestinal tract and skin

LYMPHOCYTES iTreg



Induced Treg cells have functions in the suppression of Th1 and Th2 cell immune responses. Whether Treg cells also suppress Th17 cell responses is less clear.

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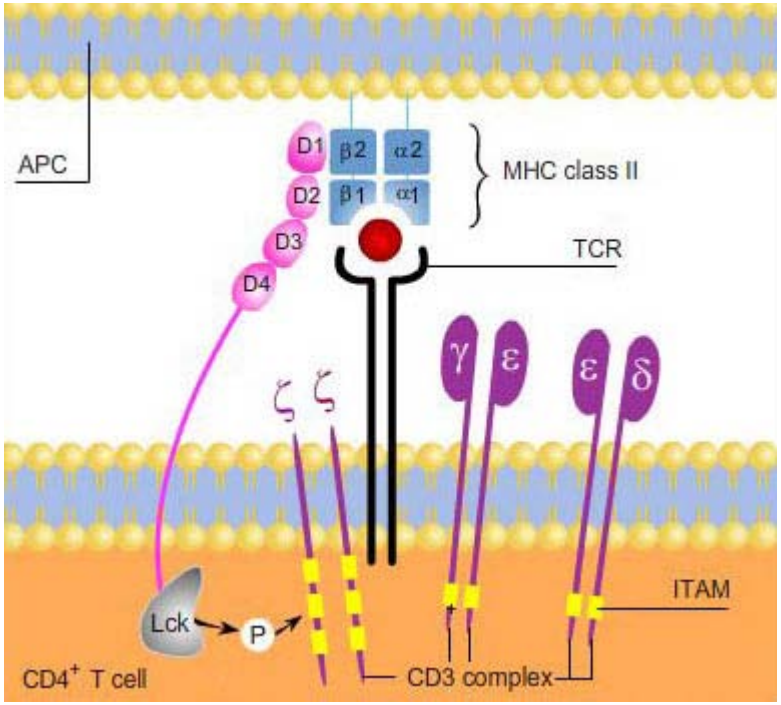
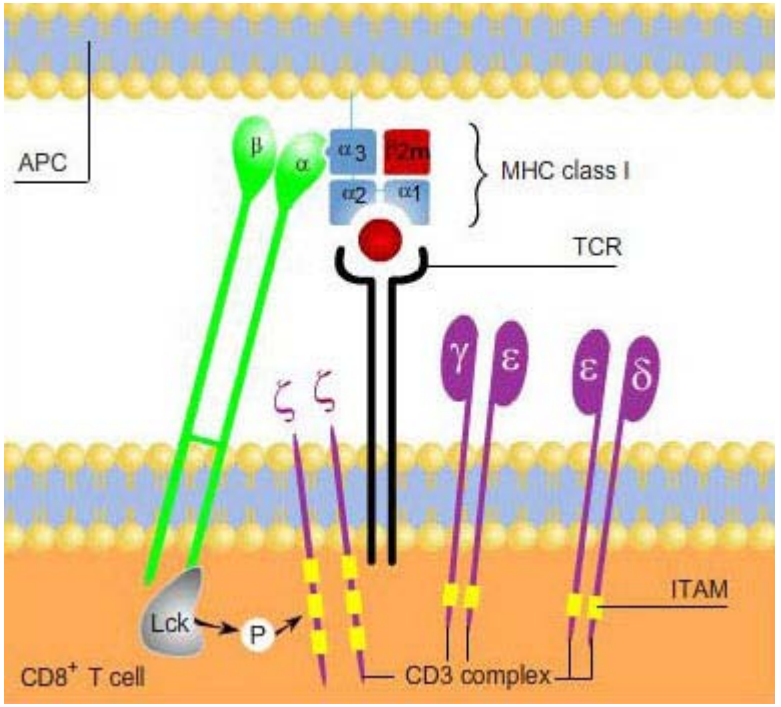
LYMPHOCYTES / IMMUNE RESPONSE (4)

CD 4 ET CD 8 CO-RECEPTORS OF T-LYMPHOCYTES





CD4 is a monomer that interacts via its two distal Ig domains (D1 and D2) with the b2 domain of MHC class II

CD8 is a dimer (either homodimer $\alpha\alpha$ or hetedimer $\alpha\beta$) that interacts via its α chain with the α3 domain of MHC class I

APC : Antigen Presenting Cell

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LYMPHOCYTOSIS / LYMPHOPENIA

LYMPHOCYTOSIS

RELATIVE : > 40%

ABSOLUTE : > 4.0 G / L

REACTIVE

- Infection : viral
  bacterial *(pertussis, tuberculosis, brucellosis, syphilis)*
- Thyrotoxicosis
- Hyposplenism

MALIGNANT

- Lymphoid leukemia

ABSOLUTE LYMPHOPENIA < 1.5 G / L

ACQUIRED

- HIV, Hodgkin lymphoma, chemotherapy, radiotherapy, steroids, ATG (Antithymocyte Globulin), autoimmune disorder

CONGENITAL

- SCID (Severe Combined Immune Deficiency)

IDIOPATHIC

PLASMACYTOSIS / MONONUCLEOSIS SYNDROME

PLASMACYTOSIS

REACTIVE : Rubella *(German measles)*
Other viral infection

MALIGNANT : Plasma cell leukemia
Plasma cell myeloma

MONONUCLEOSIS SYNDROME

Absolute lymphocytosis with polymorphic lymphocytes *(T-lymphocytes reactive to the infected B-lymphocytes)*

Etiology : EBV[1] *(infectious mononucleosis)*

Lymphadenopathy	100%
Fatigue	90%
Pharyngitis syndrome	80%
Splenomegaly	> 50%

Possibly hemolytic anemia and / or autoimmune thrombocytopenia, agranulocytosis, cardiac / neurological / respiratory complications, splenic rupture

CMV *(cytomegalovirus infection, frequently promoted by immunosuppression)*

HIV *(primary infection)*

Other virus *(e.g. hepatitis)*

Toxoplasmosis

[1] Also involved in the pathogenesis of certain lymphoid neoplasms (African Burkitt, Hodgkin lymphoma, lymphoid neoplasms + HIV)

TUMORS OF HEMATOPOIETIC AND LYMPHOID TISSUES
WHO CLASSIFICATION 2008 (1)

MYELOID NEOPLASMS (cf. p. 117-156)

LYMPHOID NEOPLASMS (cf. p. 157-192)

B-CELL NEOPLASMS

PRECURSOR B-CELL NEOPLASMS

B-lymphoblastic leukemia / lymphoma

MATURE B-CELL NEOPLASMS

Chronic lymphocytic leukemia / small lymphocytic lymphoma
B-cell prolymphocytic leukemia
Splenic B-cell marginal zone lymphoma
Hairy cell leukemia
Splenic B-cell lymphoma / leukemia, unclassifiable
Splenic diffuse red pulp small B-cell lymphoma
Hairy cell leukemia-variant
Lymphoplasmacytic lymphoma
Heavy chain diseases
Plasma cell neoplasms
Extranodal marginal zone lymphoma of Mucosa-Associated Lymphoid Tissues (MALT lymphoma)
Nodal marginal zone lymphoma
Follicular lymphoma
Primary cutaneous follicle centre lymphoma
Mantle cell lymphoma

Diffuse large B-cell lymphoma (DLBCL[1]), NOS[2]
Germinal Center B-cell-like : GCB
Activated B-cell-like : ABC
T-cell / histiocyte-rich DLBCL
Primary DLBCL of the CNS
Primary cutaneous DLBCL, leg type
EBV positive DLBCL of the elderly
DLBCL associated with chronic inflammation
Lymphomatoid granulomatosis
Primary mediastinal (thymic) large B-cell lymphoma
Intravascular large B-cell lymphoma
ALK[3] positive large B-cell lymphoma
Plasmablastic lymphoma
Large B-cell lymphoma arising in HHV8-associated multicentric Castleman disease
Primary effusion lymphoma
Burkitt lymphoma
B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and Burkitt lymphoma
B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and Hodgkin lymphoma

[1] DLBCL : Diffuse large B-Cell Lymphoma
[2] NOS : Not Otherwise Specified
[3] ALK : Anaplastic Lymphoma Kinase

Swerdlow S.H., Campo E., Harris N.L., Jaffe E.S., Pileri S.A., Stein H., Thiele J., Vardiman J.W. : WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues, 4th ed. 2008; IARC, Lyon.

TUMORS OF HEMATOPOIETIC AND LYMPHOID TISSUES
WHO CLASSIFICATION 2008 (2)

T-CELL AND NK-CELL NEOPLASMS

PRECURSORS T-CELL NEOPLASMS
- T-cell lymphoblastic lymphoma / leukemia

MATURE T-CELL AND NK-CELL NEOPLASMS
- T-cell prolymphocytic leukemia
- T-cell large granular lymphocytic leukemia
- *Chronic lymphoproliferative disorders of NK-cells*
- Aggressive NK-cell leukemia
- Systemic EBV-positive T-cell lymphoproliferative disorders of childhood
- Hydroa vacciniforme-like lymphoma
- Adult T-cell lymphoma / leukemia
- Extranodal NK / T-cell lymphoma, nasal type
- Enteropathy-associated T-cell lymphoma
- Hepatosplenic T-cell lymphoma
- Subcutaneous panniculitis-like T-cell lymphoma
- Mycosis fungoides
- Sézary syndrome
- Primary cutaneous CD30 positive T-cell lymphoproliferative disorders
- Primary cutaneous gamma-delta T-cell lymphoma
- Peripheral T-cell lymphoma not otherwise specified
- Angioimmunoblastic T-cell lymphoma
- Anaplastic large cell lymphoma (ALCL), ALK[1] positive
- *Anaplastic large cell lymphoma (ALCL), ALK[1] negative*

[1]ALK : Anaplastic Lymphoma Kinase

HODGKIN LYMPHOMA (HODGKIN DISEASE) (cf. p. 189-192)

Swerdlow S.H., Campo E., Harris N.L., Jaffe E.S., Pileri S.A., Stein H., Thiele J., Vardiman J.W. : WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues, 4th ed. 2008; IARC, Lyon.

TUMORS OF HEMATOPOIETIC AND LYMPHOID TISSUES
WHO CLASSIFICATION 2008 (3)

IMMUNODEFICIENCY-ASSOCIATED LYMPHOPROLIFERATIVE DISORDERS

- Lymphoproliferative diseases associated with primary immune disorders
- Lymphomas associated with HIV infection
- Post-Transplant Lymphoproliferative Disorders (PTLD)
  - Early lesions
    - Plasmacytic hyperplasia
    - Infectious mononucleosis-like PTLD
  - Polymorphic PTLD
  - Monomorphic PTLD (criteria for one of the B-cell or T / NK-cell neoplasms of immunocompetent host)
  - Classical Hodgkin lymphoma-type PTLD
- Other iatrogenic immunodeficiency-associated lymphoproliferative disorders

HISTIOCYTIC AND DENDRITIC CELL NEOPLASMS

- Histiocytic sarcoma
- Langerhans cell histiocytosis
- Langerhans cell sarcoma
- Interdigitating dendritic cell sarcoma
- Follicular dendritic cell sarcoma
- Fibroblastic reticular cell tumor
- Indeterminate dendritic cell tumor
- Disseminated juvenile xanthogranuloma

Swerdlow S.H., Campo E., Harris N.L., Jaffe E.S., Pileri S.A., Stein H., Thiele J., Vardiman J.W. : WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues, 4th ed. 2008; IARC, Lyon.

MYELOID NEOPLASMS

MYELOPROLIFERATIVE NEOPLASMS (MPN)

MYELOID AND LYMPHOID NEOPLASMS WITH EOSINOPHILIA AND ANOMALIES OF *PDGFRA*, *PDGFRB OR FGFR1*

MYELODYSPLASTIC SYNDROMES (MDS)

MYELODYSPLASTIC / MYELOPROLIFERATIVE NEOPLASMS (MDS / MPN)

ACUTE MYELOID LEUKEMIAS (AML) AND RELATED PRECURSOR NEOPLASMS

ACUTE LEUKEMIAS OF AMBIGUOUS LINEAGE

STEM CELL PROLIFERATION AND DIFFERENTIATION IN MYELOID NEOPLASMS

	STEM CELL Genetic mutation Humoral factors Cellular interactions	
	Proliferation	Differentiation
Myeloproliferative neoplasms	+	+
Myelodysplastic syndromes Myelodysplastic / myeloproliferative neoplasms	±	±
Acute myeloid leukemias (AML) and related precursor neoplasms Acute leukemias of ambiguous lineage	+	–

MYELOPROLIFERATIVE NEOPLASMS

GENERAL CHARACTERISTICS

Stem cell somatic mutation upstream from the myeloid precursor cell
Proliferation and maturation
Increase in peripheral blood of cells arising from one or more lineages
Myeloid metaplasia *(extramedullary hematopoiesis)*
Frequent bone marrow fibrosis
Platelet function disorders
Hyperuricemia
Possible transformation in acute leukemia

WHO CLASSIFICATION 2008

Polycythemia Vera
Chronic myelogenous leukemia (CML) *BCR-ABL 1* +
Essential thrombocythemia
Primary myelofibrosis
Chronic neutrophilic leukemia
Chronic eosinophilic leukemia, NOS[1]
Mastocytosis
- Cutaneous mastocytosis
- Systemic mastocytosis
- Mast cell leukemia
- Mast cell sarcoma
- Extracutaneous mastocytoma

Myeloproliferative neoplasm, unclassifiable

[1] NOS : Not Otherwise Specified

POLYCYTHEMIA VERA (1)

SYMPTOMS AND CLINICAL SIGNS

Facial erythrocyanosis
Water pruritus
Epigastralgia
Hyperviscosity (thromboembolic manifestations, headache, dizziness paresthesias)
Splenomegaly

DIAGNOSTIC CRITERIA

MAJOR	A1	Hb > 185 g / L (men), > 165 g / L (women)[1] or increased isotopic RBC mass > 25% of predicted value
	A2	Presence of *JAK2*V617F or other functionally similar mutation such as *JAK2* exon 12 mutation
MINOR	B1	Bone marrow biopsy showing hypercellularity for age with trilineage growth (panmyelosis) with prominent erythroid, granulocytic and megakaryocytic hyperplasia
	B2	Serum erythropoietin level below the reference range for normal
	B3	Endogenous erythroid colony formation *in vitro* (without EPO)

PV established if :

A1 + A2 and one minor criterion
or :
A1 and 2 minor criteria

[1] Hemoglobin or hematocrit > 99th percentile of method-specific reference range for age, sex, altitude of residence or hemoglobin > 170 g / L in men, > 150 g / L in women if associated with a documented and sustained increase of at least 20 g / L from an individual's baseline value that cannot be attributed to correction of iron deficiency

Swerdlow S.H., Campo E., Harris N.L., Jaffe E.S., Pileri S.A., Stein H., Thiele J., Vardiman J.W. :
WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues, 4th ed. 2008; IARC, Lyon.

POLYCYTHEMIA VERA (2)

COMPLICATIONS

- Thromboembolic
- Hemorrhagic
- Evolution to myelofibrosis, ~10% (post-polycythemic phase), cf. page 128
- Transformation in myelodysplastic syndrome or acute leukemia (> 10% after treatment with cytotoxic drugs)

PROGNOSIS

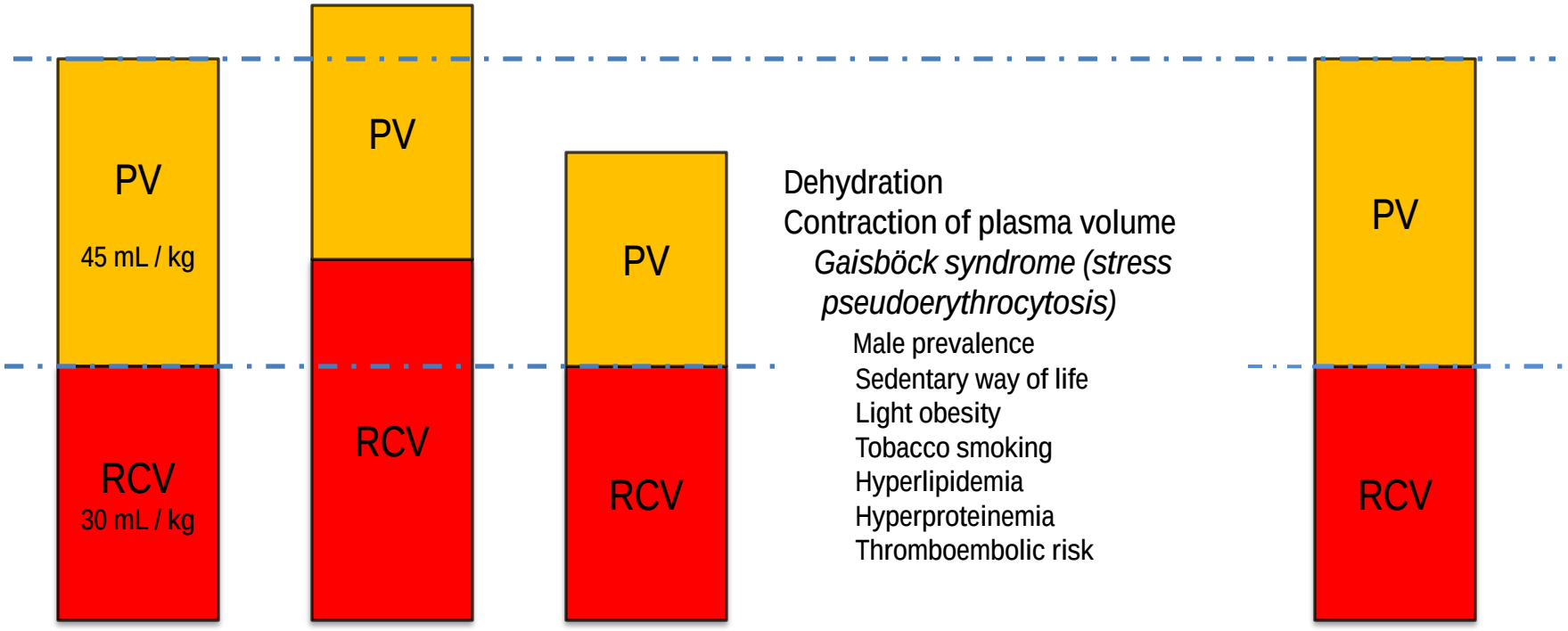
Median survival : > 10 years

TREATMENT :

- Phlebotomies
- Hydroxyurea, Pipobroman
- ^{32}P : age > 70 years (increased risk of leukemic transformation !)

DIFFERENTIAL DIAGNOSIS OF ERYTHROCYTOSIS

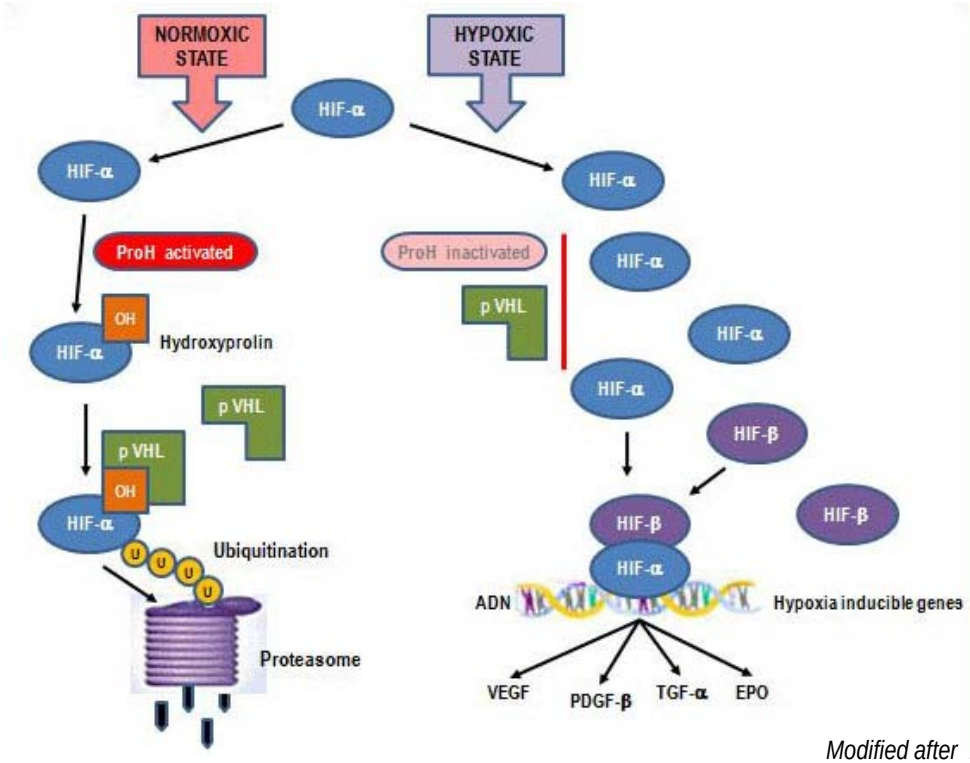
RBC VOLUME AND PLASMA VOLUME



PV : PLASMA VOLUME
RCV : RED CELL VOLUME

DIFFERENTIAL DIAGNOSIS OF TRUE ERYTHROCYTOSIS (1)

PRIMARY ERYTHROCYTOSIS	Congenital	EPO receptor mutation	EPO ↓
	Acquired	Anomaly of erythroid precursors *(Polycythemia Vera)*	
SECONDARY ERYTHROCYTOSIS	Congenital	Absence of erythroid precursors anomaly Mutations impairing the system of tissue oxygenation sensing High O_2-affinity hemoglobins	EPO ↑ or normal
	Acquired	Appropriate or abnormal EPO secretion	



SENSING PROCESS OF TISSULAR OXYGENATION

In state of normal oxygenation HIF-α protein is rapidely degraded by the action of prolin-hydroxylase and von Hippel-Lindau protein, followed by ubiquitination et destruction in the proteasome

In hypoxic state HIF-α degradation is blocked. The protein is activated by dimerization with HIF-β. The complex acts as a promoter of various genes involved in synthesis of growth factors like EPO

HIF : Hypoxia Inducible Factor
pVHL : von Hippel-Lindau protein
ProH : Prolin-Hydroxylase
U : Ubiquitin
VEGF : Vascular Endothelial Growth Factor
PDGF : Platelet-Derived Growth Factor
TGF : Tissue Growth Factor

Modified after McMullin M.F. : EHA Hematology Education, 2009; 3 : 238-241.

DIFFERENTIAL DIAGNOSIS OF TRUE ERYTHROCYTOSIS (2)

PRIMARY ERYTHROCYTOSIS

CONGENITAL

Mutation of EPO[1] receptor

ACQUIRED

Polycythemia Vera

SECONDARY ERYTHROCYTOSIS

CONGENITAL

Mutation of VHL[2] gene *(Chuvash erythrocytosis)*
Mutation of PHD2[3]
Mutation of HIF-2-α[4]
O_2 high-affinity hemoglobins
2,3-diphosphoglyceromutase deficiency

ACQUIRED

Appropriate EPO[1] production

Central hypoxia

Chronic pulmonary disorder, cardio-pulmonary right-left shunt, CO intoxication, chronic smoking, hypoventilation syndromes incl. sleep apnea, prolonged stay at high altitude

Local renal hypoxia

Renal artery stenosis, terminal renal failure, hydronephrosis, polycystic kidneys, post renal transplantation erythrocytosis

Abnormal EPO[1] production

Tumors : cerebellar hemangioblastoma, meningioma, parathyoid carcinoma / adenoma, hepatocellular carcinoma, renal cell carcinoma, pheochromocytoma, uterine leiomyoma

Drugs : androgens

Exogenous EPO[1] application

Therapeutical indication
Illicit application *(doping !)*

IDIOPATHIC ERYTHROCYTOSIS

[1] *EPO : Erythropoietin*
[2] *VHL : Von Hippel-Lindau (recessive mutations)*
[3] *PHD2 : Prolyl-Hydroxylase Domain (dominant mutations)*
[4] *HIF : Hypoxia Inducible Factor (dominant mutations)*

Modified after McMullin M.F. : EHA Hematology Education, 2009; 3 : 238-241.

CHRONIC MYELOGENOUS LEUKEMIA (CML) (1)

SYMPTOMS AND CLINICAL FEATURES

Fortuitous diagnosis - asymptomatic patient
Digestive symptoms *(abdominal heaviness, bloating)*
Splenomegaly
Thrombosis
Hemorrhage
Leucostasis *(CML with very high leukocyte count)*

BLOOD PICTURE

Leukocytosis with neutrophilia
Neutrophil left shift
Myelocytosis (20-50%)
Basophilia
Frequent thrombocytosis
Low leukocyte alkaline phosphatase score (obsolete test)

CYTOGENETIC

Philadelphia chromosome (Ph) = t(9;22)(q34;q11.2) : 90-95% of cases
BCR-ABL 1 fusion gene : 100% of cases

CHRONIC MYELOGENOUS LEUKEMIA (CML) (2)

COURSE IN 3 PHASES

CHRONIC : 4-5 years

ACCELERATION : < 6-8 months

- Persistent increase of WBC counts (> 10 G / L) and / or of spleen size unresponsive to therapy
- Persistent thrombocytosis (> 1'000 G / L) uncontrolled by therapy
- Persistent thrombocytopenia (< 100 G / L) unrelated to therapy
- Clonal cytogenetic evolution
- Basophils ≥ 20% in peripheral blood
- Blast cells : 10-19% in peripheral blood and / or of the nucleated cells of bone marrow
- Often hypercellular bone marrow, morphological signs of myelodysplasia
- Large clusters or sheets of small, abnormal megakaryocytes + reticulin or collagen fibrosis

TRANSFORMATION

- Blast cells : ≥ 20% of peripheral blood cells and / or of the nucleated bone marrow cells
- Extramedullary blast proliferation

CHRONIC MYELOGENOUS LEUKEMIA (CML) (3)

TREATMENT

Imatinib mesylate *(Glivec®)* : Tyrosine Kinase inhibitor (TK)

↘ proliferation and apoptosis induction of the *BCR-ABL 1* + cell lineages
In case of primary or secondary drug resistance : *Dasatinib (Sprycel®), Nilotinib (Tasigna®) (other TK inhibitors)*

Hydroxyurea (HU)

α-Interferon (α-IFN)

Allogeneic hemopoietic stem cell / bone marrow transplantation

AGE BASED THERAPEUTIC SELECTION

< 60 years : in case of insufficient response toTK inhibitor allogeneic hemopoietic stem cell / bone marrow transplantation *(only curative treatment)*. Probability of HLA compatible sibling donor 20-30%. Possible graft from unrelated donor. 5 year survival rate : 50-70%. Relapse after transplantation treated by infusion of donor lymphocytes (GVL effect[1])

> 60 years : and for patient not suitable for transplantation : Imatinib mesylate, α-Interferon (+ ARA-C[2]), Hydroxyurea

[1] GVL : Graft-Versus-Leukemia [2] ARA-C : Cytosine Arabinoside

ESSENTIAL THROMBOCYTHEMIA (1)

SYMPTOMS AND CLINICAL FEATURES

Arterial or venous thrombosis
Hemorrhage through thrombopathy
Erythromelalgia
Splenomegaly (< 50%)

DIAGNOSTIC CRITERIA

1	Sustained platelet count ≥ **450 G / L**[1]
2	Bone marrow biopsy : proliferation mainly of megakaryocytic lineage with increased numbers of enlarged mature megakaryocytes No significant increase or left-shift of neutrophil granulopoiesis or erythropoiesis
3	Exclusion of : Polycythemia Vera[2], primary myelofibrosis[3], *BCR-ABL1* positive CML[4], myelodysplastic syndrome[5] or other myeloid neoplasm
4	*JAK2*V617F mutation present or other clonal marker In absence of *JAK2*V617F mutation, exclusion of reactive thrombocytosis[6]

DIAGNOSIS REQUIRES MEETING ALL 4 CRITERIA

[1] Sustained during the work-up process

[2] Requires failure of iron replacement therapy to increase Hb level to PV range if decreased serum ferritin
Exclusion of PV based on Hb and Hct levels. Measure of RBC mass not required

[3] Absence of relevant reticulin fibrosis, collagen fibrosis, peripheral blood leukoerythroblastosis or hypercellular marrow with megakaryocyte morphology typical for primary myelofibrosis
(small to large megakaryocytes in dense clusters with aberrant nuclear / cytoplasmic ratio and hyperchromatic, bulbous or irregularly folded nuclei)

[4] Absence of *BCR-ABL 1*

[5] Absence of dyserythropoiesis and dysgranulopoiesis

[6] Exclusion of secondary thrombocytosis (cf. page 129)
(The presence of a condition associated with secondary thrombocytosis may not exclude the diagnosis of ET if the first 3 criteria are met)

Swerdlow S.H., Campo E., Harris N.L., Jaffe E.S., Pileri S.A., Stein H., Thiele J., Vardiman J.W. :
WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues, 4th ed. 2008; IARC, Lyon.

ESSENTIAL THROMBOCYTHEMIA (2)

POSSIBLE COURSE

Polycythemia Vera
Primary myelofibrosis
Acute leukemia (3-10%)

TREATMENT

Hydroxyurea
Pipobroman
Anagrelide (could potentially favor evolution to myelofibrosis)
Aspirin (platelet antiaggregant)

Diagnostic criteria for post-PV and post-ET myelofibrosis (MF)

REQUIRED CRITERIA	1	Documentation of a previous diagnosis of WHO-defined (2008) PV or ET
	2	Bone marrow fibrosis grade 2-3 (on 0-3 scale). See page 131
ADDITIONAL CRITERIA (2 required)	1	Post-PV MF : Anemia[1] or sustained loss of either phlebotomy alone or cytoreductive treatment requirement for erythrocytosis Post-ET MF : Anemia[1] or ≥ 20 g / L decrease from baseline hemoglobin level
	2	Leukoerythroblastic peripheral blood picture
	3	Increasing palpable splenomegaly of > 5cm from baseline (distance from the left costal margin) or newly palpable splenomegaly
	4	Post-ET MF : Increased LDH
	5	Development of > 1 of 3 constitutional symptoms : > 10% weight loss in 6 months, night sweats, unexplained fever (> 37.5°C)

[1] Below the reference range for appropriate age, gender and altitude

Swerdlow S.H., Campo E., Harris N.L., Jaffe E.S., Pileri S.A., Stein H., Thiele J., Vardiman J.W. : WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues, 4th ed. 2008; IARC, Lyon.

DIFFERENTIAL DIAGNOSIS OF THROMBOCYTOSIS

DEFINITION

Platelet count > 350 - 400 G / L

CAUSE OF ERROR

Important RBC microcytosis, presence of numerous schistocytes

CLASSIFICATION

PRIMARY THROMBOCYTOSIS

Myeloproliferative neoplasm *(cf. pages 118-132)*

Essential thrombocytosis, Polycythemia Vera, chronic myelogenous leukemia, primary myelofibrosis

Myelodysplastic syndrome *(cf. pages 134-141)*

5q- syndrome

SECONDARY THROMBOCYTOSIS

Iron deficiency
Splenectomy, asplenia[1]
Surgery
Infection, inflammation
Autoimmune disorder
Metastatic cancer
Lymphoid neoplasm
Acute phase / regeneration of acute hemorrhage or hemolysis

[1] Presence of Howell-Jolly bodies in RBC

PRIMARY MYELOFIBROSIS (1)
DIAGNOSIS

MAJOR CRITERIA	1	Proliferation of atypical megakaryocytes[1] with either reticulin and / or collagen fibrosis or : In absence of significant reticulin fibrosis, megakaryocyte changes + increased marrow cellularity with granulocytic proliferation and often decreased erythropoiesis *(i.e. prefibrotic cellular-phase disease)*
	2	Exclusion of : PV[2], *BCR-ABL1* positive CML[3], MDS[4] or other myeloid neoplasms
	3	Presence of J*AK2*V617F mutation or other clonal marker (e.g. *MPL* W515K/L) *or* In absence of clonal marker, exclusion of bone marrow fibrosis or changes secondary to infection, autoimmune disorder or other chronic inflammatory condition, hairy cell leukemia or other lymphoid neoplasm, metastatic malignancy or toxic (chronic) myelopathy[5]
MINOR CRITERIA	1	Leukoerythroblastosis
	2	Increased serum lactate dehydrogenase (LDH) level
	3	Anemia[6]
	4	Splenomegaly[6]

[1] Small to large megakaryocytes in dense clusters with aberrant nuclear / cytoplasmic ratio and hyperchromatic, bulbous, or irregularly folded nuclei

[2] Requires failure of iron replacement therapy to increase Hb level to the PV range if ferritin level is decreased. Exclusion of PV is based on Hb and Hct levels. RBC mass measure not required

[3] Absence of *BCR-ABL1*

[4] Absence of dyserythropoiesis and dysgranulopoiesis

[5] Conditions associated with reactive myelofibrosis do not exclude PMF. Diagnosis to be considered if other criteria are met

[6] Degree of anomaly borderline or marked

DIAGNOSIS : ALL 3 MAJOR + 2 MINOR CRITERIA

Swerdlow S.H., Campo E., Harris N.L., Jaffe E.S., Pileri S.A., Stein H., Thiele J., Vardiman J.W. :
WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues, 4th ed. 2008; IARC, Lyon.

PRIMARY MYELOFIBROSIS (2)

BLOOD COUNT : RBC, WBC and platelet counts in relation with disease stage
Tear drop RBC (*dacryocytes)*
Erythroblastosis and myelocytosis
Platelet anisocytosis

SEMIQUANTITATIVE GRADING OF BONE MARROW FIBROSIS (MF)

MF - 0	Scattered linear reticulin with no intersections (cross-overs), corresponding to normal bone marrow
MF - 1	Loose network of reticulin with many intersections, especially in perivascular areas
MF - 2	Diffuse and dense increase in reticulin with extensive intersections, occasionally with focal bundles of collagen and / or focal osteosclerosis
MF - 3	Diffuse and dense increase in reticulin with extensive intersections and coarse bundles of collagen, often associated with osteosclerosis

COMPLICATIONS : Splenic infarction
Infection (neutropenia)
Hemorrhage (thrombocytopenia and platelet function disorder)
Acute leukemia (5-30%)

PROGNOSIS : Mean survival : 2-15 years (depends of the stage in which PMF is first diagnosed)

TREATMENT : No treatment *("wait and watch")*
Hydroxyurea
Transfusion support
Sectorial splenic radiotherapy
Splenectomy

CHRONIC NEUTROPHILIC LEUKEMIA

1	Peripheral blood : WBC ≥ 25 G / L, neutrophils > 80% WBC, immature granulocytes < 10% WBC, myeloblasts < 1% WBC
2	Bone marrow : percentage and number of neutrophilic granulocytes increased, normal maturation, myeloblasts < 5% of nucleated marrow cells, megakaryocytes normal or left shifted
3	Hepatosplenomegaly
4	No cause of physiological neutrophilia. If present, demonstration of clonality of myeloid cells
5	No *BCR-ABL1* fusion gene, no rearrangement of *PDGFRA, PDGFRB, FGFR1*
6	No evidence of other myeloproliferative neoplasm, or myelodysplastic syndrome or myelodysplastic / myeloproliferative neoplasm. Monocytes < 1 G / L

CHRONIC EOSINOPHILIC LEUKEMIA, NOS[1]

1	Eosinophilia ≥ 1.5 G / L
2	No *BCR-ABL1* fusion gene or other myeloproliferative neoplasm or myelodysplastic / myeloproliferative neoplasm
3	No *FIP1L1-PDGFRA* fusion gene (or other rearrangement of *PDGFRA*), no rearrangement of *PDGFRB* or *FGFR1*
4	Blast cell count in peripheral blood and bone marrow < 20%, no inv(16)(p13.1q22), t(16;16)(p13.1;q22), no other feature diagnostic of acute myeloid leukemia (AML)
5	Presence of a clonal or molecular genetic abnormality or blasts > 2% in PB or > 5% in bone marrow

[1] If these criteria are not met, the diagnosis may be reactive eosinophilia, idiopathic hypereosinophilia or idiopathic hypereosinophilic syndrome (HES). (See page 98)

[1]NOS : Not Otherwise Specified

MYELOID AND LYMPHOID NEOPLASMS WITH EOSINOPHILIA AND ANOMALIES OF *PDGFRA*, *PDGFRB* OR *FGFR1*

MYELOID AND LYMPHOID NEOPLASMS WITH *PDGFRA* REARRANGEMENT	
1	Myeloproliferative neoplasm with prominent eosinophilia
2	Presence of *FIP1L1-PDGFRA* fusion gene
Acute myeloid leukemia and lymphoblastic leukemia / lymphoma with eosinophilia and *FIP1L1-PDGFRA* are also assigned to this category. If molecular analysis is not available, diagnosis is suspected if : 1) Ph-negative myeloproliferative neoplasm with features of chronic eosinophilic leukemia; 2) splenomegaly; 3) high level of vitamin B_{12}; 4) increase of serum tryptase; 5) increase of BM mast cells	
Tyrosine Kinase activity : disease is responsive to TK- inhibitors (Imatinib mesylate)	

MYELOID NEOPLASMS WITH *PDGFRB* REARRANGEMENT	
1	Myeloproliferative neoplasm often with prominent eosinophilia, sometimes neutrophilia or monocytosis
2	Presence of t(5;12)(q31~q33;p12) or variant translocation. Demonstration of *ETV6-PDGFRB* fusion gene or of rearragement of *PDGFRB*
Hematological features : chronic eosinophilic leukemia, chronic basophilic leukemia, chronic myelomonocytic leukemia with / without eosinophilia, Ph-negative chronic myeloid leukemia with eosinophilia, myelodysplastic / myeloproliferative neoplasm with eosinophilia	

MYELOID AND LYMPHOID NEOPLASMS WITH *FGFR1* ANOMALIES	
1	Myeloproliferative neoplasm with prominent eosinophilia and sometimes neutrophilia or monocytosis or acute myeloid leukemia or precursor T- or B-cell lymphoblastic leukemia / lymphoma (often associated with peripheral blood or bone marrow eosinophilia)
2	Presence of t(8;13)(p11;q12) or variant translocation with *FGFR1* rearrangement in myeloid cells, lymphoblasts or both

MYELODYSPLASTIC SYNDROMES (MDS)
GENERAL FEATURES

Somatic mutation of a hemopoietic stem cell upstream of myeloid precursor cells

Myelodyplasia (*dysmyelopoiesis) :*

Proliferation + / -

Maturation + / -

Apoptosis +

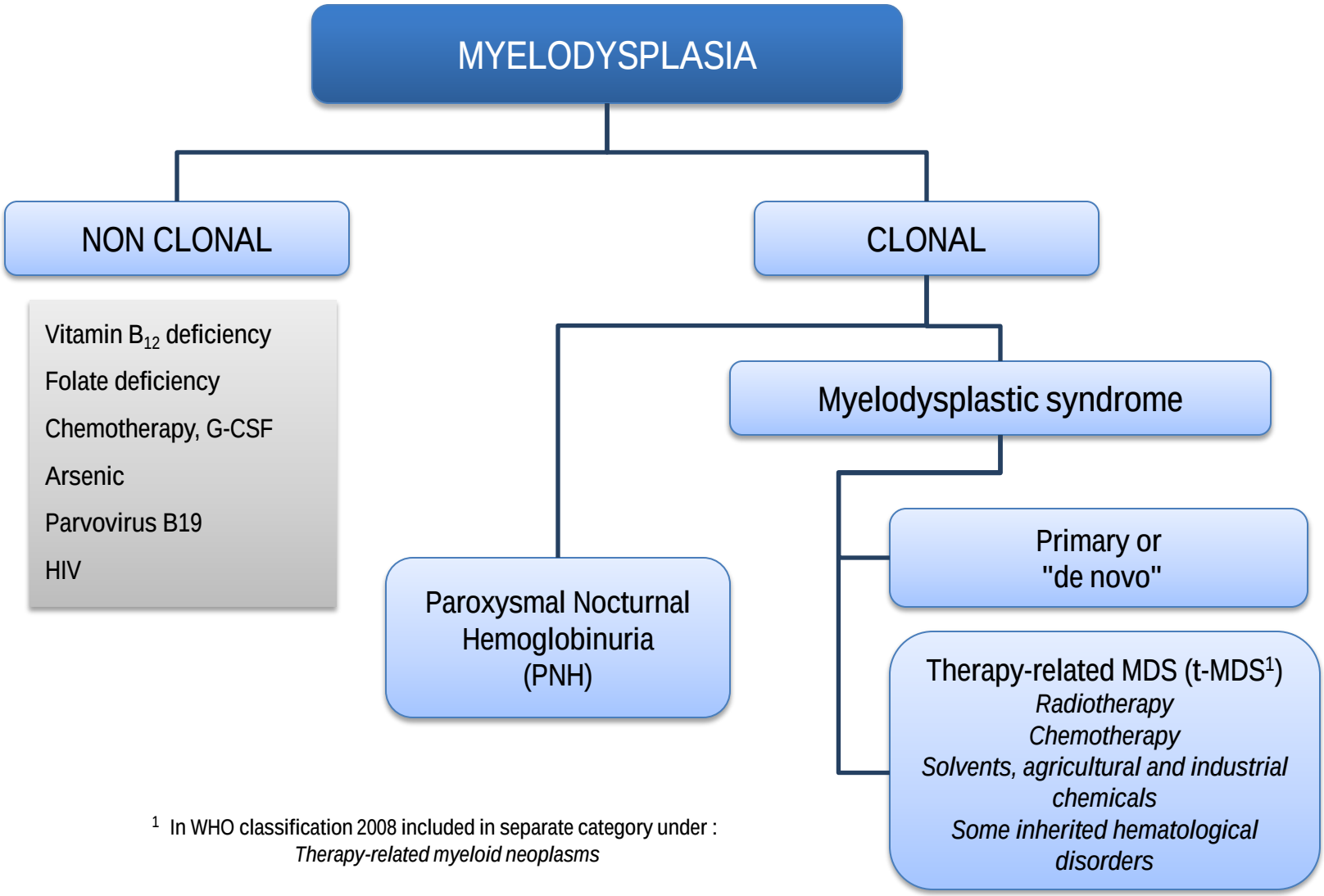
Peripheral blood with 1-3 cytopenia(s)

WHO classification considering :

- Presence of signs of dysplasia affecting only one ("unilineage") or more cell lineages ("multilineage")
- Blast cells in peripheral blood or bone marrow : < 20%
- Presence or absence of Auer rods
- Presence or absence of ring sideroblasts : < 15% or ≥ 15% (bone marrow)
- Peripheral blood monocytosis < 1 G / L

Possible transformation in acute leukemia

MYELODYSPLASIA



[1] In WHO classification 2008 included in separate category under : *Therapy-related myeloid neoplasms*

MORPHOLOGICAL SIGNS OF MYELODYSPLASIA
DYSMYELOPOIESIS

	PERIPHERAL BLOOD	BONE MARROW
Dyserythropoiesis	Macrocytosis (frequent) Anisocytosis Poikilocytosis Anisochromasia Coarse basophilic granules	Nuclear Megaloblastic changes Nuclear budding, internuclear bridging Karyorrhexis, hyperlobation Cytoplasmic Vacuolization Ring Sideroblasts (RS) Periodic acid-Schiff (PAS) staining +
Dysgranulopoiesis	Small or unusually large size Pseudo-Pelger Irregular hypersegmentation Decreased granules or agranularity Pseudo Chediak-Higashi granules Auer rods	
Dysmegakaryopoiesis (platelets)	Giant platelets Lack of granules	Micromegakaryocytes Hypolobated nuclei Multinucleated megakaryocytes

CLASSIFICATION OF MDS

PERIPHERAL BLOOD AND BONE MARROW FEATURES

DISEASE	BLOOD FEATURES	BONE MARROW FEATURES
Refractory Cytopenias with Unilineage Dysplasia (RCUD) : RA, RN, RT[1]	Unicytopenia (rarely bicytopenia) no or rare blasts (< 1%)[2]	Unilineage dysplasia : ≥ 10% of cells in one myeloid lineage; blasts < 5% Ring Sideroblasts (RS) : < 15%
Refractory Anemia with Ring Sideroblasts (RARS)	Anemia no blasts	Erythroid dysplasia only Ring Sideroblasts ≥ 15%, blasts < 5%
Refractory Cytopenia with Multilineage Dysplasia (RCMD)	Cytopenia(s), no or rare blasts (< 1%)[2] no Auer rods monocytes < 1 G / L	Dysplasia in ≥ 10% of cells in ≥ 2 myeloid lineages, blasts < 5%, no Auer rods Ring Sideroblasts ± 15%
Refractory Anemia with Excess Blasts-1 (RAEB-1)	Cytopenia(s), blasts : < 5%, no Auer rods monocytes < 1 G / L	Uni- or multilineage dysplasia, blasts : 5-9% no Auer rods
Refractory Anemia with Excess Blasts-2 (RAEB-2)	Cytopenia(s), blasts : 5-19%, Auer rods ±[3] monocytes < 1 G / L	Uni- or multilineage dysplasia blasts : 10-19%, Auer rods ±[3]
Myelodysplastic Syndrome - Unclassified (MDS-U)	Cytopenias blasts : **≤ 1%**	Evident dysplasia in less than 10% of cells in one or more myeloid cell lines with MDS cytogenetic anomaly, blasts < 5%
Myelodysplastic Syndrome associated with isolated del(5q)	Anemia, normal or increased platelet count no or rare blasts (< 1%)	Normal or increased megakaryocytes with hypolobulated nuclei, blasts : < 5%, no Auer rods, isolated del(5q)

[1] RA : Refractory Anemia; RN : Refractory Neutropenia; RT : Refractory Thrombocytopenia

[2] If bone marrow blast percentage < 5%, but 2-4% blasts are present in the blood, the diagnostic is RAEB-1. RCUD and RCMD with 1% blasts in blood are classified as MDS-U

[3] Cases with Auer rods and < 5% blasts in blood and < 10% in bone marrow are classified as RAEB-2

DIFFERENTIAL DIAGNOSIS OF MYELODYSPLASTIC SYNDROME AND ACUTE MYELOID LEUKEMIA

IMPORTANCE OF BONE MARROW ERYTHROBLASTS PERCENTAGE

ERYTHROBLASTS (in % of total nucleated bone marrow cells)			
< 50%		≥ 50%	
Blasts in % of total nucleated bone marrow cells		Blasts in % of non erythroid nucleated bone marrow cells	
≥ 20%	< 20%	< 20%	≥ 20%
AML	MDS	MDS	AML

AML : Acute Myeloid Leukemia

MDS : Myelodysplastic Syndrome

Modified from Bennett J.M. & al. : Proposed revised criteria for the classification of acute myeloid leukemia. Ann Intern Med 1985; 103 : 620-625. Modifications according to WHO classification 2008.

ANOMALIES RELATED TO MYELODYSPLASTIC SYNDROME

FUNCTIONAL ALTERATIONS

Neutrophils : Motility, adhesion, phagocytosis, bactericidal ability
Platelets : Aggregation

IMMUNOLOGICAL DISORDERS

Polyclonal gammopathy
Hypogammaglobulinemia
Paraprotein
Autoantibodies
Decreased counts of CD4 + and NK lymphocytes

MYELODYSPLASTIC SYNDROME
INTERNATIONAL PROGNOSTIC SCORE

Score	0	0.5	1.0	1.5	2.0
Cytopenia(s)	0 – 1	2 – 3			
Blasts[1] (%)	< 5	5 – 10	–	11 – 19	20 – 30[2]
Karyotype	Favorable	Intermediate	Unfavorable		

[1] Blasts in bone marrow
[2] This group is classified as AML according to WHO 2008

Cytopenia(s) :
Hemoglobin < 100 g / L
Neutrophils < 1.8 G / L
Platelets < 100 G / L

Karyotype :
Favorable : Normal karyotype, -Y, del(5q), del(20q)
Unfavorable : Chromosome 7 anomalies, complex anomalies (≥ 3)
Intermediate : Other anomalies

Risk groups	Score
Low	0
Intermediate-1	0.5 – 1.0
Intermediate-2	1.5 – 2.0
High	**≥ 2.5**

Greenberg P., Cox C., LeBeau M.M., Fenaux P., Morel P., Sanz G., Sanz M., Vallespi T., Hamblin T., Oscier D., Oyashiki K., Toyama K., Aul C., Mufti G., Bennett J. : Blood 1997; 89 : 2079-2088 & Erratum, Blood 1998; 91 : 1100.

OTHER ADVERSE PROGNOSTIC FACTORS IN MDS[1]

- Age > 60 years
- Performance status
- White blood cells > 20 G / L
- Transfusion dependence
- High percentage of CD34 positive bone marrow precursor cells
- MCV > 100 fL
- ⇗ expression of WT1 (Wilms' tumor gene)
- Serum β_2-microglobulin concentration
- Mutations of FLT3 gene
- ⇗ levels of TNF- α
- ⇗ platelet mass (mean platelet volume x platelet count)
- Presence of bone marrow fibrosis
- Lower levels of circulating endothelial cells
- Abnormal Localization of Immature Precursors (ALIP) on bone marrow histology

COMPLICATIONS / EVOLUTION / SURVIVAL

Complications : Recurrent infection
Bleeding manifestation
Immunological disorder

Evolution to acute leukemia : RA : 2% (at 5 years), RAS : 1-2%, RCMD : ~10% (at 2 y) , RAEB-1 : 25%, RAEB-2 : 33%

Survival related to prognostic scores[1] : score 0 : 5.7 years, score 0.5-1 : 3.5 y, score 1.5-2.0 : 1.2 y, score ≥ **2.5** : 0.4 y

RA : Refractory Anemia; RAS : Refractory Anemia with Ringed Sideroblasts; RCMD : Refractory Cytopenia with Multilineage Dysplasia; RAEB : Refractory Anemia with Excess Blasts

[1]Estey E.H., Schrier S.L. : Treatment and Prognosis of the Myelodysplastic Syndromes; October 2008, UpToDate.

TREATMENT OF MYELODYSPLASTIC SYNDROME

SYMPTOMATIC TREATMENT

Transfusional supportive care (RBC, platelets)
Iron chelators
Antibiotics
Erythropoietin + G-CSF, IL-11 (⇗ platelets)

CHEMOTHERAPY

Antimetabolites : Cytarabine, Azacitidine, Decitabine
Antiangiogenic, anticytokine drugs : Thalidomide, Lenalidomide (5q- syndrome)

IMMUNOSUPPRESSIVE THERAPY (Hypocellular MDS) : ATG (Anti-Thymocyte-Globulin) ± cyclosporin

ALLOGENEIC STEM CELL / BONE MARROW TRANSPLANTATION

(< 60 years, HLA identical donor)

Investigational[1] :

Histone deacetylase inhibitors (valproic acid)
Farnesyltransferase inhibitors
Tyrosine kinase receptor inhibitors

[1] Myelodysplastic Syndrome : Etiology, Natural History, Current and Future Therapies, Rowe J.M. ed., Clinical Haematology 2004; 17 : 535-661.

MYELODYSPLASTIC / MYELOPROLIFERATIVE NEOPLASMS

CLASSIFICATION

CHRONIC MYELOMONOCYTIC LEUKEMIA
ATYPICAL CHRONIC MYELOID LEUKEMIA, *BCR-ABL1* NEGATIVE
JUVENILE MYELOMONOCYTIC LEUKEMIA
MYELODYSPLASTIC / MYELOPROLIFERATIVE NEOPLASM, UNCLASSIFIABLE

Refractory anemia with ring sideroblasts (RARS) associated with marked thrombocytosis

CHRONIC MYELOMONOCYTIC LEUKEMIA

DIAGNOSTIC CRITERIA

1. Persistent peripheral blood monocytosis > 1.0 G / L
2. Absence of Philadelphia chromosome or *BCR-ABL1* fusion gene
3. No rearrangement of *PDGFRA*, *PDGFRB* (should be specifically excluded in cases with eosinophilia)
4. < 20% blasts (myeloblasts, monoblasts and promonocytes) in peripheral blood and in the bone marrow
5. Signs of dysplasia in one or more myeloid lineage(s)

If dysplasia minimal or absent : 1 + 2 + 3 + 4 with :
Presence of acquired cytogenetic or molecular anomaly or :
persisting monocytosis (> 3 months) and exclusion of any other cause of monocytosis (see p. 101)

VARIANTS : CMML-1 : blasts (and promonocytes) < 5% (peripheral blood), < 10% (bone marrow)
CMML-2 : blasts (and promonocytes) 5-19% (peripheral blood), 10-19% (bone marrow) or presence of Auer rods

UNFAVORABLE PROGNOSTIC CRITERIA : Severe anemia + high leukocytosis (leukostasis !) + splenomegaly

EVOLUTION : Progression to acute myeloid leukemia : 15-30%
Median survival : 20-40 months

ACUTE MYELOID LEUKEMIA (AML)

EPIDEMIOLOGY

IONIZING RADIATION

ALKYLATING AGENTS

BENZENE AND DERIVATIVES

MYELOPROLIFERATIVE NEOPLASMS (MPN)

MYELODYSPLASTIC SYNDROMES (MDS)

MYELODYSPLASTIC / MYELOPROLIFERATIVE NEOPLASMS (MDS / MPN)

TRISOMY 21

PRIMITIVE IMMUNODEFICIENCY

FANCONI ANEMIA (bone marrow aplasia of genetic origin)

PAROXYSMAL NOCTURNAL HEMOGLOBINURIA

CLINICAL FEATURES OF ACUTE MYELOID LEUKEMIA (1)

SIGNS OF BONE MARROW FAILURE

Anemia	→	fatigue, dyspnea
Neutropenia	→	infection
Thrombocytopenia	→	hemorrhage

TUMORAL SIGNS DUE TO BLASTIC INFILTRATION

Frequently absent
Gingival involvement[1]
Cutaneous involvement[1]
Neuromeningeal involvement[1]
Lymphadenopathy, splenomegaly

OTHER DISORDERS

Lysozyme tubulopathy[1]
Uric nephropathy
Electrolytic disorder (⇗ K^+, ⇗ Ca^{++})

[1] Acute myelomonocytic leukemia, acute monoblastic and monocytic leukemia

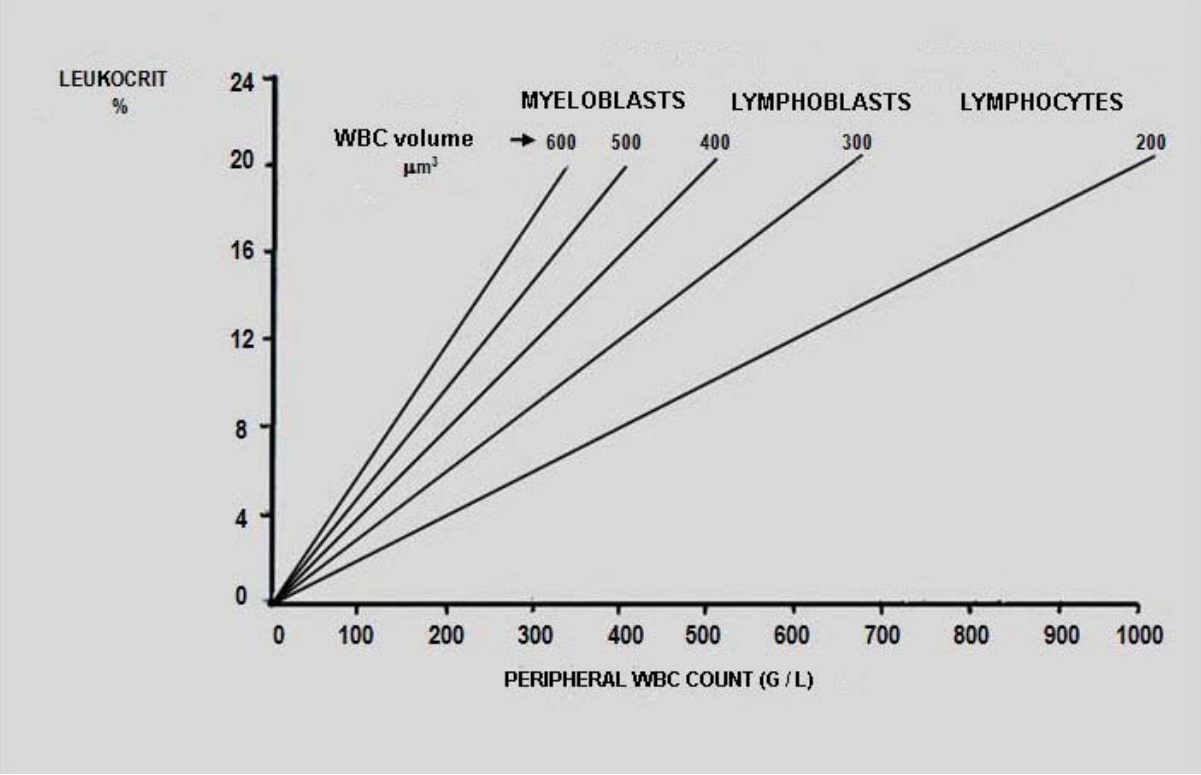
CLINICAL FEATURES OF ACUTE MYELOID LEUKEMIA (2)

DISSEMINATED INTRAVASCULAR COAGULATION : DIC

Mainly acute promyelocytic leukemia with t(15;17)(q22;q12); *PML-RARA*

LEUKOSTASIS

Mainly acute myelomonocytic, acute monoblastic and monocytic leukemia



Modified from Lichtman M.A., Baillière's Clinical Haematology 1987; 1 : 725-746.

ACUTE MYELOID LEUKEMIA
BONE MARROW AND PERIPHERAL BLOOD

BONE MARROW

≥ 20 % BLASTS

PERIPHERAL BLOOD

PERIPHERAL BLOOD		1	2	3	4	5
HEMOGLOBIN	g / L	78	117	82	97	56
MCV	fL					112
WBC	G / L	320	0.9	7.6	115	3.1
PLATELETS	G / L	12	12	97	426	76

1. Acute myeloid leukemia with very high WBC count (hyperleukocytosis)
2. Aleukemic acute myeloid leukemia (absence of blasts in peripheral blood)
3. Acute myeloid leukemia with normal WBC count (blasts : 85% in peripheral blood)
4. Acute transformation of myeloproliferative neoplasm (persisting thrombocytosis)
5. Acute transformation of myelodysplastic syndrome (macrocytosis !)

ACUTE MYELOID LEUKEMIA (AML)
WHO CLASSIFICATION 2008 (1)

CRITERIA

- CYTOLOGY
- CYTOCHEMISTRY
- IMMUNOPHENOTYPING
- CYTOGENETIC
- MOLECULAR BIOLOGY

CLASSIFICATION

ACUTE MYELOID LEUKEMIA WITH RECURRENT CYTOGENETIC ABNORMALITIES

- AML with t(8;21)(q22;q22); *RUNX1-RUNX1T1 (generally with neutrophil lineage maturation)*
- AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22); *CBFB/MYH11 (myelomonocytic with abnormal eosinophils)*
- Acute promyelocytic leukemia[1] with t(15;17)(q22;q12); *PML-RARA* and variant *(microgranular variant)*
- AML with t(9;11)(p22;q23); *MLLT3-MLL (generally associated with monocytic features)*
- AML with t(6;9)(p23;q34); *DEK-NUP214 (often associated with basophilia, multilineage dysplasia ± monocytosis)*
- AML with inv(3)(q21q26.2) or t(3;3)(q21;q26.2); *RPN1-EVI1 (often normal or ⇗ platelets in peripheral blood, ⇗ atypical bone marrow megakaryocytes, multilineage dysplasia)*
- AML (megakaryoblastic) with t(1;22)(p13;q13); *RBM15-MKL1 (peripheral blood and bone marrow similar to acute megakaryoblastic leukemia NOS[2])*, c.f. page 150
- *AML with molecular genetic alterations : mutations / overexpression of genes, cf. "Prognostic factors" p. 151*

[1] Former FAB M3

[2] NOS : Not Otherwise Specified

ACUTE MYELOID LEUKEMIA (AML)
WHO CLASSIFICATION 2008 (2)

ACUTE MYELOID LEUKEMIA WITH MYELODYSPLASIA RELATED CHANGES
- AML from previous MDS or MDS / MPN
- AML with MDS-related cytogenetic anomaly
- AML with multilineage dysplasia

THERAPY-RELATED MYELOID NEOPLASMS (t-AML, t-MDS, t-MDS / MPN)
- Alkylating agents, ionizing radiation therapy, topoisomerase II inhibitors, antimetabolites, antitubulin agents

ACUTE MYELOID LEUKEMIA, NOS[1]
- cf. pages 149-150
- Acute basophilic leukemia
- Acute panmyelosis with myelofibrosis

MYELOID SARCOMA

MYELOID PROLIFERATIONS RELATED TO DOWN SYNDROME

BLASTIC PLASMACYTOID DENDRITIC CELL NEOPLASM

ACUTE LEUKEMIAS OF AMIBIGUOUS LINEAGE
- Acute undifferentiated leukemia
- Mixed phenotype acute leukemia with t(9;22)(q34;q11.2); *BCR-ABL1* : B (or T) and myeloid lineages
- Mixed phenotype acute leukemia with t(v;11q23); *MLL rearranged*
- Mixed phenotype acute leukemia B / myeloid, NOS[1]
- Mixed phenotype acute leukemia T / myeloid, NOS[1]

[1] NOS : Not Otherwise Specified

ACUTE MYELOID LEUKEMIA (AML)
WHO CLASSIFICATION 2008 (3)

ACUTE MYELOID LEUKEMIA , NOS* (1)

With minimal differentiation :	Blasts ≥ **20**% of NMC[1], P[2] + and SB[3] + < 3%, presence of myeloid markers : CD13 and / or CD117, CD33 (60%); T-marker : CD7 (40%)
Without maturation :	Blasts ≥ 90% of NENC[4], P + and SB + ≥ **3**%, promyelocytes → neutrophils ≤ **10% of** NENC, CD13 +, CD33 +, CD117 +, generally CD15 -, CD65 -
With maturation :	Blasts 20-89% of NENC, P +, SB +, promyelocytes → neutrophils ≥ 10% of NENC, CD13 +, CD33 +, CD65 +, CD11b +, CD15 +
Acute myelomonocytic leukemia :	Blasts 20-79% of NENC. Monoblasts → monocytes ≥ **20**% of NENC and / or monocytosis in peripheral blood ≥ **5** G / L, P +, ANBE[5] +, DE[6] +, CD13 +, CD33 +, CD65 +, CD15 + (monocytic differentiation : CD14 +, CD4 +, CD11b +, CD11c +, CD64 +, CD36 +, CD68 + (PGM1[7]), CD163 +, lysozyme +)

[1] NMC : Nucleated Marrow Cells; [2] P : Peroxydase; [3] SB : Sudan Black; [4] NENC : Non Erythroid Nucleated Cells
[5] ANBE : α-naphtyl-butyrate esterase; [6] DE : double esterase ANBE + CAE (chloroacetate esterase); [7] PGM1 : phosphoglucomutase 1

* Former FAB M0-M2, M4

ACUTE MYELOID LEUKEMIA (AML)
WHO CLASSIFICATION 2008 (4)

ACUTE MYELOID LEUKEMIA, NOS* (2)

Acute monoblastic and monocytic leukemia :	*Monoblastic :* Monoblasts ≥ 80% of NENC[1] *Monocytic :* Monoblasts < 80% of NENC, presence of promonocytes and monocytes, P[2] ±, ANBE[3] +, CD13 +, CD33 +, CD15 +, CD65 +, CD14 +, CD4 +, CD11b +, CD11c +, CD64 +, CD68 +, CD36 +, lysozyme +
Acute erythroid leukemia :	*Erythroleukemia (Erythroid / myeloid) :* ≥ **50**% erythroid precursors (with signs of dysplasia, PAS[4] ±, glycophorin +) of NMC[5], ≥ **20**% myeloblasts of NENC (myeloid markers of AML minimal / without differentiation) *Pure erythroid leukemia :* ≥ **80**% of dysplastic erythroid precursors (basophilia, vacuoles, PAS +, glycophorin +), without myeloblastic component
Acute megakaryoblastic leukemia :	Blasts ≥ **20**% of NMC; ≥ **50**% of blasts must express markers of megakaryocytic lineage : CD41 + (glycoprotein IIb/IIIa) and / or CD61 + (glycoprotein IIIa), CD42 ± (glycoprotein Ib), vW[6] +. Other markers : CD13 ±, CD33 ±, CD36 +

[1] NENC : Non Erythroid Nucleated Cells; [2] P : Peroxydase; [3] ANBE : α-naphtyl-butyrate esterase; [4] PAS : Periodic acid-Schiff)
[5] NMC : Nucleated Marrow Cells; [6] vW : von Willebrand

* Former FAB M5-M7

PROGNOSTIC FACTORS IN ACUTE MYELOID LEUKEMIA (AML)

		FAVORABLE	UNFAVORABLE
Age		< 50 y	> 60 y
Karnofsky[1] Index		> 60%	< 60%
Phenotype		CD34 - MDR1[2] neg	CD34 + MDR1 pos
Leukocytes (WBC)		< 30 G / L	> 30 G / L
Post chemo- and / or radiotherapy Prior hematological disorder (MPN, MDS, other)		No	Yes
Genetic		t(8;21), inv(16) / t(16;16), t(15;17)	Complex karyotypic anomalies, -5, -7, 3q26 aberrations, t(6;9), 11q23 aberrations except t(9;11)
Molecular genetic alterations	Mutations	*NPM1*[3] ,*CEBPA*[4] *NPM1* & *FLT3*-ITD	*KIT* : t(8;21), exon 17; inv16, t(16;16), exon 8, *FLT3*-ITD[5] , *FLT3*-TKD[6], *WT1*[7] & *FLT3*-ITD *MLL*-PTD[8]
	Overexpression		*BAALC*[9], *ERG*[10], *MN1*[11]

[1] Karnofsky Index : patient performance index cf. next page; [2] MDR : Multidrug Resistance; [3] *NPM1:* Nucleophosmin, Member 1; [4] *CEBPA* : CCAAT / Enhancer Binding Protein α ; [5] *FLT3*-ITD : Fms-Like Tyrosine Kinase 3-Internal Tandem Duplication (Tyrosine Kinase Receptor); [6] *FLT3*-TKD : *FLT3*-2nd TK Domain; [7] *WT1* : Wilms' Tumor; [8] *MLL*-PTD : Myeloid / Lymphoid or Mixed Lineage Leukemia-Partial Tandem Duplication; [9] *BAALC* : Brain and Acute Leukemia, Cytoplasmic; [10] *ERG* : ETS (Erythroblast Transformation Specific)-Related Gene; [11]*MN1* : Meningioma 1

Schiffer C.A. : Prognosis of acute myeloid leukemia; August 2008, UpToDate Modified from Mrozek & al. : Blood 2007; 109 : 431-448.

KARNOFSKY PERFORMANCE STATUS

	%	CRITERIA
Normal activity No assistance needed	100	Normal, no complaints; no evidence of disease
	90	Able to carry on normal activity; minor signs or symptoms of disease
	80	Normal activity with effort; some signs or symptoms of disease
Impaired activity Ambulatory Assistance needed	70	Cares for self; unable to carry on normal activity or to do active work
	60	Requires occasional assistance but is able to care for most of his / her needs
	50	Requires considerable assistance and frequent medical care
Assistance dependant Hospital care desirable	40	Disabled; requires special care and assistance
	30	Severely disabled; hospitalization is indicated although death not imminent
	20	Very sick; hospitalization necessary; active supportive treatment necessary
Terminal care	10	Moribund; fatal processes progressing rapidly
	0	Dead

ACUTE MYELOID LEUKEMIA
THERAPEUTICAL PRINCIPLES

SUPPORTIVE CARE

INFECTION TREATMENT
TRANSFUSION SUPPORT (RBC, platelets)

CHEMOTHERAPY

INDUCTION
CONSOLIDATION
INTENSIFICATION

HEMOPOIETIC STEM CELL / BONE MARROW TRANSPLANTATION

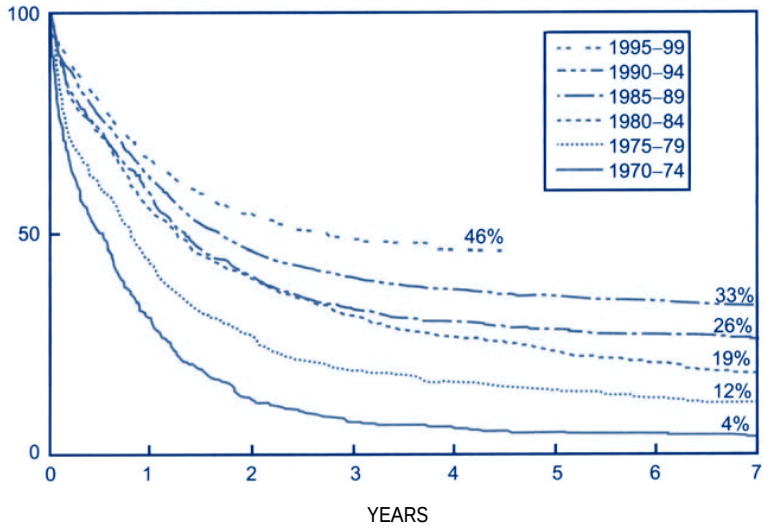
ALLOGENEIC (→ 60 y)
MINI-ALLO TRANSPLANT

Reduced intensity conditioning transplant

Compatible sibling donor : 20-30% of patients have an HLA identical sibling donor

Unrelated donor

AUTOLOGOUS (peripheral blood stem cells / BM)



Survival improvement for patients 15-59 years of age from 1970-1999 (UK MRC : United Kingdom Medical Research Council)

Burnett A.K. : Treatment of acute myeloid leukaemia in younger patients. Clinical Haematology 2001; 14 : 95-118.

TREATMENT OF ACUTE MYELOID LEUKEMIA
CHEMOTHERAPY

ARA-C (Cytosine arabinoside)

ANTHRACYCLINES (Daunorubicin, Idarubicin, Mitoxantrone, Amsacrine)

6-THIOGUANINE

ETOPOSIDE

60-70% Complete Remissions (CR)
(30-40% with 2nd induction cycle)
Then, 2-3 consolidation cycles
5 years relapse free survival rate : 20-25%
Improvement of survival rate with intensification, autologous or allogeneic transplantation
(5 years relapse free survival rate : 40-50%)

ATRA (aII-trans retinoic acid) + ARA-C and Anthracycline :

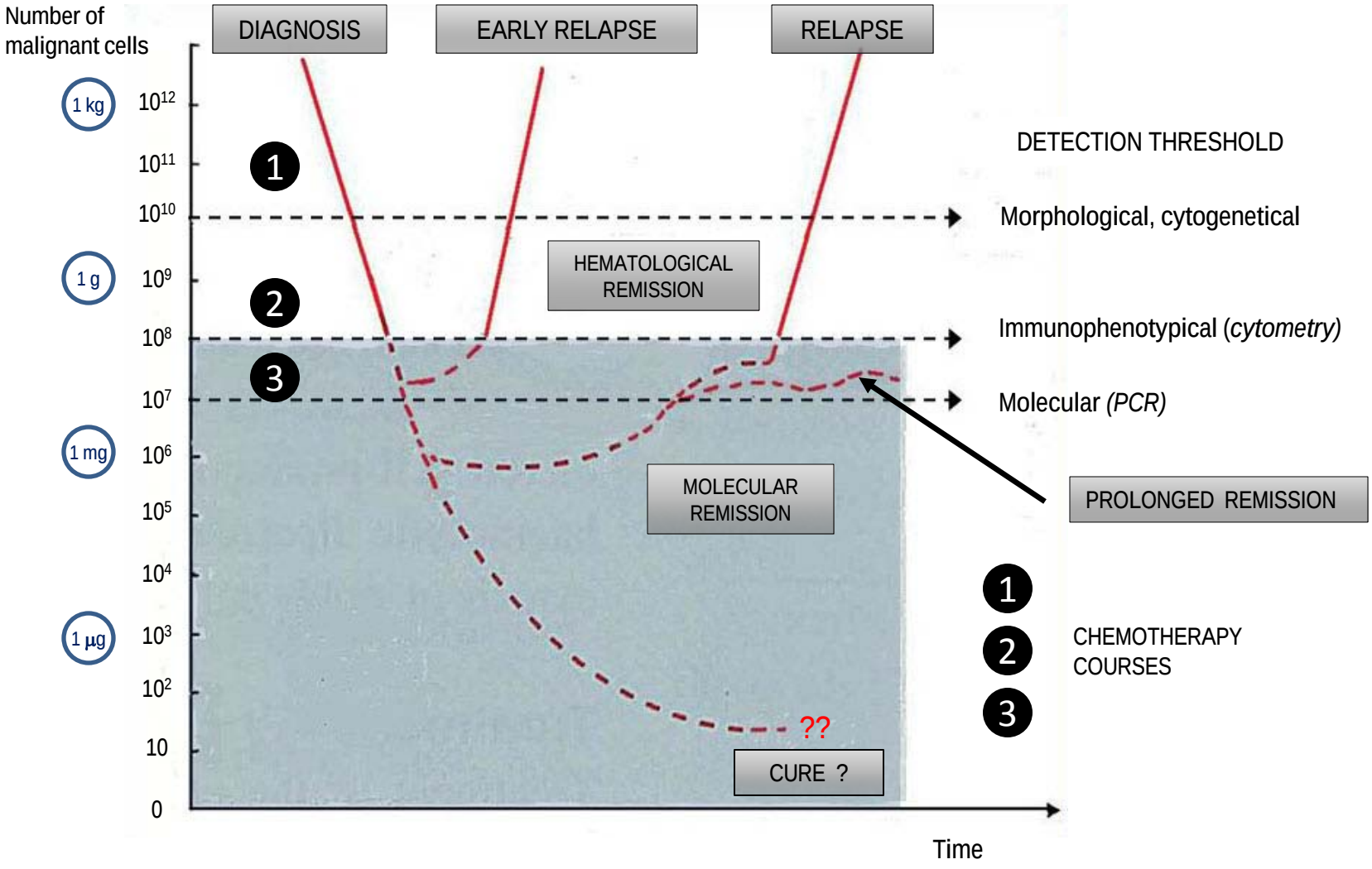
Acute promyelocytic leukemia t(15;17)(q22;q12); *PML-RARA*

Investigational :

Stratification by risk factors
Treatment of relapse : Arsenic trioxyde; Farnesyltransferase inhibitors, MDR1[1], BCL2[2] , FLT3[3] and Tyrosine Kinases inhibitors, antiangiogenic drugs, anti-CD33 (Gemtuzumab)

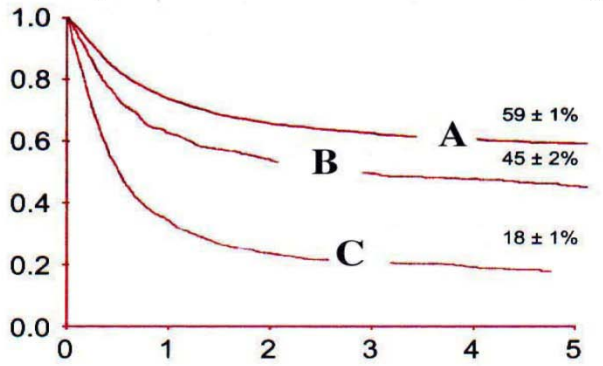
[1] MDR : Multidrug Resistance
[2] BCL2 : B-Cell Leukemia / Lymphoma 2 (protooncogene, inhibitor of apoptosis)
[3] FLT3 : Fms-Like Tyrosine Kinase 3 (Tyrosine Kinase receptor)

KINETICS OF LEUKEMIC CELLS RELATED TO TREATMENT



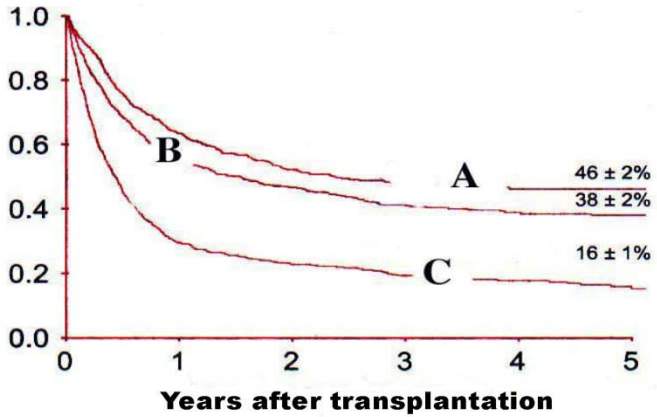
ACUTE MYELOID LEUKEMIA : ALLOGENEIC TRANSPLANTATION

Overall survival after allogeneic transplantation for HLA identical sibling



A : CR-1 (n = 5'192)
B : CR-2 (n = 1'122)
C : Advanced phase (n = 1'787)

Overall survival after allogeneic transplantation from HLA compatible unrelated donor



A : CR-1 (n = 831)
B : CR-2 (n = 880)
C : Advanced phase (n = 854)

Modified from Labopin M. : Acute Leukemia Working Party of the European Group for Blood and Marrow Transplantation (EBMT)
Chaidos A. et al. : Clinical Haematology 2007; 20 : p. 133.

LYMPHOID NEOPLASMS[1] (1)
(WHO CLASSIFICATION 2008)

SIMPLIFIED CLASSIFICATION[2]

B-CELL NEOPLASMS

PRECURSOR B-CELL NEOPLASMS

B-lymphoblastic leukemia / lymphoma (former FAB L1-L2)[2]

MATURE B-CELL NEOPLASMS

B-cell lymphoid leukemias
Burkitt leukemia variant (former FAB L3)[2]

T-CELL AND NK-CELL NEOPLASMS

PRECURSOR T-CELL

T-lymphoblastic leukemia / lymphoma (former FAB L1-L2)[2]

MATURE T-CELL OR NK-CELL NEOPLASMS

T-cell and NK-cell lymphoid leukemias

HODGKIN LYMPHOMA

IMMUNODEFICIENCY-ASSOCIATED LYMPHOPROLIFERATIVE DISORDERS

[1] Former lymphoproliferative syndromes, malignant lymphomas

[2] Lymphoblastic leukemias / lymphomas cf. pages 178-183

LYMPHOID NEOPLASMS (2)

PROOF OF MONOCLONALITY

Expression of one type only of light chain (κ or λ) on the lymphocyte surface (B)
Rearrangement of Ig genes (B)
Presence of paraprotein (B)
Rearrangement of TCR[1] genes (T)
Cytogenetics (B,T, NK)

CLINICAL CONDITION
PERFORMANCE STATUS OF THE EASTERN COOPERATIVE ONCOLOGY GROUP (ECOG)

GRADE	ECOG
0	Fully active, able to carry on all pre-disease performance without restriction
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature
2	Ambulatory and capable of all selfcare but unable to carry out any work activities. Up and about < 50% of waking hours
3	Only capable of limited selfcare, confined to bed or chair > 50% of waking hours
4	Completely disabled. Cannot carry on any selfcare.Totally confined to bed or chair

PROGNOSTIC FACTORS

Histology (low grade → high grade)
Staging
Tumor volume ("bulky")
Performance status (ECOG score)
LDH serum level
Presence or not of inflammatory syndrome

CLINICAL BEHAVIOUR *(survival without treatment)*

Indolent	*years*
Aggressive	*months*
Highly aggressive	*weeks*

[1] TCR : T-Cell Receptor

LYMPHOID NEOPLASMS (3)
STAGING (ANN ARBOR CLASSIFICATION)

STAGES	
I	Involvement of single lymph node region
IE	Limited involvement of single extralymphatic organ or site
II	Involvement of two or more lymph node regions on the same side of the diaphragm alone
IIE	With involvement of limited contiguous extralymphatic organ or tissue
III	Involvement of lymph node regions on both sides of the diaphragm
IIIS	With spleen involvement
IIIE	With limited, contiguous extralymphatic organ or site
IIIES	With limited involvement of contiguous extralymphatic organ or site and spleen
IV	Diffuse or disseminated foci of involvement of one or more extralymphatic organ(s) or tissue(s) (digestive tract, liver, lung, bone marrow, bone…) with or without associated lymphatic involvement

LYMPHOID NEOPLASMS (4)

INITIAL ASSESSMENT

Lymph node or tissue biopsy (histology, immunophenotyping, molecular biology, cytogenetics)

Staging :
- Clinical examination
- CT-scan (if indicated PET-CT)
- Bone marrow cytology and histology
- (Lumbar puncture : CSF[1] examination)

Evaluation of prognosis :

- Histological type (low grade vs. high grade malignancy)
- IPI[2] score (aggressive lymphoid neoplasms)
  - Age ≤ **60** years vs. > 60 years
  - Clinical condition (ECOG[3] score) 0 - 1 vs. ≥ **2**
  - Ann Arbor I-II vs. III-IV
  - Extranodal involvement 0 - 1 vs. ≥ 2 sites
  - LDH ≤ normal value vs. > normal level

Assessment of possible etiology :

- History of immunosuppression (EBV)
- Prior chemotherapy and / or radiotherapy
- HIV, HTLV-1 serology

Further tests : ECG, creatinin, calcemia, liver tests, search of paraprotein, β_2-microglobulin

[1] CSF : Cerebrospinal fluid

[2] IPI : International Prognostic Index

[2] ECOG : Eastern Cooperative Oncology Group

LYMPHOID NEOPLASMS (5)
TREATMENT

HIGHLY AGGRESSIVE LYMPHOID NEOPLASM (e.g. Precursor B- or T-cell lymphoblastic leukemia / lymphoma)

CHOP[1], DHAP[2]…
Intensification with autologous transplantation or stem cell reinfusion

Overall 5 years survival about 25%

AGGRESSIVE LYMPHOID NEOPLASM (e.g. diffuse large B-cell lymphoma)

CHOP, MACOP-B[3], BACOP[4], CHOP + Rituximab (anti-CD20)
Intensification + autologous transplant

Overall 5 years survival about 30-40%

INDOLENT LYMPHOID NEOPLASM (e.g. follicular lymphoma grade 1-2)

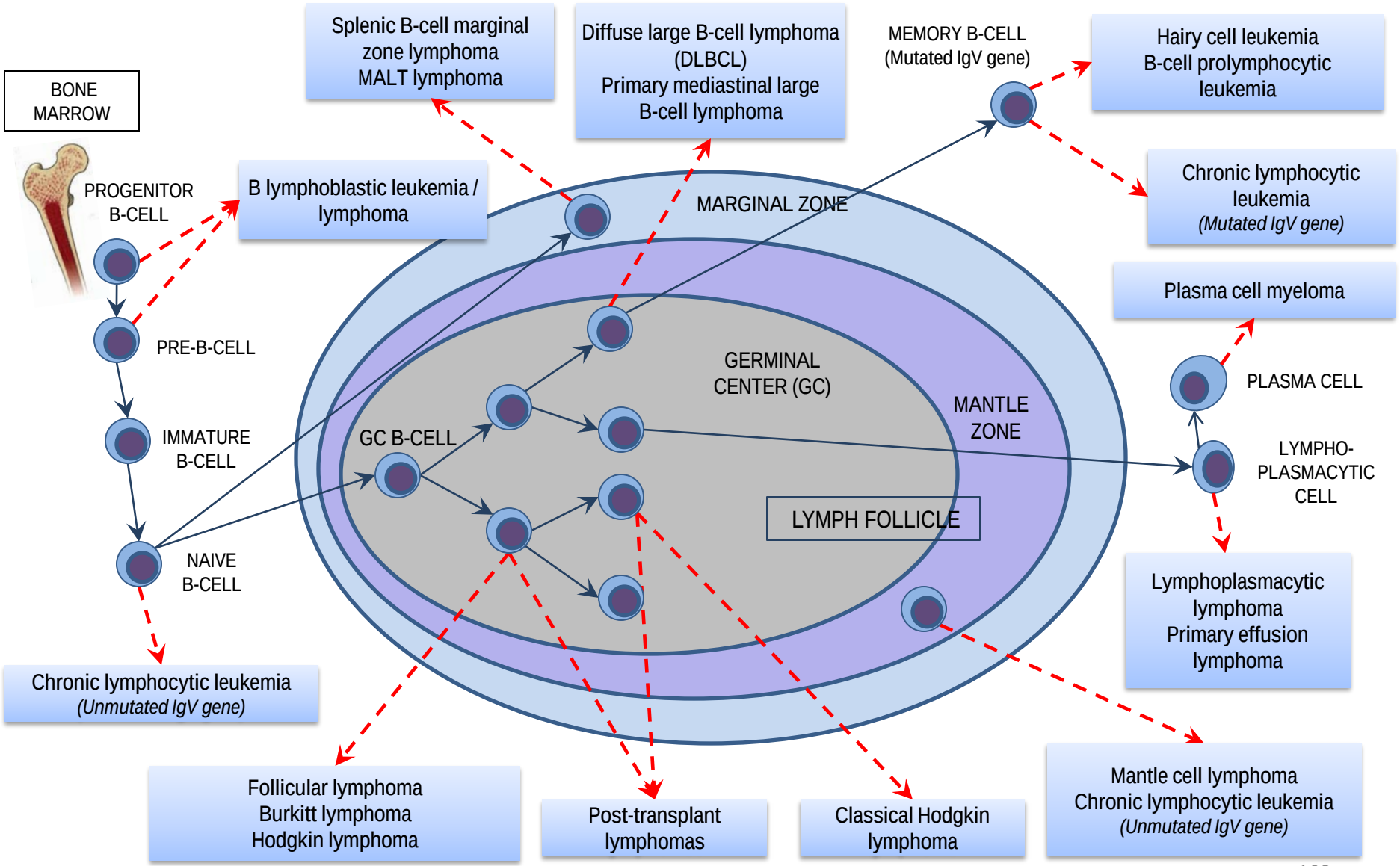
Radiation therapy, α-Interferon, purine analogues (Fludarabine, Cladribine), monoclonal antibodies :
Rituximab (Mabthera®) alone or in combination, radioimmunoconjugates : Ibritumomab (Zevalin®), CVP[5], CHOP

Overall 5 years survival about 50-70%

[1] CHOP : Cyclophosphamide + Doxorubicin + Vincristine + Prednisone
[2] DHAP : Dexamethasone + Cisplatin + Cytarabine
[3] MACOP-B : Methotrexate + Doxorubicin + Cyclophosphamide + Vincristine + Bleomycin + Prednisone
[4] BACOP : Cyclophosphamide + Doxorubicin + Vincristine + Bleomycin + Prednisone
[5] CVP : Cyclophosphamide + Vincristine + Prednisone

B-CELL DIFFERENTIATION

RELATIONSHIP TO MAJOR B-CELL NEOPLASMS



LYMPHOID LEUKEMIAS

B-CELL PROLIFERATION

Chronic lymphocytic leukemia (CLL)

B-cell prolymphocytic leukemia (B-PLL)

Hairy cell leukemia and variant (HCL, HCL-v)

Splenic B-cell marginal zone lymphoma (SMZL)

Splenic B-cell marginal zone lymphoma / leukemia, unclassifiable

Lymphoplasmacytic lymphoma (LPL) - Waldenström macroglobulinemia (WM)

Leukemic form of follicular lymphoma (FL)

Leukemic form of mantle cell lymphoma (MCL)

Plasma cell leukemia (PCL)

T- AND NK-CELL PROLIFERATION

T-cell prolymphocytic leukemia (T-PLL)

T-cell large granular lymphocytic leukemia (T-LGL)

Chronic lymphoproliferative disorders of NK-cells (CLPD-NK)

Aggressive NK-cell leukemia

Adult T-cell leukemia / lymphoma (ATLL)

Sézary syndrome (SS)

B-CELL LYMPHOID LEUKEMIAS

CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) (1)

DEFINITION

Monoclonal B-cell lymphoid proliferation

SYMPTOMS AND CLINICAL FEATURES

Fortuitous diagnosis
Lymph node enlargement
Splenomegaly
Relapsing infections
Severe anemic syndrome
Hemorrhagic manifestations

BLOOD PICTURE

Relative and absolute lymphocytosis
Monoclonality shown by cell surface markers :
- Coexpression of CD5 / CD19
- κ or λ expression

CLASSIFICATION (cf. next page)

Rai
Binet

CHRONIC LYMPHOCYTIC LEUKEMIA (2)

RAI CLASSIFICATION (1975)

STAGE	CRITERIA	MEDIAN SURVIVAL (MONTHS)
0	Isolated monoclonal lymphocytosis (peripheral blood and bone marrow)	150
I	0 + lymphadenopathies[1]	101
II	0 and I + splenomegaly[2] and / or hepatomegaly[2]	71
III	0 and Hb < 100 g / L ± tumoral syndrome	19
IV	0 and platelets < 100 G / L ± tumoral syndrome	19

BINET CLASSIFICATION (1981)

STAGE	LYMPHOID SITES[3]	Hb AND PLATELETS	MEDIAN SURVIVAL (MONTHS)
A	< 3	Hb ≥ **100** g / L Platelets ≥ **100** G / L	Comparable to age-matched control
B	≥ **3**		84
C	Irrelevant	Hb < 100 g / L or Platelets < 100 G / L	24

[1] Cervical, axillary, inguinal lymph nodes on clinical examination
[2] On abdominal palpation
[3] Cervical, axillary, inguinal lymph nodes, splenomegaly and hepatomegaly on clinical examination

Rai K.R., Keating M.J. : Staging and prognosis of chronic lymphocytic leukemia; october 2008, UpToDate.

CHRONIC LYMPHOCYTIC LEUKEMIA (3)

COURSE AND COMPLICATIONS

Infection secondary to :

- B-cell immunological defect
- Potential neutropenia (mainly secondary to chemotherapy)

Autoimmune manifestation

- Hemolytic anemia with positive direct Coombs test (10-15%)
- Immune thrombocytopenia (5%)
- Pure red cell aplasia (Erythroblastopenia)

Transformation to diffuse large B-cell lymphoma (Richter syndrome)

DIFFERENTIAL DIAGNOSIS

Viral or bacterial lymphocytosis (cf. page 112)

Other lymphoid leukemia

CHRONIC LYMPHOCYTIC LEUKEMIA (4)
PROGNOSTIC FACTORS

	FAVORABLE	UNFAVORABLE
Bone marrow lymphocytic infiltration	Focal	Diffuse
Peripheral lymphocytosis doubling time		< 12 months
Serum markers of rapid cell turnover		⇗ Thymidine kinase ⇗ sCD23 ⇗ β_2-microglobulin
Immunophenotype		CD38 +, ZAP-70 +[1]
Cytogenetics	Normal karyotype isolated del 13q14.3 (20%)	del 11q22-23 (17-20%) del 17p (7-10%), del 6q Mixed anomalies
IgV genes (variable region of immunoglobulins)	Mutated	Unmutated

[1] ZAP-70 : Zeta chain-Associated Protein : tyrosine kinase restricted to T- and NK-lymphocytes under normal physiological conditions

Rai K.R., Keating M.J. : Pathophysiology and cytogenetics of chronic lymphocytic leukemia; October 2008, UpToDate.
Müller-Hermelink H.K., Montserrat E., Catovsky D., Campo E., Harris N.L., Stein H. : Chronic lymphocytic leukemia / small lymphocytic lymphoma, in Swerdlow S.H., Campo E., Harris N.L., Jaffe E.S., Pileri S.A., Stein H., Thiele J., Vardiman J.W. : WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues, 4th ed. 2008; IARC, Lyon, p. 180-182.

CHRONIC LYMPHOCYTIC LEUKEMIA (5)
TREATMENT

"Wait and watch" as long as possible

Alkylating agents *(Chlorambucil)*

Purine analogs *(Fludarabine, Cladribine)*

Polychemotherapy *(CVP[1], CHOP[1])*

Proapoptotic drugs (monoclonal antibodies) : *Rituximab : anti-CD20, Alemtuzumab (MabCampath) : humanized anti-CD52, Ofatumumab : humanized anti-CD20 (↑affinity for CD20)*

Lenalidomide *(relapsing or refractory CLL)*

Polyvalent immunoglobulin concentrates *(in case of relapsing infections related to B immunological defect)*

Allogeneic transplantation
(< 50 years, HLA identical donor, disease with rapid evolution. 5 years relapse free survival : 40%)

[1] See p. 161

OTHER B-CELL LYMPHOID LEUKEMIAS (1)

B-CELL PROLYMPHOCYTIC LEUKEMIA

Large splenomegaly, few or absent lymphadenopathies

Lymphocytosis > 100 G / L, anemia and thrombocytopenia (50% of cases)

Large cells with prominent nucleolus : CD19 +, CD20 +, CD22 +, CD79a +, CD79b +, CD5 + (20-30%), CD23 + (10-20%)

Treatment : CHOP (see p. 161), purine analogs (fludarabine, cladribine), chemotherapy + Rituximab, splenectomy

Median survival : 30-50 months

HAIRY CELL LEUKEMIA

Splenomegaly without lymphadenopathy

Pancytopenia

WBC usually < 4 G / L, > 10 G / L (10-20%), rarely > 200 G / L, monocytopenia

Presence of hairy cells (TRAP +), CD19 +, CD11c +, CD25 +, CD103 +, CD123 +

Bone marrow fibrosis

Complications :	Recurrent opportunistic Infections Vasculitis or other immune dysfunction Neurologic disorders Bleeding disorders Bone lesions
Treatment :	Purine analogs (+ Rituximab), α-Interferon, splenectomy, anti-CD22, anti-CD25 immunotoxins

Overall 10-year survival rate : > 90%

OTHER B-CELL LYMPHOID LEUKEMIAS (2)

SPLENIC B-CELL MARGINAL ZONE LYMPHOMA (SMZL)

Splenomegaly
Variable presence in peripheral blood of villous lymphocytes CD20 +, CD79a +, CD5 -, CD25 + / -, CD11c + / -, CD103 usually -, CD123 rarely +
Occasionally autoimmune thrombocytopenia or anemia
Small monoclonal serum paraprotein (1/3 of cases)
Clinical course indolent
Treatment : splenectomy

SPLENIC B-CELL MARGINAL ZONE LYMPHOMA / LEUKEMIA, UNCLASSIFIABLE

Splenic diffuse red pulp small B-cell lymphoma (SMZL-diffuse variant)

Frequently massive splenomegaly
Usually low lymphocytosis, presence of villous lymphocytes
Sometimes cutaneous infiltration (pruritic papules)

Hairy cell leukemia-variant (HCL-v) - "Prolymphocytic variant of HCL"

Average WBC count ~ 35 G / L, ↓ platelets (~ 50%), ↓ RBC (~ 25%)
Lymphocytes : hybrid features of prolymphocytic leukemia and classical hairy cell leukemia
Absence of monocytopenia

Treatment : Rituximab, anti-CD22 immunotoxin
Usually no response to purine analogues and to α-Interferon

OTHER B-CELL LYMPHOID LEUKEMIAS (3)

LYMPHOPLASMACYTIC LYMPHOMA - WALDENSTRÖM MACROGLOBULINEMIA (WM)

Lymphoplasmacytic bone marrow infiltration

Lymphocytosis : generally < 10 G / L (mixture of small and large lymphocytes, sometimes with eccentric nucleus and pronounced cytoplasmic basophilia)

Lymphadenopathies : 40%, splenomegaly or hepatomegaly : 30%, hepatosplenomegaly : 25%

Mainly IgM paraproteinemia (WM) : hyperviscosity syndrome (IgM > 30 g / L)

Possible cryoglobulinemia (Raynaud phenomenon, vasculitis)

Anemia of variable severity

- Hemodilution
- Bone marrow failure
- Autoimmune hemolytic anemia (cold agglutinins)

Polyneuropathy with sensory and motor defect (anti-MAG[1] antibodies)

Bleeding tendency (thrombocytopenia + thrombopathy)

Indolent lymphoid neoplasm

Treatment :	Plasmapheresis if hyperviscosity syndrome Alkylating agents, Rituximab, purine analogs, CHOP[2] + Rituximab, corticosteroids, splenectomy
Median survival :	5-10 years

[1] Myelin Associated Glycoprotein

[2] See p. 161

B-CELL LYMPHOID LEUKEMIA

Contribution of immunological markers, cytogenetics and molecular biology

	sIg	CD19	CD5	CD23	CYTOGENETICS	OTHERS
CLL	+ / -	+	+	+		
B-PLL	+	+	- / +	- / +		
HCL	+	+	-	-		TRAP + CD11c + CD25 + CD103 +
SMZL	+	+	- / +	-		
MCL	+	+	+	-	t(11;14)	Cyclin D1
FL	+	+	-	-	t(14;18)	CD10 + BCL2

CLL : Chronic lymphocytic leukemia
B-PLL : B-cell prolymphocytic leukemia
HCL : Hairy cell leukemia
SMZL : Splenic marginal zone lymphoma
MCL : Mantle cell lymphoma
FL : Follicular lymphoma
BCL2 : B-cell Leukemia / Lymphoma 2 Protooncogene, inhibitor of apoptosis or cell death

	CD123[1]	CD25	CD11c	CD103
HCL	22 / 23 95%	24 / 25 96%	25 / 25 100%	25 / 25 100%
HCL VARIANT	1 / 11 9%	0 / 11 0%	11 / 11 100%	4 / 11 36%
SMZL	1 / 29 3%	18 / 28 64%	10 / 26 38%	0 / 25 0%

The contribution of morphology remains paramount for the differential diagnosis of B-cell prolymphocytic leukemia, hairy cell leukemia and its variant form as for splenic marginal zone lymphoma

[1] *Del Giudice I. et coll. : The diagnostic value of CD123 in B-cell disorders with hairy or villous lymphocytes. Haematologica 2004; 89 : 303-308.*

T-CELL AND NK-CELL LYMPHOID LEUKEMIAS (1)

T-CELL PROLYMPHOCYTIC LEUKEMIA (T-PLL)

Hepatosplenomegaly, generalized lymphadenopathy

High WBC count > 100 G / L (> 200 G / L in 50% of patients)

Skin involvement (20% of cases)

CD2 +, CD3 + / -, CD7 +, usual expression of CD4 (60%), CD8 + / -, CD52 +

Cytogenetic anomalies : inv(14), t(14;14), idic(8p11), t(8;8), trisomy 8q, del 12p13, del 11q23

Aggressive disease, median survival < 1 year

Treatment : anti-CD52 (alemtuzumab)

T-CELL LARGE GRANULAR LYMPHOCYTE LEUKEMIA (T-LGL)

Serious neutropenia, variable anemia (sometimes severe due to red cell aplasia)

Moderate splenomegaly

Frequent autoantibodies, immune complexes and hypergammaglobulinemia

Association with rheumatoid arthritis

CD3 +, $TCR_{\alpha\beta}$ +, CD4 - / +, CD8 +; CD57 +, CD 16 + : > *80% of cases*

Indolent clinical course, median survival ~ 13 years

T-CELL AND NK-CELL LYMPHOID LEUKEMIAS (2)

CHRONIC LYMPHOPROLIFERATIVE DISORDERS OF NK-CELLS (CLPD-NK)

Usually asymptomatic, some cases with systemic symptoms, cytopenia(s)

Sometimes in association with solid tumors, vasculitis, neuropathy, autoimmune disorders

CD3 -, CD4 -, CD8 -, $TCR_{\alpha\beta}$ -, CD16 +, CD56 + (usually weak), CD57 -

AGGRESSIVE NK-CELL LEUKEMIA

Rare, prevalent Asians, median age : 42 years

Principal involved sites : peripheral blood, bone marrow, spleen, liver

CD2 +, CD3 -, CD56 +

Fulminant clinical course (coagulopathy, hemophagocytic syndrome)

Median survival : < 2 months

T-CELL AND NK-CELL LYMPHOID LEUKEMIAS (3)

ADULT T-CELL LEUKEMIA / LYMPHOMA (ATLL)

Japan (1977), Caribbean region, Central Africa

Clinical variants : 1) acute (most common); 2) lymphomatous; 3) chronic; 4) smoldering

Lymphadenopathy, hepatosplenomegaly

Skin involvement (erythematous rushes, papules, nodules)

Leukocytes : 5 – 100 G / L

Lymphocytes with lobated nucleus, CD2 +, CD3 +, CD5 +, usually CD4 +, CD 7 -, CD8 -

Association with HTLV-1 virus

Hypercalcemia

Survival for acute and lymphomatous variants : 2 weeks to > 1 year

T AND NK LYMPHOID LEUKEMIAS (4)

SEZARY SYNDROME (SS)

Skin involvement (Mycosis fungoides)

Erythema, pruritus, generalized erythroderma
Pautrier's microabscesses (epidermotropism)

Presence of Sézary cells in peripheral blood (> 5%)

Lymphocytes with convoluted, cerebriform nucleus (cleft)
Variable phenotype : CD2 +, CD3 +, TCR_{β} +, CD5 +, usually CD4 +, CD8 -

Secondary infiltration of tissues and organs

Lymph nodes, bone marrow, lungs, heart, kidneys, bone

Aggressive disease

Overall survival rate : 10-20% at 5 years

Stages of mycosis fungoides and Sézary syndrome

Stages	Extension
I A / B	Exclusive skin involvment (patch / plaque) A : skin < 10% of cutaneous surface B : skin > 10% of cutaneous surface
II A / B	Stage I with : A : clinical lymph node involvment or : B : cutaneous tumors
III	Erythrodermia : > 80% of cutaneous surface
IV A / B	A : histological lymph node involvment or Sézary cells in peripheral blood B : secondary infiltration of tissues and organs

Modifié d'après Baumann Conzett K. et coll. : Lymphomes cutanés. Classification et recommandations thérapeutiques. Forum Med Suisse 2009, 42 : 744-749.

T-CELL AND NK-CELL LYMPHOID LEUKEMIAS (5)

Contribution of immunological markers, cytogenetics and molecular biology

	CD4	CD8	CD56	RTCR	OTHERS
T-PLL	+	+ / -	-	+	inv(14)
T-LGL	- / +	+	-	+	CD3 +
CLPD-NK	-	-	+	-	CD3 -
ATLL	+	-	-	+	-
SS	+	-	-	+	-

RTCR : Rearrangement of genes coding for variable part of TCR (T-Cell Receptor)

T-PLL : T-cell prolymphocytic leukemia

T-LGL : T-cell large granular lymphocytic leukemia

CLPD-NK : Chronic lymphoproliferative disorders of NK-cells

ATLL : Adult T-cell leukemia / lymphoma

SS : Sézary syndrome

LYMPHOBLASTIC LEUKEMIA / LYMPHOMA (1)

WHO CLASSIFICATION 2008

PRECURSOR B and T LYMPHOID NEOPLASMS

B lymphoblastic leukemia / lymphoma, not otherwise specified (B-ALL / B-LBL)

B lymphoblastic leukemia / lymphoma with recurrent genetic anomalies

T lymphoblastic leukemia / lymphoma

MATURE B CELL NEOPLASMS

Acute lymphoblastic leukemia Burkitt type

LYMPHOBLASTIC LEUKEMIA / LYMPHOMA (2)

CLINICAL FEATURES (1)

B ACUTE LYMPHOBLASTIC LEUKEMIA (B-ALL)

Bone marrow usually involved, peripheral blood frequently

Extramedullary involvement

- Central nervous system
- Lymph nodes, spleen, liver
- Testes

Pancytopenia

Leukocyte count decreased, normal or highly elevated

B LYMPHOBLASTIC LYMPHOMA (B-LBL)

Most frequent sites of involvement

- Skin
- Soft tissues
- Bone marrow
- Lymph nodes

LYMPHOBLASTIC LEUKEMIA / LYMPHOMA (3)
RELATION BETWEEN GENETIC ANOMALY AND PROGNOSIS

LYMPHOBLASTIC LEUKEMIA / LYMPHOMA WITH RECURRENT GENETIC ANOMALIES

CYTOGENETICS	FUSION TRANSCRIPT	PROGNOSIS
t(9;22)(q34;q11.2)	*BCR-ABL 1*	worst of ALL
t(v;11q23)	*MLL rearranged*	poor
t(12;21)(p13;q22)	*TEL-AML1 (ETV6-RUNX1)*	good[1]
Hyperdiploidy (50-66 chromosomes)		good[1]
Hypodiploidy (< 45 chromosomes)		poor
t(5;14)(q31;q32)	*IL3-IGH*	intermediate
t(1;19)(q23;p13.3)	*E2A-PBX1 (TCF3-PBX1)*	poor

[1] In absence of adverse prognostic factors : age > 10 years, higher initial WBC count, slow response to initial therapy, minimal residual disease after therapy, CNS involvment at diagnosis

LYMPHOBLASTIC LEUKEMIA / LYMPHOMA (4)

CLINICAL FEATURES (2)

T LYMPHOBLASTIC LEUKEMIA / LYMPHOMA

Frequent mediastinal (thymic) involvement

Lymphadenopathies

Extranodal sites : skin, tonsils, liver, spleen, central nervous system, testes

High leukocyte count

High risk disease in childhood (induction failure, early relapse, isolated CNS relapse)

In adults, better prognosis than for B-ALL with prognostic adverse cytogenetic anomalies

MATURE B-CELL BURKITT LEUKEMIA VARIANT *(former FAB L3)*

Frequent involvement of CNS at diagnosis

Blasts with deeply basophilic cytoplasm with prominent vacuoles

Extreme chemosensitivity

(risk of acute tumor lysis syndrome)

LYMPHOBLASTIC LEUKEMIA / LYMPHOMA (5)

IMMUNOLOGICAL MARKERS

B-ALL :

PRO-B or EARLY PRE-B CD10 -

EARLY PRE-B or EARLY PRE-B CD10 + or COMMON PRE-B ALL

PRE-B

B MATURE ALL (type Burkitt leukemia variant, former FAB L3)

MARKERS	PRO-B	EARLY PRE-B	PRE-B	B MATURE
CD19	+	+	+	+
CD10	-	+	+	-
CD20	-	+ / -	+	+
CD22	+ cyto	+	+	+
CD34	+ +	+	-	-
HLA-DR	+	+	+	+
TdT	+ + +	+ +	+	+ / -
cIgM[1]	-	-	+	
sIgM[2]	-	-	-	+

T-ALL :

PRE-T

EARLY-T

T CORTICAL

T MATURE OR MARROW T

MARKERS	PRE-T	EARLY-T	T CORTICAL	T MATURE
CD7	+	+	+	+
CD2	-	+	+	+
CD5	-	+	+	+
CD1a	-	-	+	-
cCD3[1]	+	+	-	-
CD3	-	-	+ / -	+
CD4 & CD8	-	-	+	-
CD4 or CD8	-	-	-	+
TdT	+	+	+	+

[1] cIgM, cCD3 : Intracytoplasmic IgM, CD3

[2] sIgM : IgM expressed on cell surface

TREATMENT OF LYMPHOBLASTIC LEUKEMIA / LYMPHOMA

PREDNISONE - VINCRISTINE - ANTHRACYCLINES - ASPARAGINASE

PRINCIPLES : Induction - Consolidation - Maintenance

RESULTS : Adults[1] (1991-2000) : CR*: 69-91%

DFS**: 17- 42%

Children[2] : CR* : 88-96% *(2 children / 3 cured at 5 years)*

ALL *BCR-ABL 1* +	Chemottherapy alone (historical controls)[3]	Chemotherapy + Imatinib (%) (n = 45)[4]
Hematological CR	71	96
Molecular CR		29
Overall survival (at 18 months)	39	65
DFS * (at 18 months)	31	51

Followed, if possible, (age ≤ **55 years, related or** unrelated donor) by bone marrow / stem cell transplantation in CR

*CR : Complete Remission
**DFS : Disease Free Survival

Developments of therapeutical possibilities :

Stratification for risk factors

Allograft in patient with unfavorable risk factors, early autologous transplantation with peripheral blood progenitor cells

Clofarabine, Nelarabine, FMdC, Trimetrexate, liposomal Vincristine, Flavopiridol, Bryostatin, monoclonal antibodies (anti-CD20, anti-CD52)

Arsenic trioxide, proteasome or tyrosine kinase inhibitors[5]

[1] *Hoelzer D., Gökbuget N. : Acute lymphocytic leukemia in adults, in Hoffman R. et al., Hematology : Basic Principles and Practice 2005; Elsevier : p. 1181.*

[2] *Rivera G.K., Crist W.M. : Acute Lymphoblastic Leukemia, in Handin R.I. et al., Blood : Principles & Practice of Hematology 1995; J.P. Lippincott : p. 758.*

[3] *Larson R.A. : Induction therapy for acute lymphoblastic leukemia. UpToDate, mai 2009.*

[4] *Labarthe A. et al. : Imatinib combined with induction or consolidation chemotherapy in patients with de novo Philadelphia chromosome-positive acute lymphoblastic leukemia : results of the GRAAPH-2003 study. Blood 2007; 109 : 1408-1413*

[5] *Thomas D.A. et al. : New agents in the treatment of acute lymphocytic leukaemia. Clinical Haematology 2002; 15 : 771-790.*

PLASMA CELL MYELOMA (MULTIPLE MYELOMA) (1)

DEFINITION	Monoclonal plasma cell proliferation
CLINICAL FEATURES	Asymptomatic Bone pain Pathological fractures Plasmatic hyperviscosity syndrome
BLOOD PICTURE	Rouleaux formation of RBC Signs of bone marrow failure
BIOLOGY	Plasmatic and / or urinary paraprotein High sedimentation rate (ESR) Hypercalcemia Hyperuricemia Renal failure signs

CLINICAL VARIANTS

- Asymptomatic (smoldering) plasma cell myeloma
- Non-secretory myeloma
- Plasma cell leukemia
- Solitary plasmacytoma of bone
- Extraosseous plasmacytoma
- Monoclonal immunoglobulin deposition diseases
  - Primary amyloidosis
  - Monoclonal light and heavy chain deposition diseases
- Osteosclerotic myeloma (POEMS syndrome)
  - Polyneuropathy
  - Organomegaly : *spleen, liver, lymph nodes*
  - Endocrinopathy : *diabetes mellitus, gynecomastia, testicular atrophy*
  - Monoclonal gammopathy
  - Skin : *hyperpigmentation, hypertrichosis*

PLASMA CELL MYELOMA (MULTIPLE MYELOMA) (2)

DIAGNOSTIC CRITERIA OF SYMPTOMATIC PLASMA CELL MYELOMA

1) Paraprotein in serum or urine. No level included. In most cases, IgG > 30 g / L, IgA > 25 g / L, urine light chain > 1 g / 24 hr. Some patients have lower levels
2) Clonal plasma cells in bone marrow or plasmacytoma. No minimal level. Usually > 10% of nucleated BM cells. 5% patients with < 10% BM plasma cells
3) Related organ or tissue impairment
   CRAB : Hypercalcemia, renal insufficiency, anemia, bone lesions
   Hyperviscosity, amyloidosis, recurrent infections

Modified from : Criteria for the classification of monoclonal gammopathies, multiple myeloma and related disorders : a report of the International Myeloma Working Group. Br J Haematol 2003; 121 : 749-757.

INTERNATIONAL STAGING SYSTEM FOR PLASMA CELL MYELOMA

STAGE I (< 0.6 x 10^{12} cells/m^2)	STAGE II (0.6 – 1.2 x 10^{12} cells/m^2	STAGE III (> 1.2 x 10^{12} cells/m^2)
IgG < 50 g / L, IgA < 30 g / L, urinary light chains < 4.0 g / 24 hr Absent or solitary bone lesion Normal hemoglobin, serum calcium, Ig levels (non-M protein)	Intermediate	IgG > 70 g / L, IgA > 50 g / L, urinary light chains > 12 g / 24 hr Multiple lytic bone lesions Hemoglobin < 85 g / L Serum calcium > 3 mmol / L

A : Creatinin < 177 µmol / L B : Creatinin ≥ 177 µmol / L

Modified from : Durie B.G., Salmon S.E. : A clinical staging system for multiple myeloma. Correlation of measured myeloma cell mass with presenting clinical features, response to treatment and survival. Cancer 1975; 36 : 842-854.

PLASMA CELL MYELOMA (MULTIPLE MYELOMA) (3)

PARAPROTEINS = MONOCLONAL IMMUNOGLOBULINS

TYPE	%	TYPE	%
IgG	50	IgD, IgM Biclonal	< 10
IgA	20	No monoclonal Ig	~ 3
Light chain	20	IgE	< 1

FACTORS OF POOR PROGNOSIS

⇗ Creatinin, ⇗ β2-m[1], ⇘ Albumin, ⇗ CRP, ⇗ LDH, ⇘ Hb, ⇗ Ca^{++}

Important plasmacytic infiltration (bone marrow)

Poorly differentiated plasmacytes

Cytogenetic anomalies :

del 13, hypodiploidy

FISH : t(4;14)(p16.3;q32), t(14;16)(q32;q23), t(14;20)(q32;q12)

del 17p13 (p53)

Deletions of Rb (retinoblastoma) gene

Ki-67 expression

Presence of MDR (Multidrug Resistance)

[1] β_2-m : β_2-microglobulin

COMPLICATIONS

Hyperviscosity syndrome (mostly IgA, IgG3)

Neurological : nerve compression (radicular or spinal)

Renal : light chain, calcium or uric nephropathy, amyloidosis, plasmacytic infiltration

Infectious

Hematological : bone marrow failure, thrombopathy

SURVIVAL : ISS (International Staging System) 8'449 patients[2]

STAGE	PARAMETERS	MEDIAN SURVIVAL (MONTHS)
1	β_2-m < 3.5 mg / L Albumin ≥ 35 g / L	62
2	β_2-m < 3.5 mg / L Albumin < 35 g / L or β_2-m ≥ 3.5 - < 5.5 mg / L	44
3	β_2-m ≥ 5.5 mg / L	29

[2] Modified from : Greipp P.R. et al. : International staging system for multiple myeloma. J Clin Oncol 2005; 23 : 3412-3420.

PLASMA CELL MYELOMA (MULTIPLE MYELOMA) (4)

DIFFERENTIAL DIAGNOSIS

1. MGUS : Monoclonal Gammopathy of Undetermined Significance (evolution to plasma cell myeloma, lymphoplasmacytic lymphoma - Waldenström macroglobulinemia or amyloidosis : 22% after 19 years)

Differential diagnosis between MGUS and smoldering myeloma

	MGUS	SMOLDERING MYELOMA
Plasma cells (bone marrow)	< 10%	≥ 10%
Serum monoclonal Ig	< 30 g / L ↘ of uninvolved Ig : 30-40% of cases	> 30 g / L ↘ of uninvolved Ig : > 90% of cases
Lytic bone lesions	0	0
Symptoms / Infections	0	0

For both entities, levels of hemoglobin, creatinin and calcium within normal range

2. Primary amyloidosis (amyloidosis AL)
3. Lymphoplasmacytic lymphoma - Waldenström macroglobulinemia, cf. page 171
4. Heavy chain diseases

	HISTOLOGY	CLINICAL LOCALISATION
γ heavy chain disease	Lymphoplasmacytic lymphoma	Lymph nodes, Waldeyer's ring, BM, spleen, liver, blood
μ heavy chain disease	Chronic lymphocytic leukemia	Spleen, liver, BM, blood
α heavy chain disease (IPSID)[1]	Extranodal marginal zone lymphoma (MALT)[2]	Small bowel, mesenteric lymph nodes

5. Reactional plasmacytosis : German measles, HIV, tuberculosis, immunological disorders, alcoholism, see page 113

[1] IPSID : Immunoproliferative small intestinal disease

[2] MALT : Mucosa-Associated Lymphoid Tissue

PLASMA CELL MYELOMA (MULTIPLE MYELOMA) (5)
TREATMENT

Plasmapheresis (in case of hyperviscosity syndrome)

Melphalan + Prednisone, VBAP[1], VMCP[2] (60% of responses, no complete remission)

VAD (Vincristine + Doxorubicin + Dexamethasone high dose)

Radiotherapy (solitary plasmacytoma)

Supportive care (RBC, platelet transfusions, antibiotics, analgesics, bisphosphonates)

Intensification with autologous transplant (PB stem cells or BM)

Allogeneic transplantation : PB stem cells or BM (< 50 years, possible cure, important transplant related mortality, GvH +++)

Thalidomide, Lenalidomide, Bortezomib (proteasome inhibitor), reduced intensity conditioning transplant (mini-allotransplant with non myeloablative conditioning)

[1] VBAP : Vincristine + BCNU + Doxorubicin + Prednisone
[2] VMCP : Vincristine + Melphalan + Cyclophosphamide + Prednisone

HODGKIN LYMPHOMA (1)

SYMPTOMS AND CLINICAL FEATURES

B symptoms:

- Unexplained persistent and recurrent fever > 38°C during the previous month
- Recurrent drenching nights sweats during the previous month
- Unexplained loss of > 10% of body weight during the 6 months before initial staging

Other symptoms : pruritus
alcohol-induced pain (usually abdominal)

Lymphadenopathy(-ies)

- Mediastinal involvement mainly in nodular sclerosis subtype
- Abdominal (and splenic) involvement mainly in mixed cellularity subtype

HISTOLOGY

Reed-Sternberg cells (most often of B-cell origin)

5 histological types :
- Nodular lymphocyte predominant Hodgkin lymphoma
- Classical Hodgkin lymphoma
  - Nodular sclerosis classical Hodgkin lymphoma
  - Lymphocyte-rich classical Hodgkin lymphoma
  - Mixed cellularity classical Hodgkin lymphoma
  - Lymphocyte-depleted classical Hodgkin lymphoma

HODGKIN LYMPHOMA (2)

STAGING - COTSWOLDS REVISION (1989) OF THE ANN ARBOR CLASSIFICATION

STAGE	DESCRIPTION
I	Involvement of a single lymph node region or lymphoid structure (e.g. spleen, thymus, Waldeyer ring)
II	Involvement or two or more lymph node regions on the same side of the diaphragm (the mediastinum is a single site; hilar lymph nodes are lateralized). The number of anatomic sites involved should be indicated by suffix (e.g. II_3)
III	Involvement of lymph nodes regions or structures on both sides of the diaphragm If together with spleen involvement : III_s
III_1	With or without spleen involvement (III_s) and with hilar splenic, coeliac or portal nodes involvement
III_2	With paraaortic, iliac or mesenteric nodes involvement
IV	Diffuse or disseminated involvement of one or more extranodal organs or tissues, with or without associated lymph node involvement

At any disease stage :	A	No symptoms
	B	Fever, sweats, loss of weight
	X	Bulky disease (widening of the mediastinum ≥ 1/3 of the internal transverse diameter of the thorax at the level of T 5/6 interspace or > 10 cm maximum dimension of a nodal mass)
	E	Involvement of a single extranodal site, contiguous or proximal to the known nodal site

Modified from : Lister T.A. et al. : Report of a committee convened to discuss the evaluation and staging of patients with Hodgkin's Disease : Cotswolds meeting. J Clin Oncol 1989; 7 : 1630-1636.

HODGKIN LYMPHOMA (3)

DIFFERENTIAL DIAGNOSIS

Anaplastic large T cell lymphoma : t(2;5)

UNFAVORABLE PROGNOSTIC FACTORS

Large tumor mass (e.g. : bulky mediastinal)

Presence of B symptoms

Primary refractory form

IPS = International Prognostic score (advanced stages of disease)

Serum albumin < 40 g / L
Hemoglobin < 105 g / L
Male gender
Stage IV disease
Age ≥ 45 years
WBC count > 15 G / L
Lymphocyte count < 0.6 G / L (or < 8% of leukocyte differential count)

COMPLICATIONS

Immediate, treatment related
Infection(s)
Azoospermia, early menopause
Secondary leukemia / cancer

HODGKIN LYMPHOMA (4)

TREATMENT

Radiotherapy
Chemotherapy

M(C)OPP, ABVD, M(C)OPP + ABVD

MIME, CEP, DHAP, BEACOPP
Autologous / allogeneic transplant

PROGNOSIS AND PREDICTIVE FACTORS

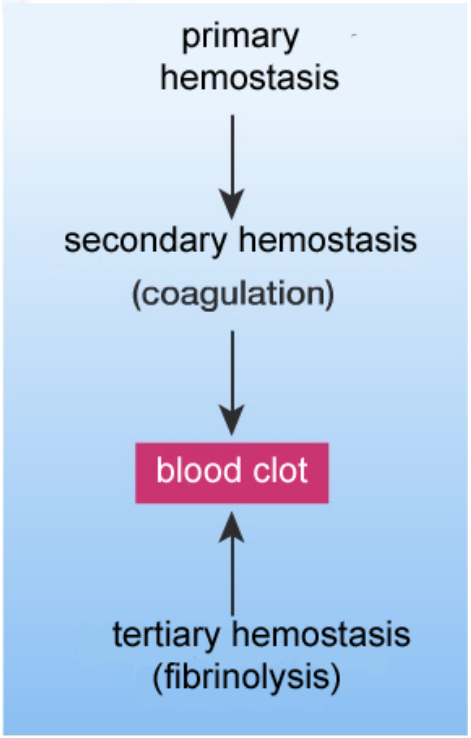
Curable disease in more than 85% of cases by modern radiation and chemotherapy
Prognosis is function of staging, clinical and laboratory parameters
Response after 2 courses of ABVD by FDG-PET imaging is a relevant prognostic indicator in advanced stage disease[1]

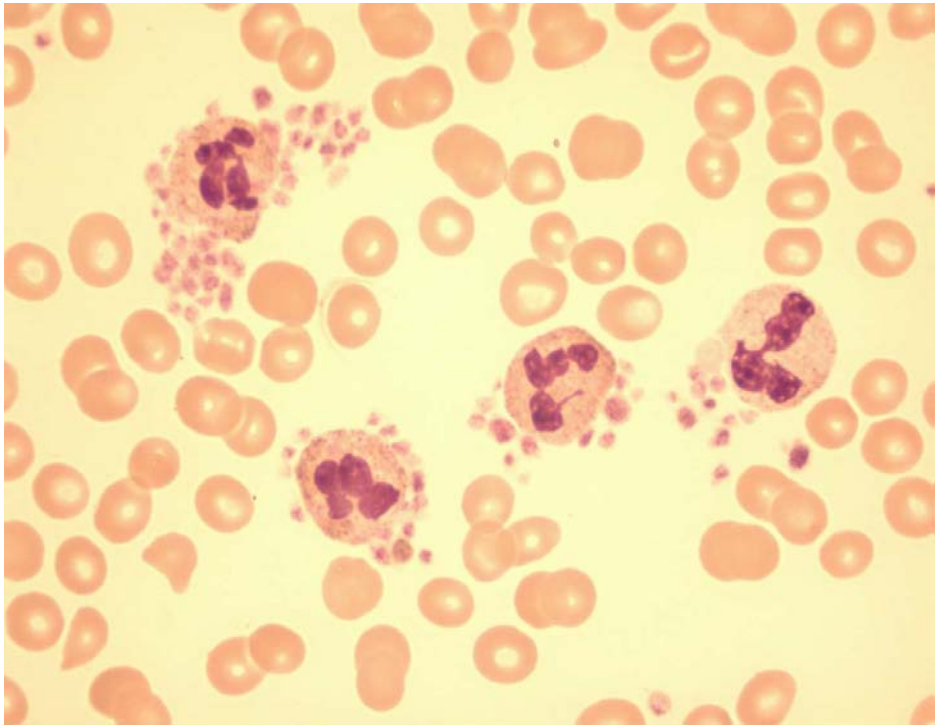
M(C)OPP : Mustard gas analog (Cyclophosphamide) + Vincristine + Procarbazine + Prednisone
ABVD : Adriamycin + Bleomycin + Vinblastine + Dacarbazine (DTIC)
MIME : Mitoguazone + Ifosfamid + Methotrexate + Etoposide
CEP : Lomustine + Etoposide + Prednimustine
DHAP : Dexamethasone + Cisplatin + Cytarabine
BEACOPP : Bleomycin + Etoposide + Doxorubicin + Cyclophosphamide + Vincristine + Procarbazine + Prednisone

[1] *Gallamani A. et al. : Early interim 2-(^{18}F)fluoro-2-deoxy-D-glucose positron emission tomography is prognostically superior to international prognostic score in advanced-stage Hodgkin's lymphoma : a report from a joint Italian-Danish study. J clin Oncol 2007; 25 :3746-3752.*

Part 3

HEMOSTASIS



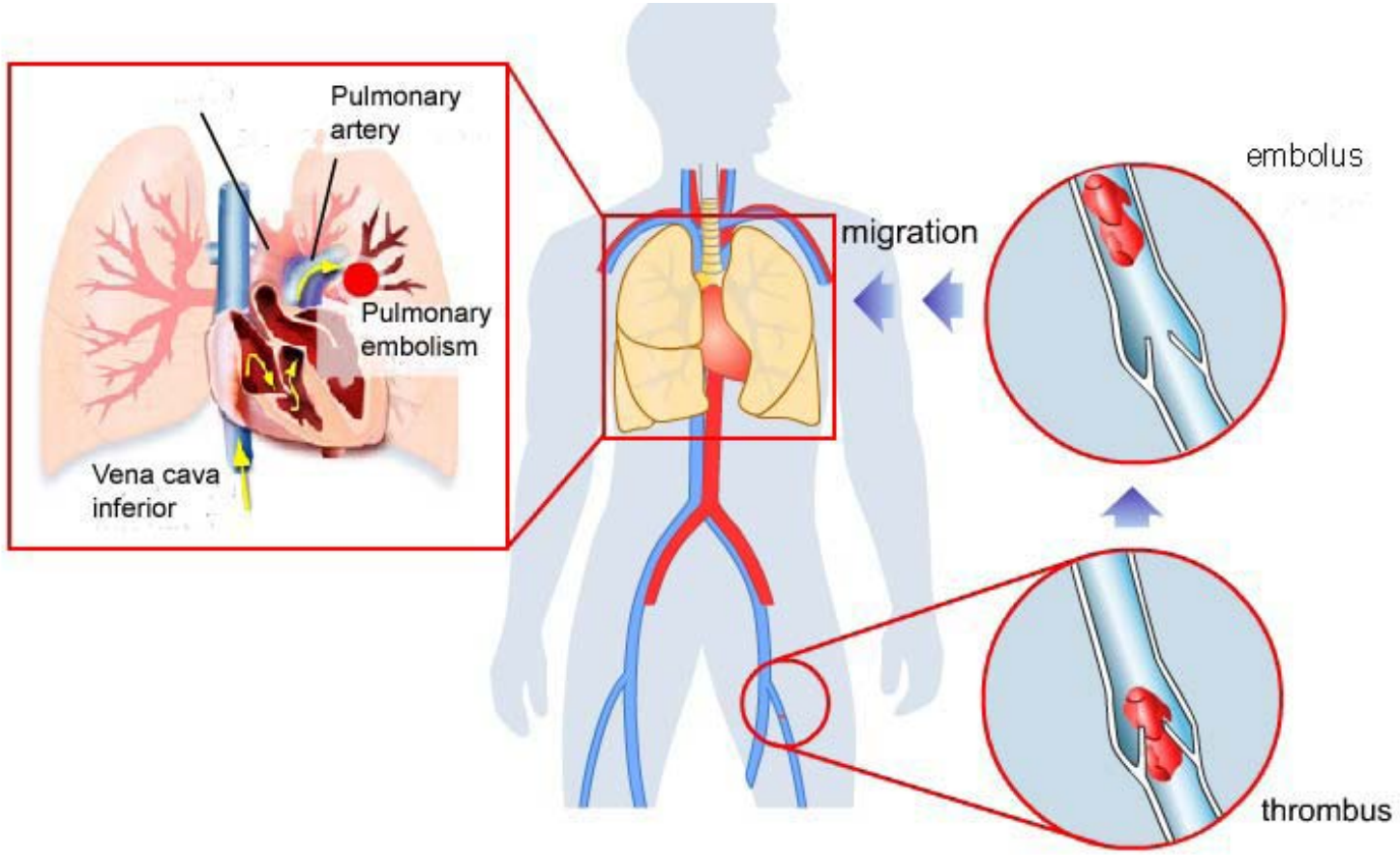


HEMOSTASIS
EXPLORATION METHODS

PRIMARY HEMOSTASIS	Capillary resistance
	Platelet count (RI : 150 – 350 G / L)
	PFA-100™ [1]
	Platelet functions (ADP, arachidonic acid, adrenalin-heparin, collagen, ristocetin)
SECONDARY HEMOSTASIS *(Coagulation)*	Prothrombin time (PT, Quick) *(Exploration of extrinsic pathway)*
	Activated partial thromboplastin time (aPTT) *(Exploration of intrinsic pathway)*
	Thrombin time (TT) *(Exploration of fibrin formation)*
	Fibrinogen dosage
	Investigation of factor XIII deficiency (fibrin stabilizing factor)
	Investigation of activation *(Fibrin monomers and D-dimers)*
TERTIARY HEMOSTASIS (*Fibrinolysis)*	Euglobulins lysis time

[1] PFA-100™ (Platelet Function Analyzer) : *in vitro* measure of the time to occlusion of a membrane (measure of platelet adhesion and aggregation process). Replaces, if device available, the classical bleeding time

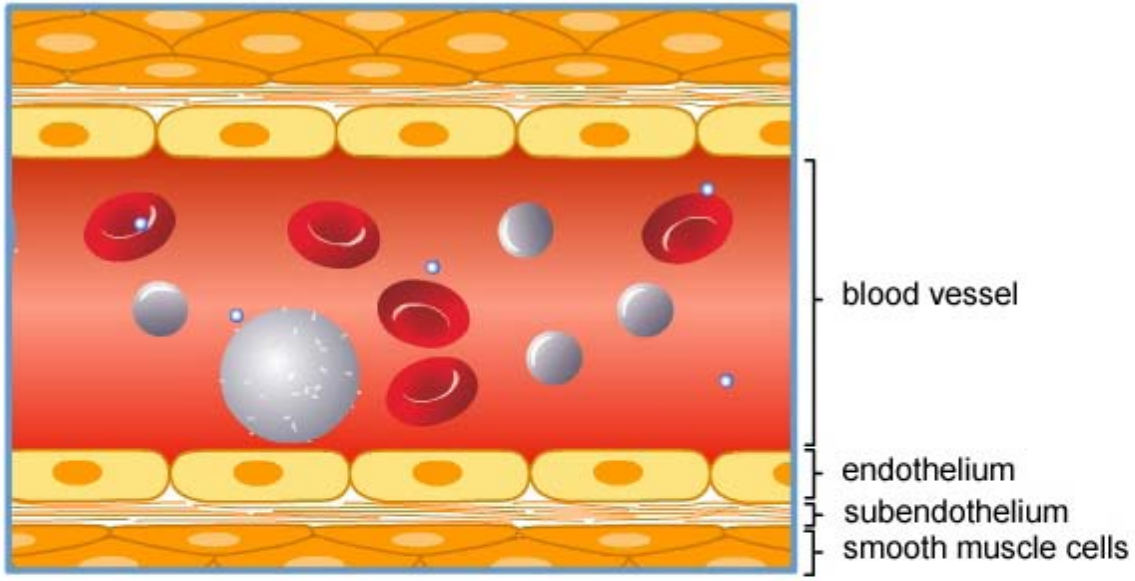
THROMBUS AND EMBOLUS



Thrombus : inappropriate clot formation in a blood vessel (artery or vein)
Embolus : migrating thrombus

MAIN ACTORS OF HEMOSTASIS

Blood vessels
Platelets
Coagulation proteins



STEPS OF HEMOSTASIS

PRIMARY HEMOSTASIS

- Vascular time
  - Vasoconstriction (vascular spasm)
- Platelet time
  - Platelet adhesion to the vessel lesion
  - Platelet plug formation

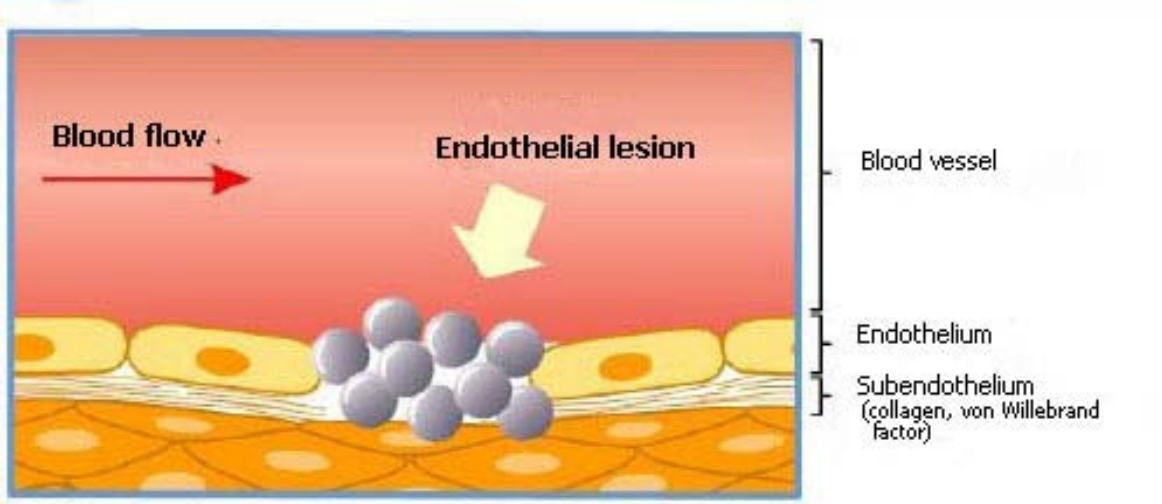
SECONDARY HEMOSTASIS (coagulation)

- Coagulation cascade
- Clot formation

TERTIARY HEMOSTASIS (fibrinolysis)

- Clot lysis

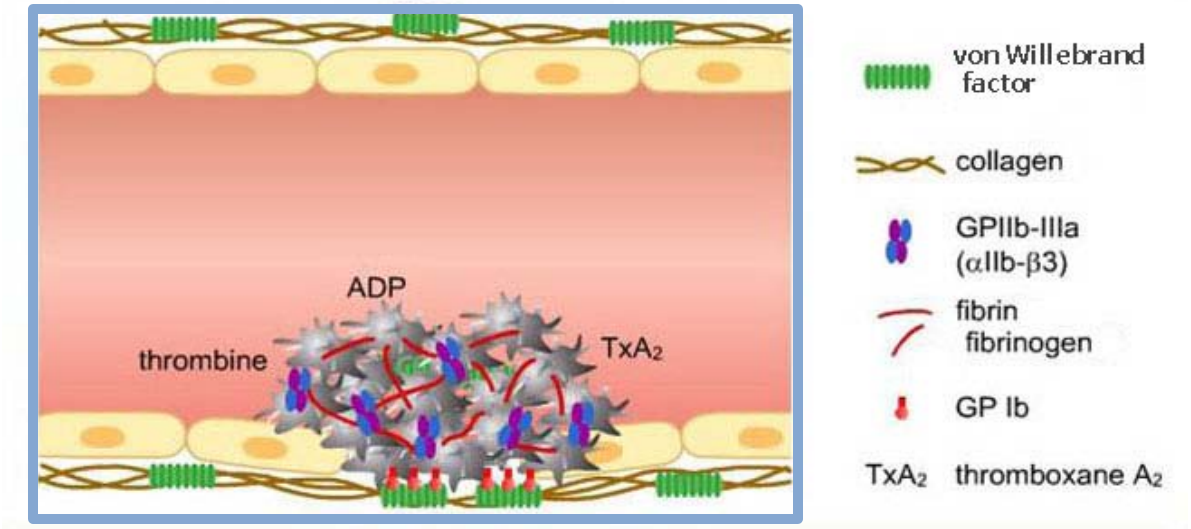
STEPS OF PRIMARY HEMOSTASIS



Platelet adhesion

Platelet activation

Platelet aggregation



Formation of platelet plug

VON WILLEBRAND FACTOR

Synthetized by endothelial cells and megakaryocytes

Composed of a series of multimers : the very high molecular weight multimers are physiologically degraded by a specific protease (ADAMTS13), leading to prevention of spontaneous platelet aggregates formation (cf. TTP, p. 86-87)

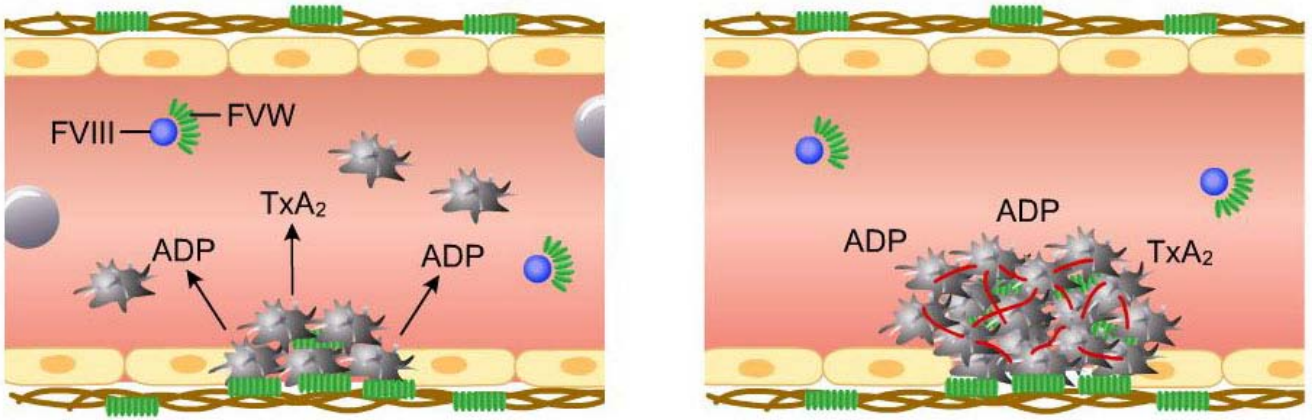
Involved, *in vitro,* in the process of platelet adhesion to subendothelial fibers

Mandatory for *in vitro* ristocetin induced platelet aggregation

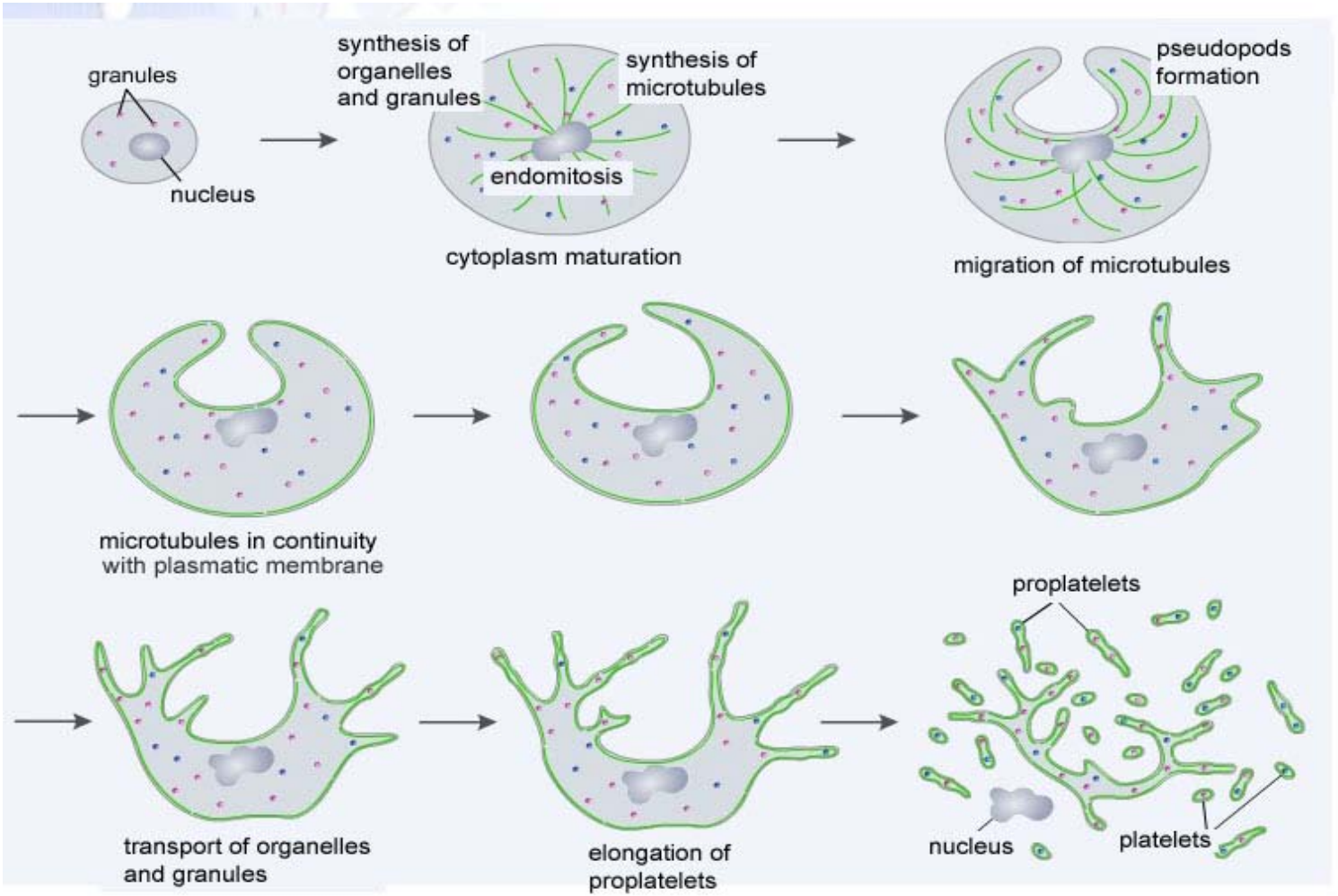
Transport of factor VIII to vascular lesion

Bound to factor VIII, it prolongs its life expectancy

TxA_2 : Thromboxane A_2
FVW : von Willebrand factor
ADP : Adenosin Diphosphate
FVIII : Factor VIII



PLATELET PRODUCTION FROM THE MEGAKARYOCYTE

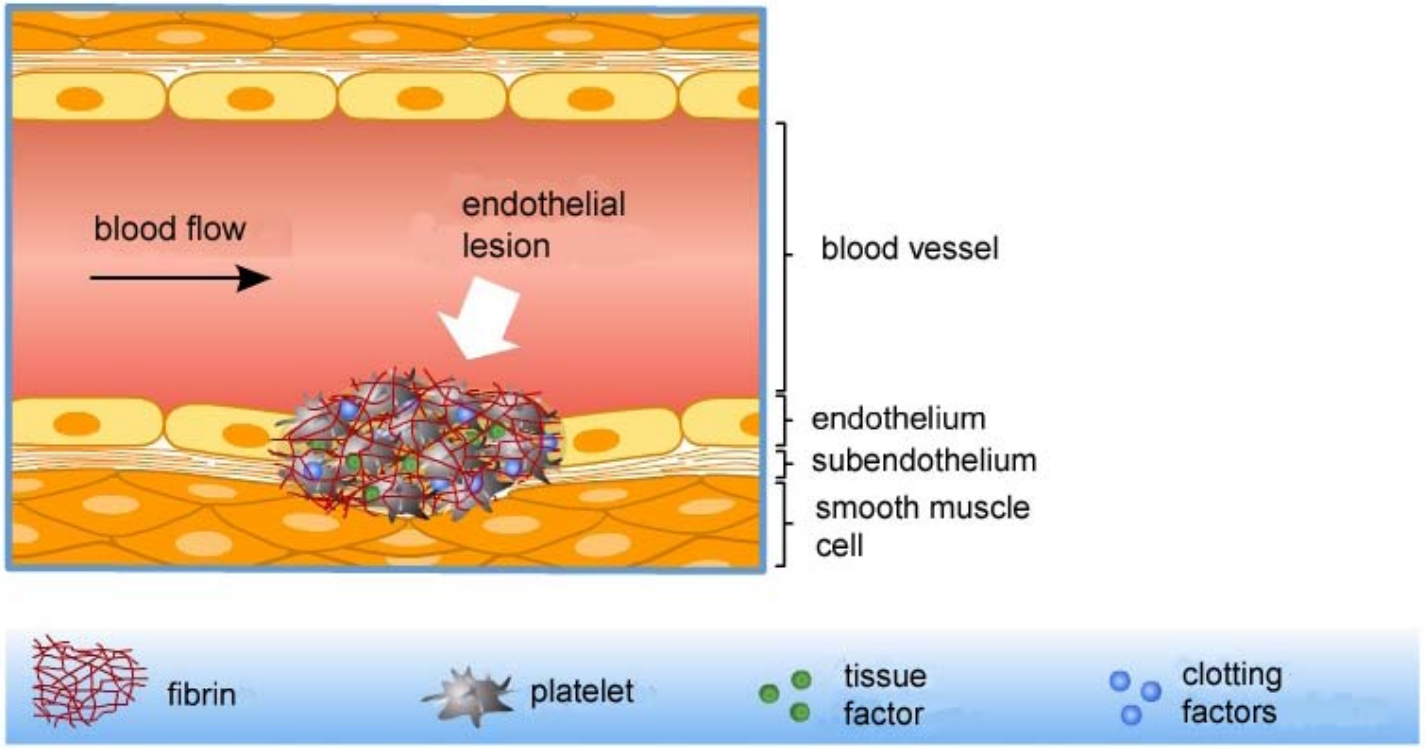


1 mature megakaryocyte produces 2'000 - 3'000 platelets

SECONDARY HEMOSTASIS
COAGULATION

Coagulation (blood clotting) needs interaction of :

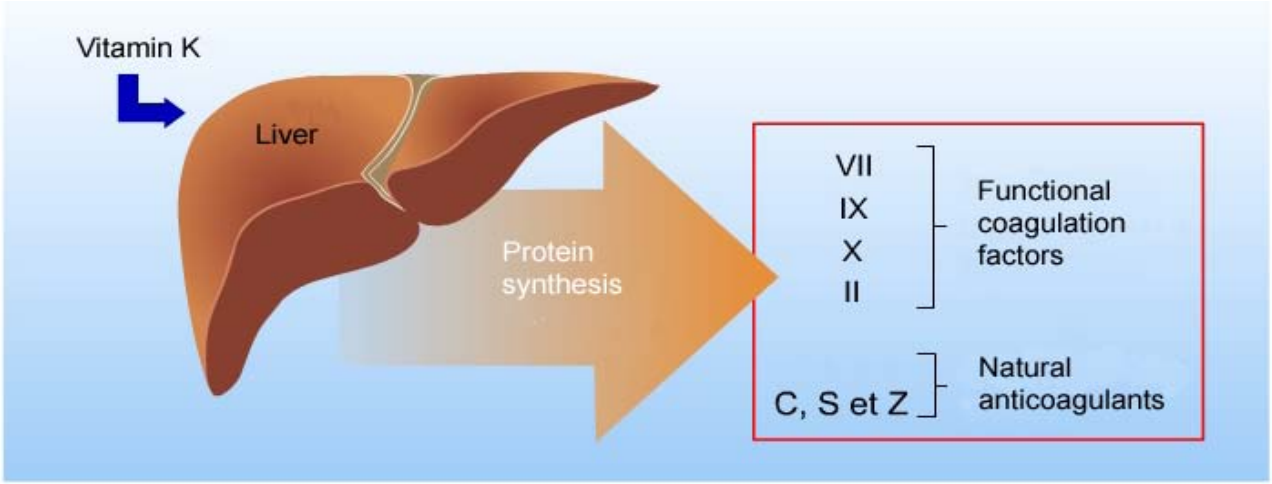
- Plasmatic proteins (coagulation factors and inhibitors)
- A tissular protein (tissue factor)
- Platelets
- Calcium



COAGULATION FACTORS

FACTOR	NAME	HALF-LIFE (hours)	PRODUCTION	VITAMINE K DEPENDENCE
High molecular weight kininogen	Fitzgerald factor	150	Liver	–
Prekallikrein	Fletcher factor	35	Liver	–
Factor I	Fibrinogen	90	Liver	–
Factor II	Prothrombin	65	Liver	+
Factor V	Proaccelerin	15	Liver	–
Factor VII	Proconvertin	5	Liver	+
Factor VIII	Antihemophilic factor A	12	Liver (sinusoidal cells)	–
Factor IX	Christmas factor or antihemophilic factor B	24	Liver	+
Factor X	Stuart-Prower factor	40	Liver	+
Factor XI	Antihemophilic factor C	45	Liver	–
Factor XII	Hageman factor	50	Liver	–
Factor XIII	Fibrin stabilizing factor	200	Liver	–
Factor vW	von Willebrand factor	15	Endothelium Megakaryocytes	–

VITAMIN K DEPENDENT FACTORS



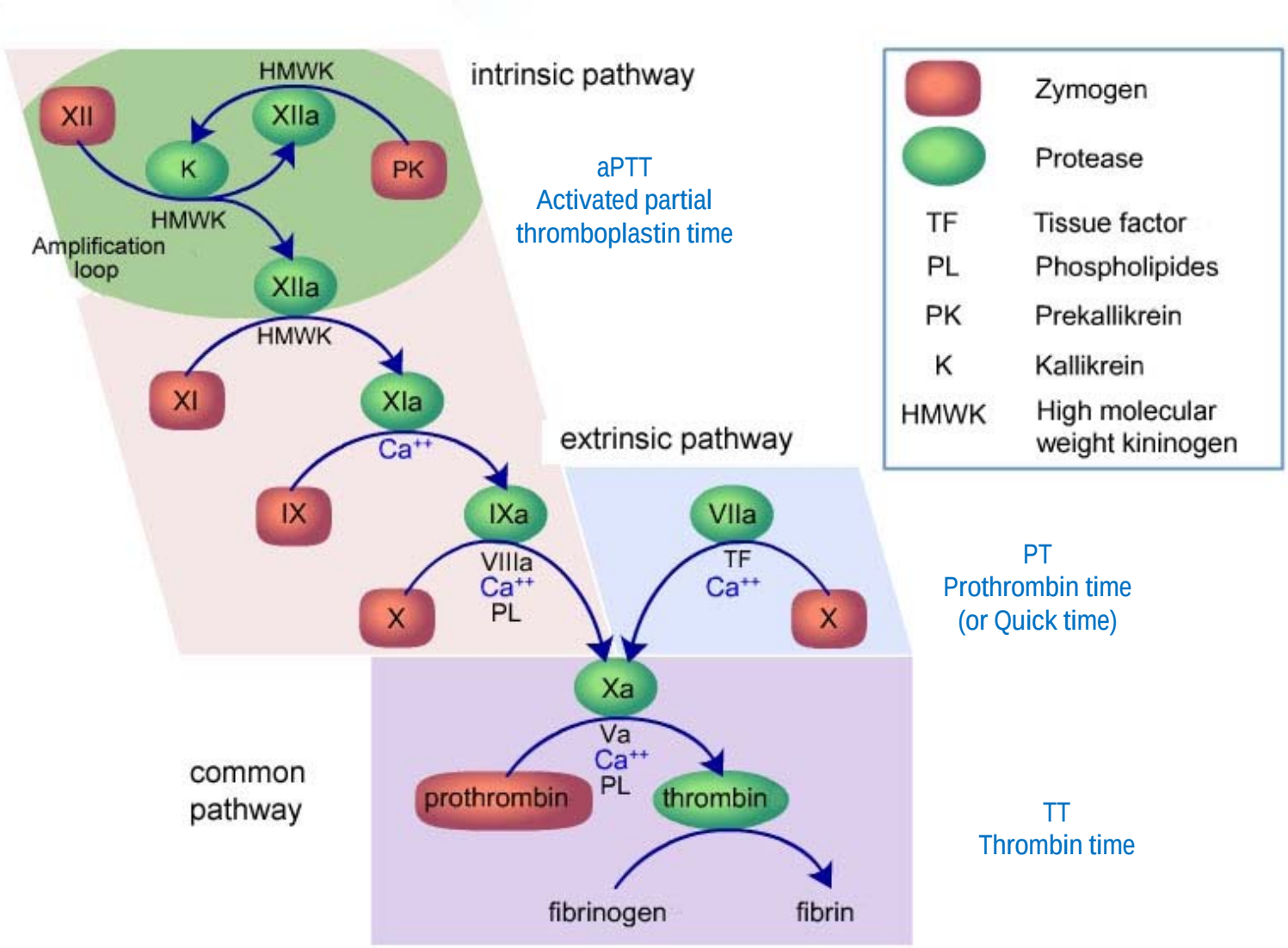
These coagulation factors are synthetized by hepatocytes

Vitamin K is necessary for complete functional synthesis

Vitamin K (liposoluble), in reduced state, works as a cofactor to a carboxylase which transforms 10-12 glutamic acid (Glu) residues in γ-carboxyglutamic acid (Gla)

Vitamin K dependent factors bind to the cell membranes through this Gla domain, in presence of Ca^{++}

COAGULATION CASCADE (1)
CLASSICAL SCHEME



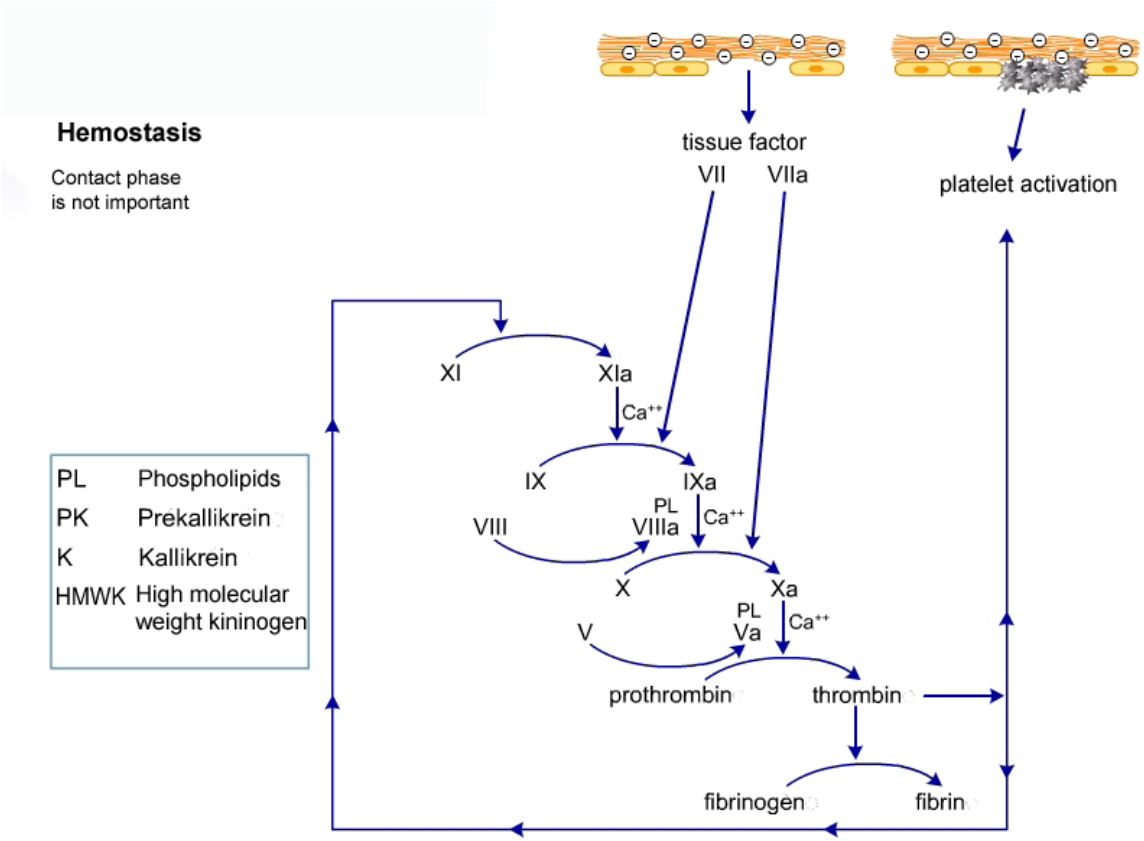
aPTT
Activated partial thromboplastin time

PT
Prothrombin time
(or Quick time)

TT
Thrombin time

Fibrinogen
Functional or quantitative dosage

COAGULATION CASCADE (2)
CONCEPTUAL CHANGES



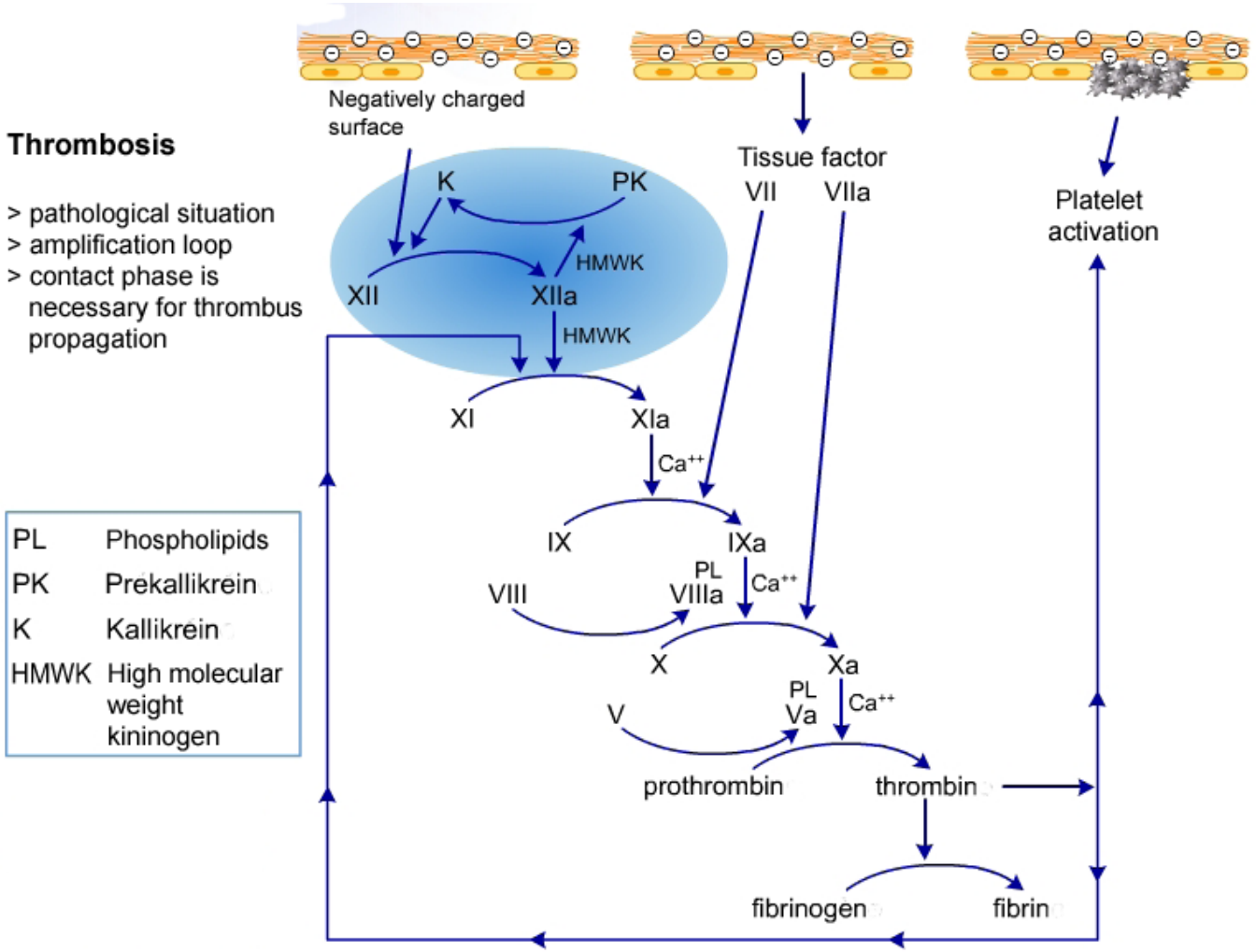
Factor XI may be activated by thrombin as well as by factor XIIa

Factor XI deficiency is responsible for bleeding whereas deficiencies in factor XII, prekallikrein or high molecular weight kininogen do not cause bleeding

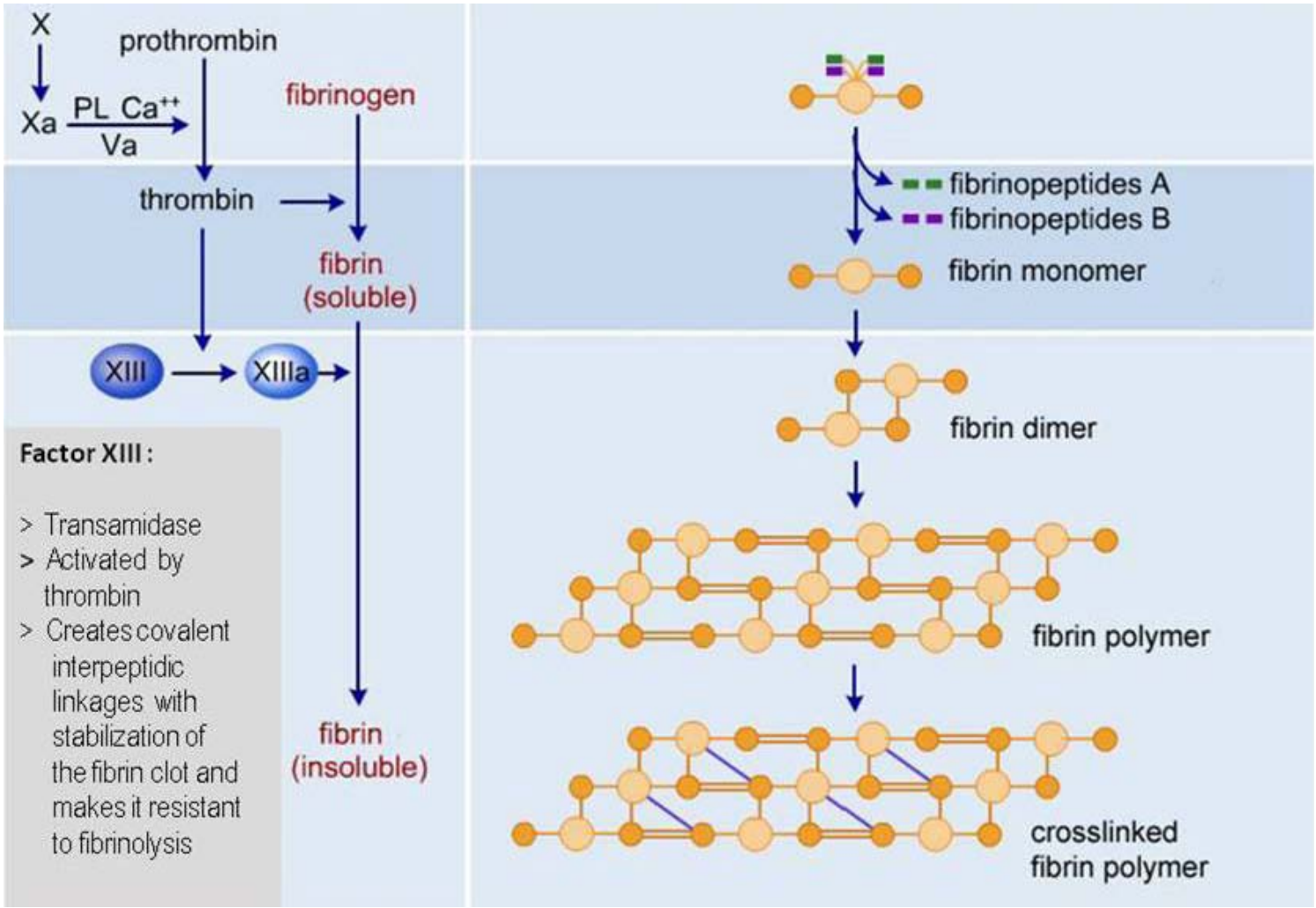
In experimental models factor XI and factor XII deficiencies have antithrombotic effect

Factor XII is activated by negatively charged surfaces, activated platelets and clot surface

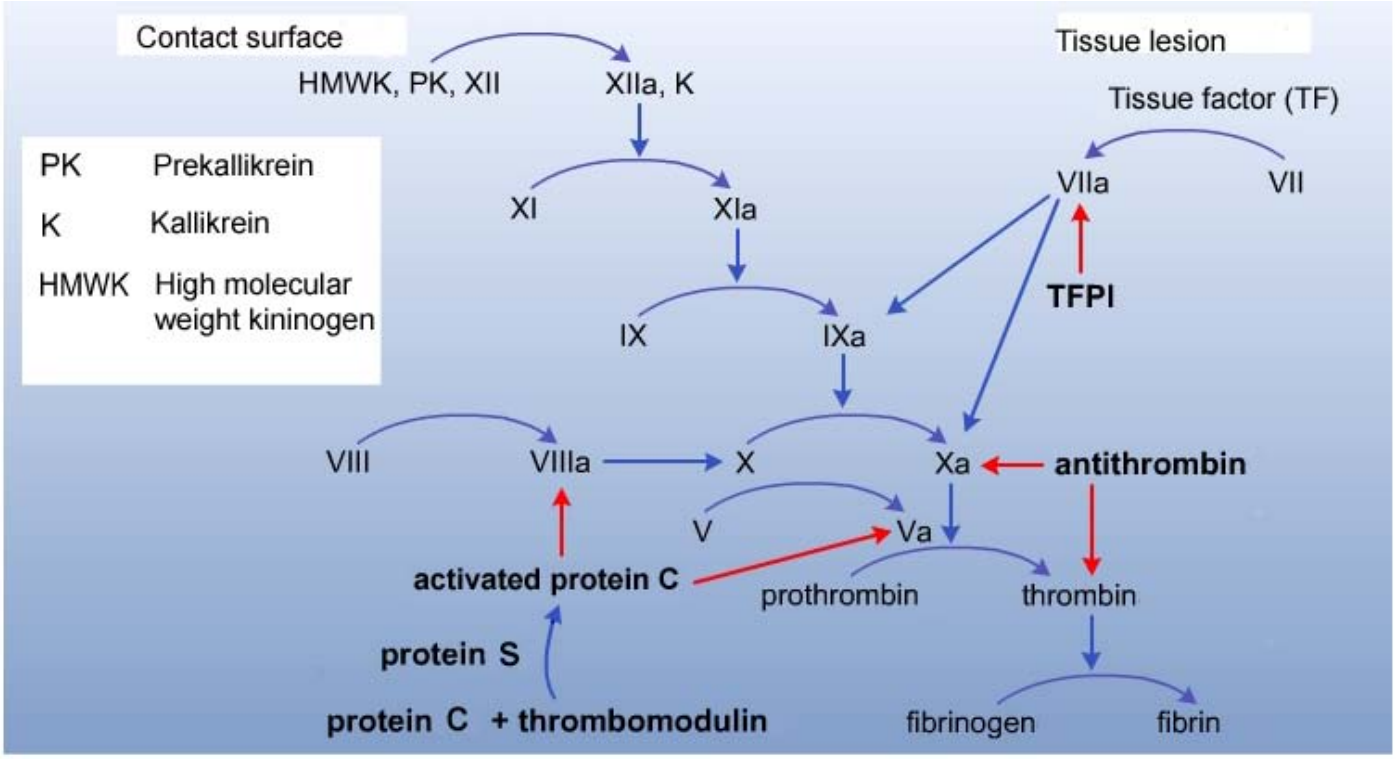
COAGULATION CASCADE (3)
CONCEPTUAL CHANGES (2)



FACTOR XIII AND FIBRIN STABILIZATION



NATURAL ANTICOAGULANTS

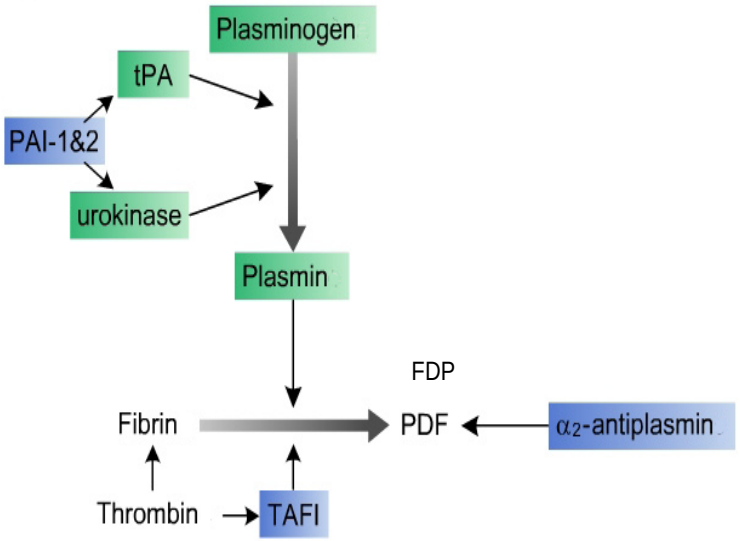


TFPI *(Tissue Factor Pathway Inhibitor)* is an effective inhibitor of factor VII - Tissue factor complex

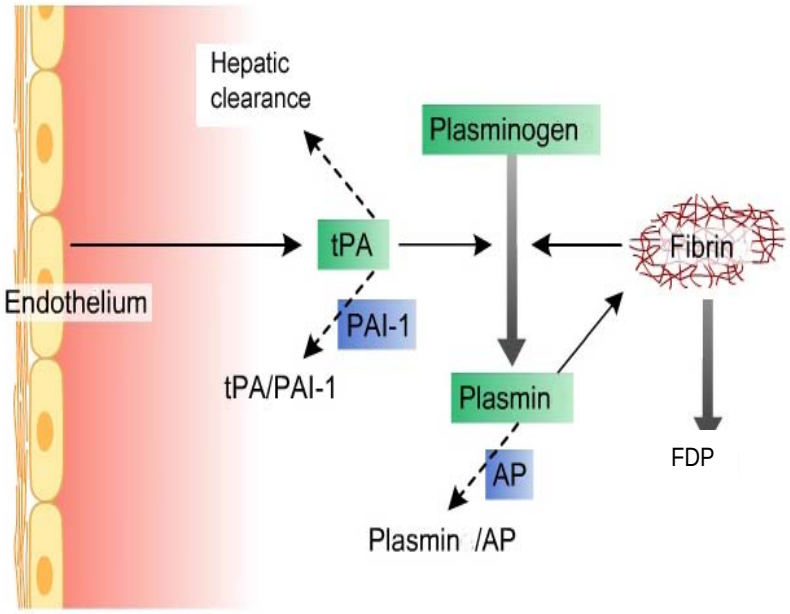
Antithrombin neutralizes all procoagulant serine proteases (thrombin, factors IXa, Xa and XIa)

The protein C - protein S system inhibits factors Va and VIIIa

TERTIARY HEMOSTASIS
FIBRINOLYSIS



Intravascular fibrinolysis



tPA :	Tissular Plasminogen Activator	Profibrinolytic proteins	
PAI :	Plasminogen Activators Inhibitors 1 and 2		
FDP :	Fibrin Degradation Products	Antifibrinolytic proteins	
TAFI :	Thrombin Activatable Fibrinolysis Inhibitor		
AP :	α_2-antiplasmin		

HEMORRHAGIC SYNDROME
PRIMARY HEMOSTASIS (1)

Reduced capillary resistance with platelet count[1], PFA-100™[2], tests of platelet function, coagulation, and fibrinolysis in normal range

VASCULAR PURPURA

NON INFLAMMATORY

- Senile purpura
- Ehlers-Danlos syndrome (collagen abnormality)
- Vitamin A deficiency
- Treatment with steroids, Cushing disease
- Chronic and pigmented dermatitis
- Osler disease (hereditary hemorrhagic telangiectasia)

INFLAMMATORY (VASCULITIS)

- Drug induced (Penicillin, non steroidal antiinflammatory drugs)
- Autoimmune disease SLE, RA, PAN, Crohn's disease)
- Bacterial infection
- Viral infection (hepatitis B, CMV, EBV, parvovirus)
- Lymphoid neoplasm
- Cancer
- Rheumatoid purpura (Henoch-Schoenlein)
- Cryoglobulinemia
- Hypergammaglobulinemia
- Idiopathic

SLE : Systemic Lupus Erythematosus
RA : Rheumatoid arthritis
PAN : Panarteritis nodosa
EBV : Epstein-Barr Virus
CMV : Cytomegalovirus

[1] In case of vasculitis, immune thrombocytopenia may be found
[2] Replaces bleeding time

HEMORRHAGIC SYNDROME
PRIMARY HEMOSTASIS (2)

Prolonged occlusion time (PFA-100™) [1]

With normal platelet function tests

Thrombocytopenia
Secondary thrombocytosis

With platelet function anomaly and aPTT within normal range

Thrombopathy : acquired
hereditary
Thrombocytosis of myeloproliferative neoplasm

With platelet function anomaly and prolonged aPTT

von Willebrand disease

[1]Occlusion time (PFA-100™)

	Normal *(seconds)*[1]	Aspirin	von Willebrand	Glanzmann[2]	Bernard-Soulier[2]
Col / EPI[3]	84 – 160	⇗	⇗	⇗	⇗
Col / ADP[4]	68 – 121	normal	⇗	⇗	⇗

LCH-CHUV, 2009

[2] cf. following page
[3] Col / EPI : Collagen / Epinephrin
[4] Col / ADP : Collagen / Adenosin-5'-diphosphate

THROMBOPATHY

ACQUIRED

DRUGS

- Aspirin : irreversible inhibition of cyclooxygenase
- Clopidogrel (Plavix®) : inhibition of ADP binding to its platelet receptor
- Abciximab (ReoPro®) : Fab fragment of humanized chimeric antibody against receptors of Glycoprotein IIb-IIIa

RENAL FAILURE

PARAPROTEINEMIA

MYELOPROLIFERATIVE NEOPLASM AND MYELODYSPLASTIC SYNDROME

HEREDITARY

THROMBASTHENIA OR GLANZMANN DISEASE

- Autosomal recessive transmission
- GP IIb-IIIa deficiency
- Pathological aggregation tests with ADP, adrenalin, and collagen
- Normal aggregation on ristocetin (primary phase only)
- Platelet count within normal range
- Absence of morphological anomaly

BERNARD-SOULIER SYNDROME

- Autosomal recessive transmission (rarely dominant)
- GP Ib / IX / V deficiency
- Absence of aggregation on ristocetin
- Thrombocytopenia of variable importance
- Presence of giant platelets

STORAGE POOL DISEASE

- Anomalies of dense granules (ADP deficiency)
- Pathological aggregation on ADP, adrenalin and collagen
- Platelet count within normal range
- Absence of morphological anomaly

GRAY PLATELET SYNDROME

- Anomalies of α granules
- Platelet aggregation tests usually within normal range
- Thrombocytopenia of variable importance
- Giant, agranular platelets, of gray color on blood smear

THROMBOCYTOPENIA (1)

DEFINITION

Platelet count < 150 G / L

HEMORRHAGIC RISK

(In case of normal platelet function)

Low if platelet count in range of 50 to 150 G / L

High by platelet count < 20 G / L

SOME RULES OR RECOMMENDATIONS

Every thrombocytopenia has to be controlled on a blood smear (eliminate pseudothrombocytopenia due to EDTA anticoagulation of the probe)

By platelet count < 50 G / L, measure of occlusion time (PFA-100™) is useless

If platelet functions are correct, the occlusion time on PFA-100™ becomes prolonged from platelet counts < 100 G / L. Platelet count at 70 G / L with normal occlusion time does not allow exclusion of hemorrhagic risk in case of surgical intervention

At similar platelet levels the hemorrhagic risk is higher in case of "central" thrombocytopenia than in thrombocytopenia of "peripheral" origin

THROMBOCYTOPENIA (2)
IN THE SETTING OF BICYTOPENIA OR PANCYTOPENIA

Hypersplenism (e.g. severe hepatic failure)

Bone marrow dysfunction

Aplasia

Infiltration : Myeloid or lymphoid neoplasm, osteomedullary cancer metastasis

Dysplasia : Reversible (Vitamin B_{12} or folate deficiency)

Refractory (myelodysplastic syndrome)

Fibrosis

Reduction of thrombopoietin synthesis (e.g. severe hepatic failure)

SOLITARY THROMBOCYTOPENIA

	CENTRAL	PERIPHERAL
Megakaryocytes	⇘	Usually ⇗
Mean platelet volume (MPV)	⇘ [1]	⇗
Etiology	Thiazide Alcohol	cf. pages 215-216

[1] *Frequently increased in myeloproliferative neoplasm and myelodysplastic syndrome*

SOLITARY PERIPHERAL THROMBOCYTOPENIA (1)
NON IMMUNOLOGICAL

BY ANOMALY OF PLATELET DISTRIBUTION

Hypersplenism

BY PLATELET DESTRUCTION

Alcohol
Disseminated Intravascular Coagulation (DIC)
Extracorporeal circulation
Thrombotic Thrombocytopenic Purpura (TTP)
Hemolytic Uremic Syndrome (HUS)
HELLP[1] syndrome (10% of preeclampsias)
Renal transplant rejection
Allogeneic stem cell or bone marrow transplantation

[1]HELLP : Hemolysis, Elevated Liver function tests, Low Platelets (in pregnancy)

SOLITARY PERIPHERAL THROMBOCYTOPENIA (2)
IMMUNE

PRIMARY

Primary immune thrombocytopenia (PIT)

SECONDARY

Due to autoantibody or immune complexes

Drugs *(Heparin, Quinine)*
Infection *(bacteria, virus, parasite)*
Autoimmune disease *(SLE[1], Evans syndrome[2])*
Lymphoid neoplasm
Cancer

Due to alloantibody

Neonatal thrombocytopenia
Posttransfusion purpura

[1] Systemic lupus erythematosus
[2] Autoimmune hemolytic anemia and thrombocytopenia

INVESTIGATION OF THROMBOCYTOPENIA

Full blood count

Blood smear examination

- Pseudothrombocytopenia
- RBC fragmentation (schistocytes)
- Toxic changes of neutrophils
- Lymphocyte stimulation
- Absolute lymphocytosis
- Erythroblastosis and / or myelocytosis
- Parasites

Complete coagulation tests with search for coagulation activation (DIC)

Bone marrow examination (cytology and histology)

Direct Coombs test (antiglobulin test)

Viral serology (HIV, EBV, CMV)

SLE[1] serology

Thyroid function tests

Anti-HLA antibodies

Antiplatelet antibodies

[1] *Systemic lupus erythematosus*

HEMORRHAGIC SYNDROME
SECONDARY HEMOSTASIS (COAGULATION)

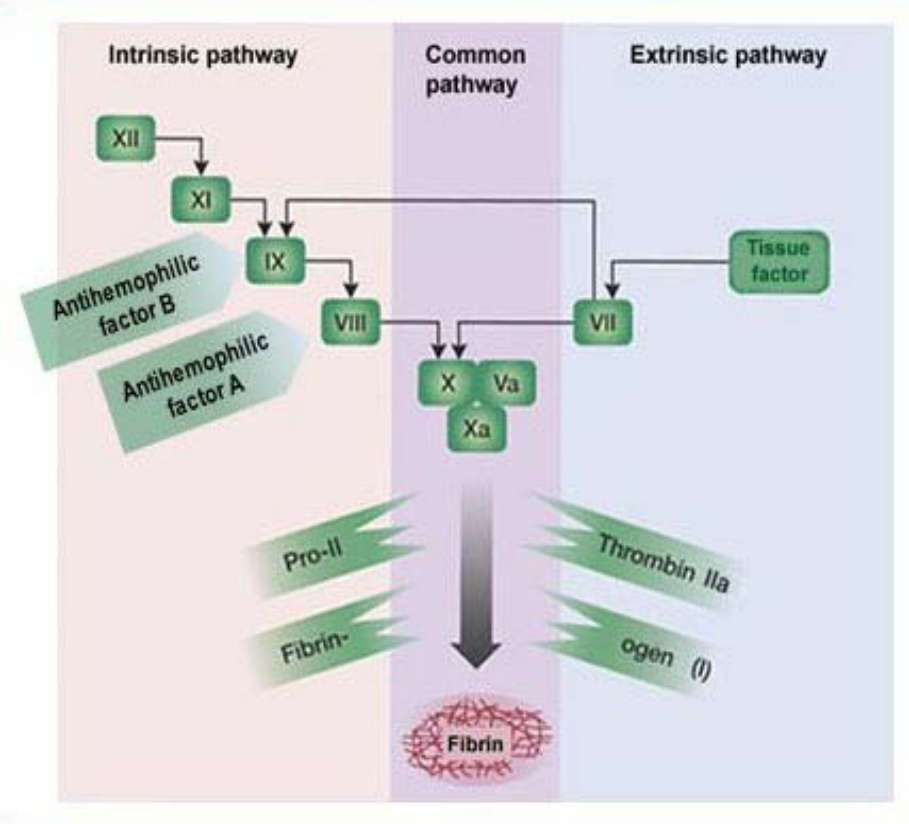
CONSTITUTIONAL ANOMALIES

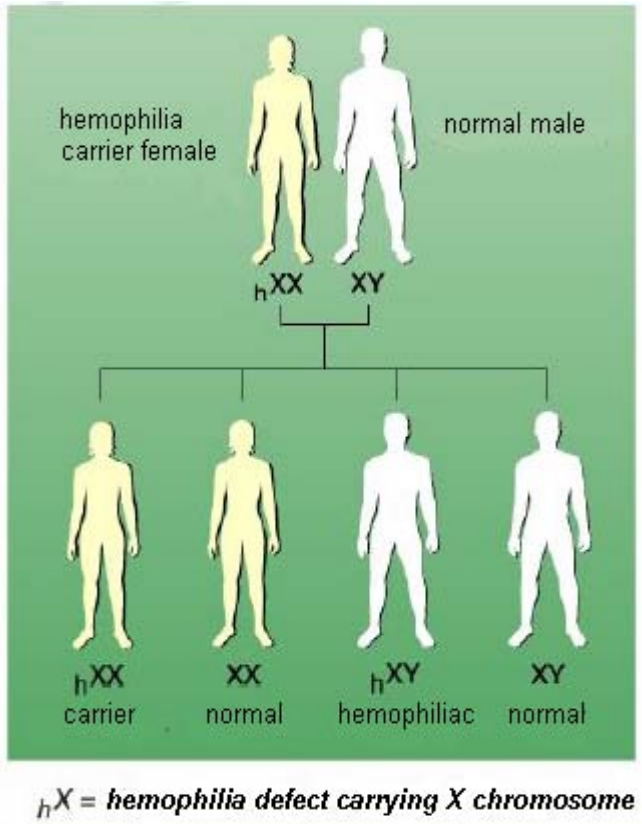
- Hemophilias (factors VIII, IX), cf. pages 219-221
- Fibrinogen, factors II, V, VII, X, XI, XIII deficiencies

ACQUIRED ANOMALIES

- Hepatocellular failure (deficiencies of fibrinogen, factors II, V, VII, X)
- Vitamin K deficiency (deficiencies of factors II, VII, IX, X)
- Disseminated intravascular coagulation (DIC)
  - Bacterial or parasitic infections
  - Cancer (lung, pancreas, prostate)
  - Acute leukemia, particularly Acute Promyelocytic Leukemia, t(15;17)(q22;q12)
  - Obstetrical complications
    - Amniotic liquid embolism
    - Placental retention
    - Eclampsia
    - Septic abortion
  - Invasive surgery
  - Extended burns
  - Transfusion complications
  - Vascular malformations (Kasabach-Merritt syndrom)
- Coagulation inhibitors (circulating anticoagulants)
  - Alloaantibodies against factor VIII (5-10% of hemophilia patients)
  - Autoantibodies against factor VIII (acquired hemophilia A) : pregnancy, postpartum
  - Rhumatoïd arthritis, lupus erythematosus, cancer, drugs

HEMOPHILIA (1)





Recessive X-linked transmission

Absence of familial context in 30% of hemophilia patients : de novo mutation

Risk for offsprings of a couple of a carrier woman and a normal man:

50% of the sons with hemophilia

50% of daughters are carriers

HEMOPHILIA (2)

INCIDENCE

Hemophilia A : 1 / 10'000, 5 x more frequent than hemophilia B

HEMOPHILIA	FACTOR LEVEL (%)	HEMORRHAGIC SYNDROME
Light[1]	5 – 40	Surgery Dental extraction Important trauma / injury
Moderate	1 – 5	Light trauma (e.g. sport)
Severe[2]	< 1%	Several bleeding episodes / month Frequent spontaneous hemorrhages Frequent hemarthrosis episodes

TREATMENT

Analgesia (*paracetamol, tramadol, codein, opiates; aspirine and NSAID[3] absolutely contra-indicated*

Factors concentrates or recombinant factors. Desmopressin (DDAVP) : light forms.

Factor VIII : distribution ½ -life 4 hours, plasmatic ½-life 12 hours
Factor IX : distribution ½-life 2 hours, plasmatic ½-life 24 hours

Orthopedic surgery : hemarthrosis

In case of inhibitors : recombinant factor VIIa, FEIBA ("Factor Eight Inhibitor By-passing Activity")

[1] Carrier female may have occasionally light symptoms
[2] Females may only have severe symptoms if the father is hemophiliac and the mother carrier
[3] NSAID : Non Steroidal Antiinflammatory Drugs

VON WILLEBRAND DISEASE

Quantitative or qualitative anomaly of von Willebrand factor

Transmission autosomal, dominant or recessive

The most common constitutional hemorrhagic disorder (incidence ~ 1% of whole population)

Mucosal and cutaneous bleeding (epistaxis, menorrhagia)

Biological signs : PFA-100™ prolonged, PT (Prothrombin time) normal, aPTT prolonged, ↓ Factor VIII

THROMBOEMBOLIC DISEASE

VIRCHOW'S TRIAD — Stasis + vascular lesion + blood hypercoagulability

MAIN RISK FACTORS

Arterial thrombosis :

Arterial hypertension
Hyperlipidemia, diabetes mellitus
Tobacco smoking

Venous thrombosis :

Stasis (bed rest, dehydration, ↗ plasma viscosity, varicose veins
Surgery (in particular hip and abdomen)
Pregnancy and post-partum
Estrogens, contraceptive pills
Cancer
Behçet disease
Constitutional coagulations anomalies *(cf. table)*

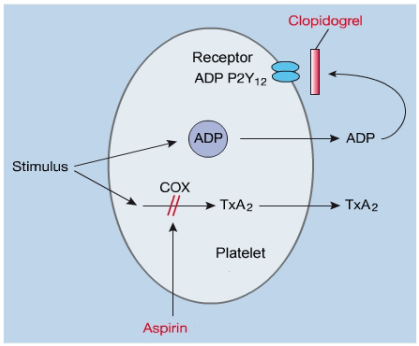
Deficiency / anomaly	Prevalence (healthy european individuals (%)	Prevalence (patients with deep vein thrombosis) (%)	Estimated relative risk
Antithrombin III, protein C, protein S	1 – 2	1 – 3	8 –10
Factor V Leiden heterogygous homozygoous	3 – 10 0,06 – 0,25	15 1.5	3 – 7 50 – 80
Mutation G20210A F. II heterozygous	1 – 3	5 – 6	2 – 4

Venous or arterial thrombosis :

Myeloproliferative neoplasm
Lupus anticoagulant, antiphospholipid syndrome
Hyperhomocysteinemia
Heparin induced thrombocytopenia (HIT)

THROMBOEMBOLIC DISEASE
TREATMENT AND PREVENTION (1)

ANTIPLATELET DRUGS



Aspirin blocks synthesis of Thromboxane A_2 by irreversible acetylation of cyclooxygenases (COX)

Clopidogrel (Plavix®) causes irreversible inhibition of $P2Y_{12}$ receptor of ADP

Dipyridamole increases platelet cyclic AMP through inhibition of phosphodiesterases *(Asasantine® : dipyridamole + aspirin)*

Abciximab (ReoPro®) is an antagonist of GP IIb/IIIa receptor

HEPARINS, THROMBIN AND FACTOR Xa INHIBITORS

Heparins Unfractioned : *Liquemin®, Calciparin®*	Fixation and activation of AT III[1], inhibition of factors Xa and IIa, inhibition of platelets, interaction with endothelium
Heparins Low molecular weight : *Nadroparin (Fraxiparin® or Fraxiforte®), Dalteparin (Fragmin®), Enoxaparin (Clexane®), Certoparin (Sandoparin®)*	Fixation and activation of AT III, inhibition of factor Xa, very low inhibition of factor IIa, absence of platelet inhibition, few interactions with endothelium
Danaparoid : *Orgaran®*	High affinity for AT III, anti-Xa activity, no effect on platelets
Hirudin analogs : *Lepirudin (Refludan®), Bivalirudin (Angiox®)*	Direct inhibition of thrombin
Argatroban : *Argatra®*	
Pentasaccharide : *Fondaparinux (Arixtra®)* Rivaroxaban *(Xarelto®)*	Pure anti-Xa activity

[1]AT III : Antithrombin III

THROMBOEMBOLIC DISEASE
TREATMENT AND PREVENTION (2)

VITAMIN K ANTAGONISTS

Therapeutic agents

Acenocoumarol (*Sintrom®*)
(½ life : 8-11 hours)

Phenprocoumon *(Marcoumar®)*
(½ life : 32-46 hours)

Inhibition of γ-carboxylation of vitamin K dependant factors (FII, FVII, FIX, FX)

Biological monitoring of treatment with vitamin K antagonists (INR : International Normalized Ratio)

$$INR = (PT\ patient\ [seconds]\ /\ PT\ control\ [seconds])^{ISI}$$

ISI = International Sensitivity Index : sensitivity index of employed reagent compared to international reference reagent

Therapeutical ranges

	Low limit	Target	High limit
Primary and secondary prevention of venous thromboembolic disease	2.0	2.5	3.0
Mechanical prosthetic cardiac valves[1]	2.5	3.0	3.5

FIBRINOLYTIC AGENTS

Tissular plasminogen activator, t-PA *(Actilyse®)*, Streptokinase *(Streptase®)*, Urokinase *(Urokinase HS medac®)*

[1] For more information : Salem D.N. et al. : Valvular and Structural Heart Disease : American College of Chest Physicians Evidence-Based Clinical Practice Guidelines (8th Edition). Chest 2008; 133 : 593-629.

VENOUS TRHOMBOEMBOLIC DISEASE

ANTICOAGULATION GUIDELINES

INITIAL (Options , depending on situation)

FONDAPARINUX (Arixtra®) :	LOW MOLECULAR WEIGHT HEPARIN	UNFRACTIONATED HEPARIN[1,2] :
7,5 mg SC / d 5 mg by body weight (BW) < 50 kg, 10 mg if BW > 100 kg. Contraindication: creatinin clearance < 30mL / min. No laboratory control test needed.	*e.g. : Enoxaparine = Clexane®* : 2 mg / kg / 24 h in 2 SC inj. In elderly patients, by BW < 50 kg or > 100 kg : dosage of plasmatic anti-Xa activity after 2nd or 3d dose, 3-5 h after SC injection. No laboratory control tests needed Caution by creatinin clearance < 30 mL / min.	Bolus IV 80 UI / kg (2'500-5'000 UI), then 400-600 UI / kg / 24 h (usually : 25'000-40'000 UI / 24 h) as continuous IV infusion. As priority in case of severe renal failure

EARLY SWITCH TO ANTIVITAMIN K DRUGS (Acenocoumarol : Sintrom®)

3 mg / d orally from the first or second treatment day (2 mg / d by age > 70 ans, BW < 50 kg or initial PT < 85%). INR control after the first 2 doses
By INR > 1.8 : ↓ dosis of 3d day
By INR between 1.2 et 1.8 : same dosis on 3d day
By INR < 1.2 : light dosis ↑ on 3d day
Target : allow stopping of the in initial anticoagulation (SC ou IV) < 5 days and / or after 2 consecutive INR at 24 h interval > 2.0

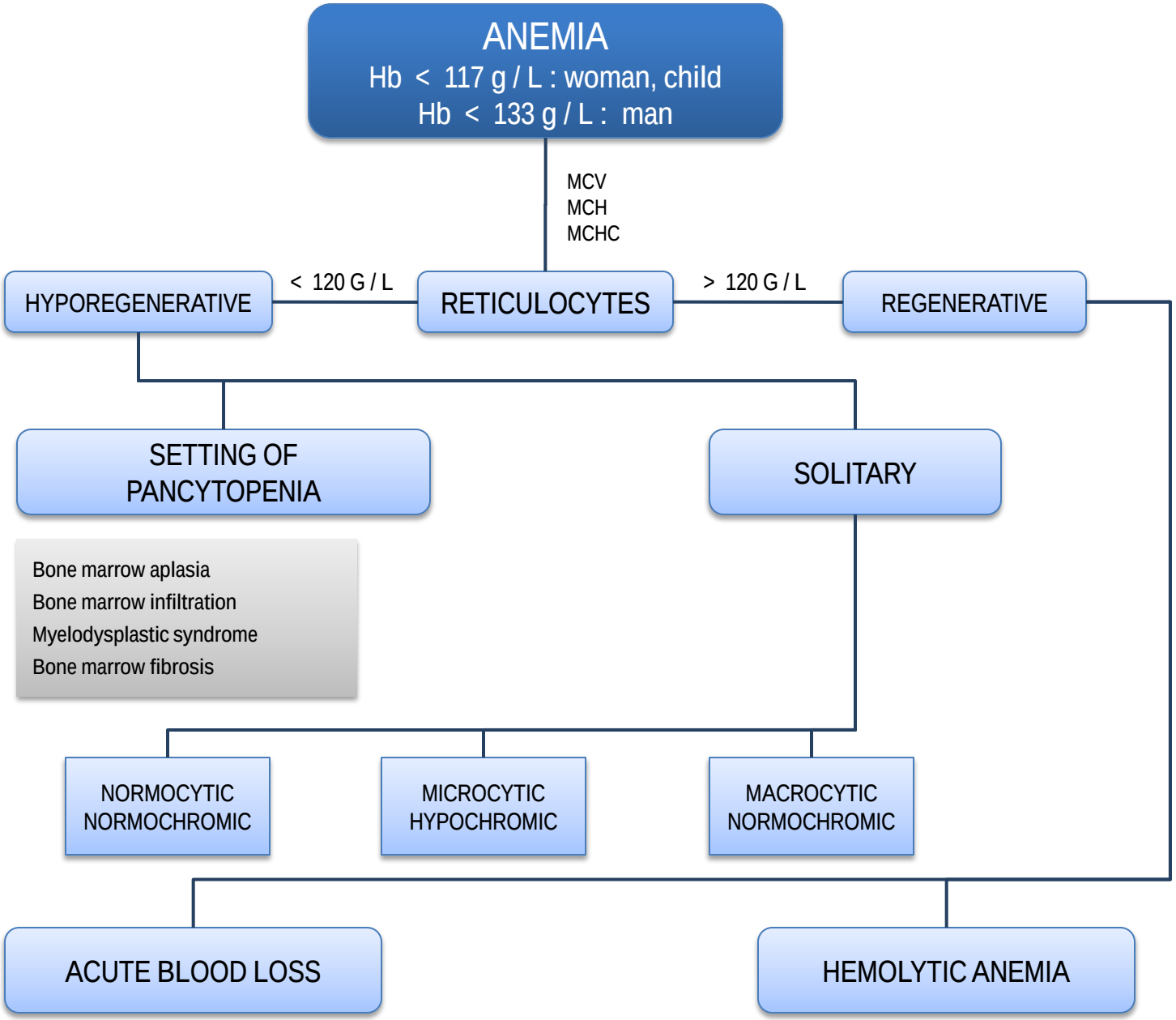
DURATION OF ANTICOAGULATION

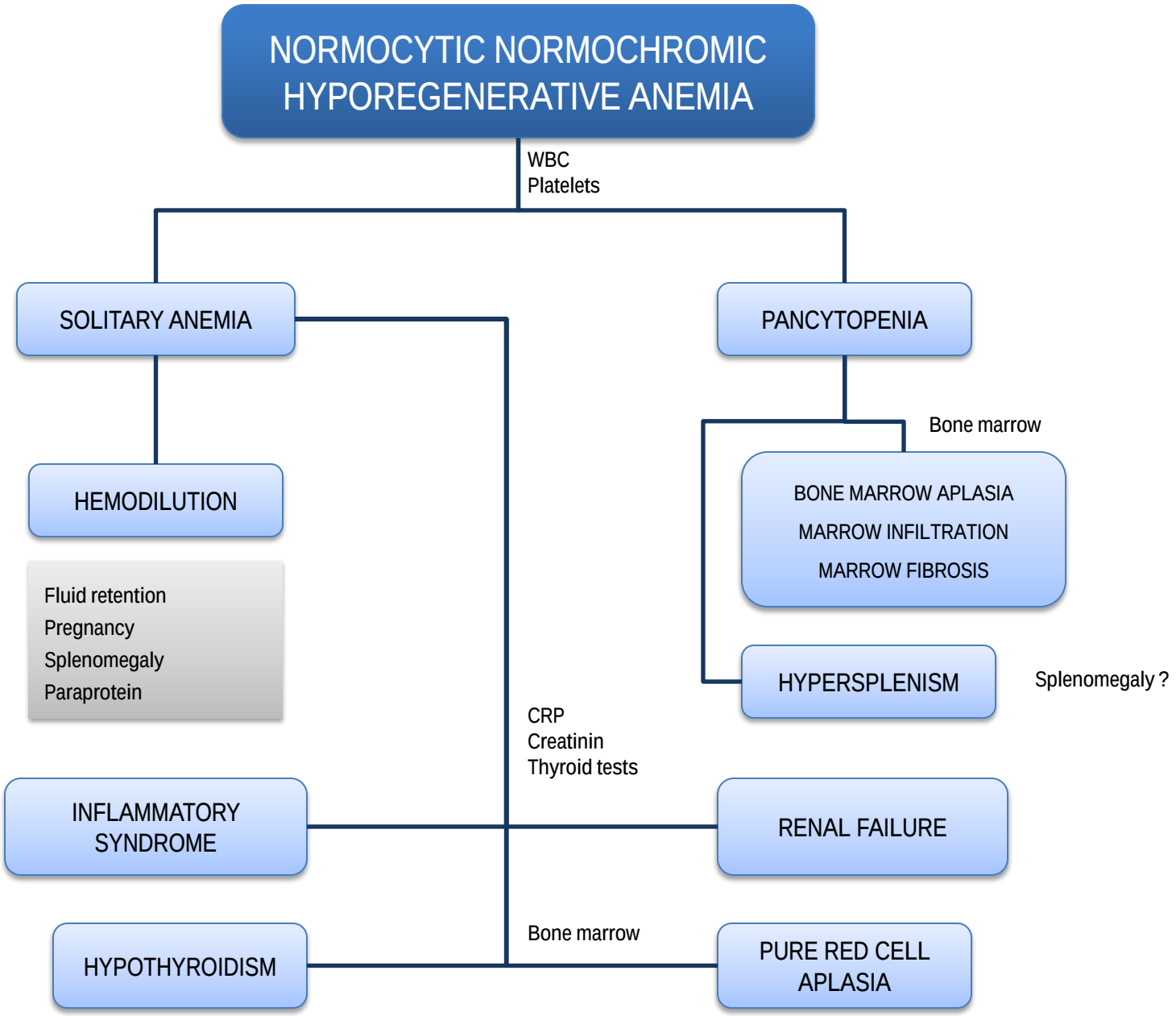
Postoperative limited deep vein thrombosis of the leg, increased bleeding risk	6 weeks
Proximal deep vein thrombosis / secondary pulmonary embolism	3 months
Deep vein thromobosis / Idiopathic pulmonary embolism	6-12 months (or more if persisting risk factor without increased bleeding risk)
Relapsing deep vein thrombosis and / or pulmonary embolism	Long term

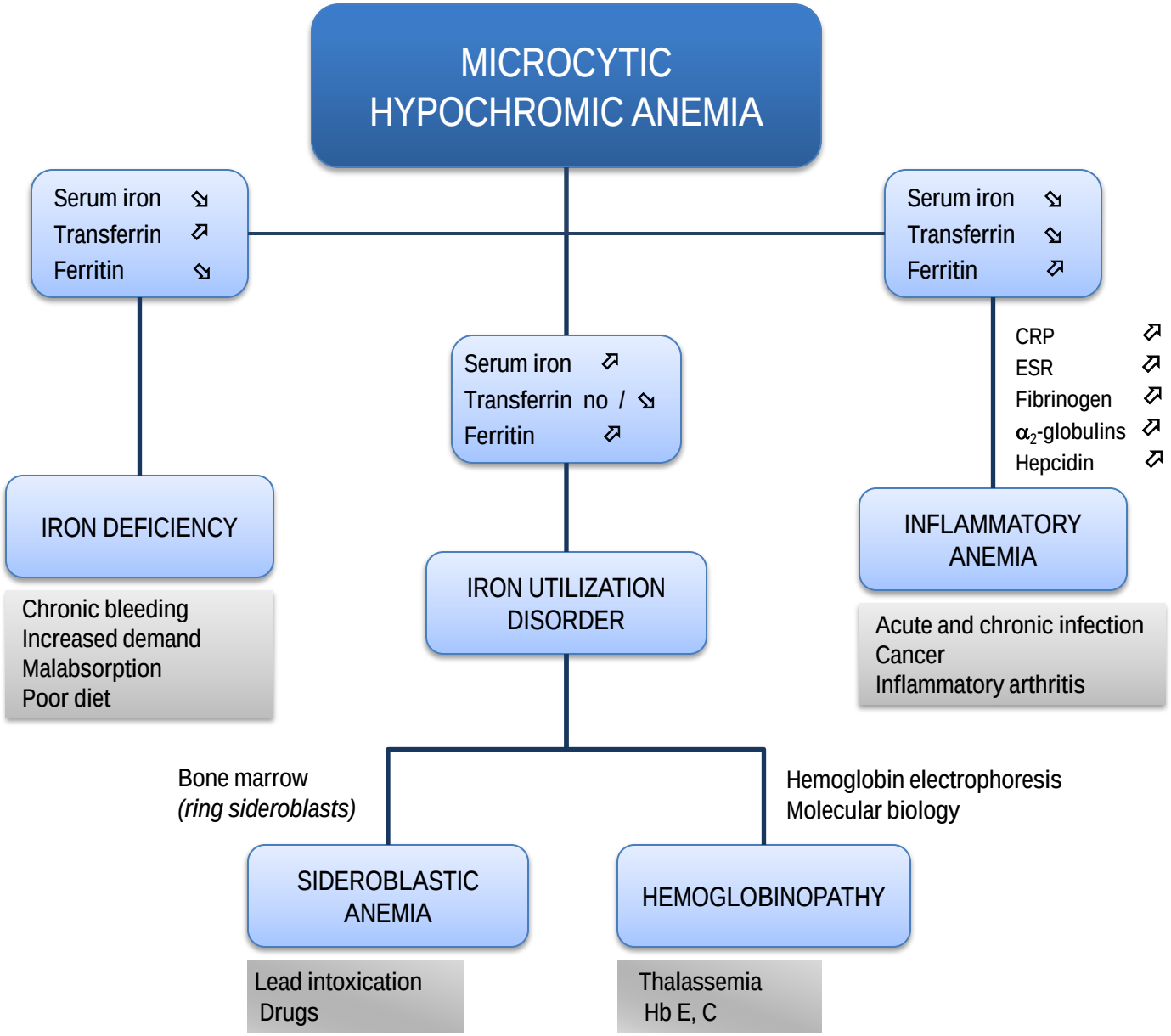
[1] Activated partial thrombopoplastin time (aPTT) controls must be 1.5 - 2.5 time over basic value. Daily heparin dosis is consequently adapted
[2] Heparin administration has to be kept as short as possible (↑ risk of heparin induced thrombocytopenia / HIT with prolonged heparin treatment)

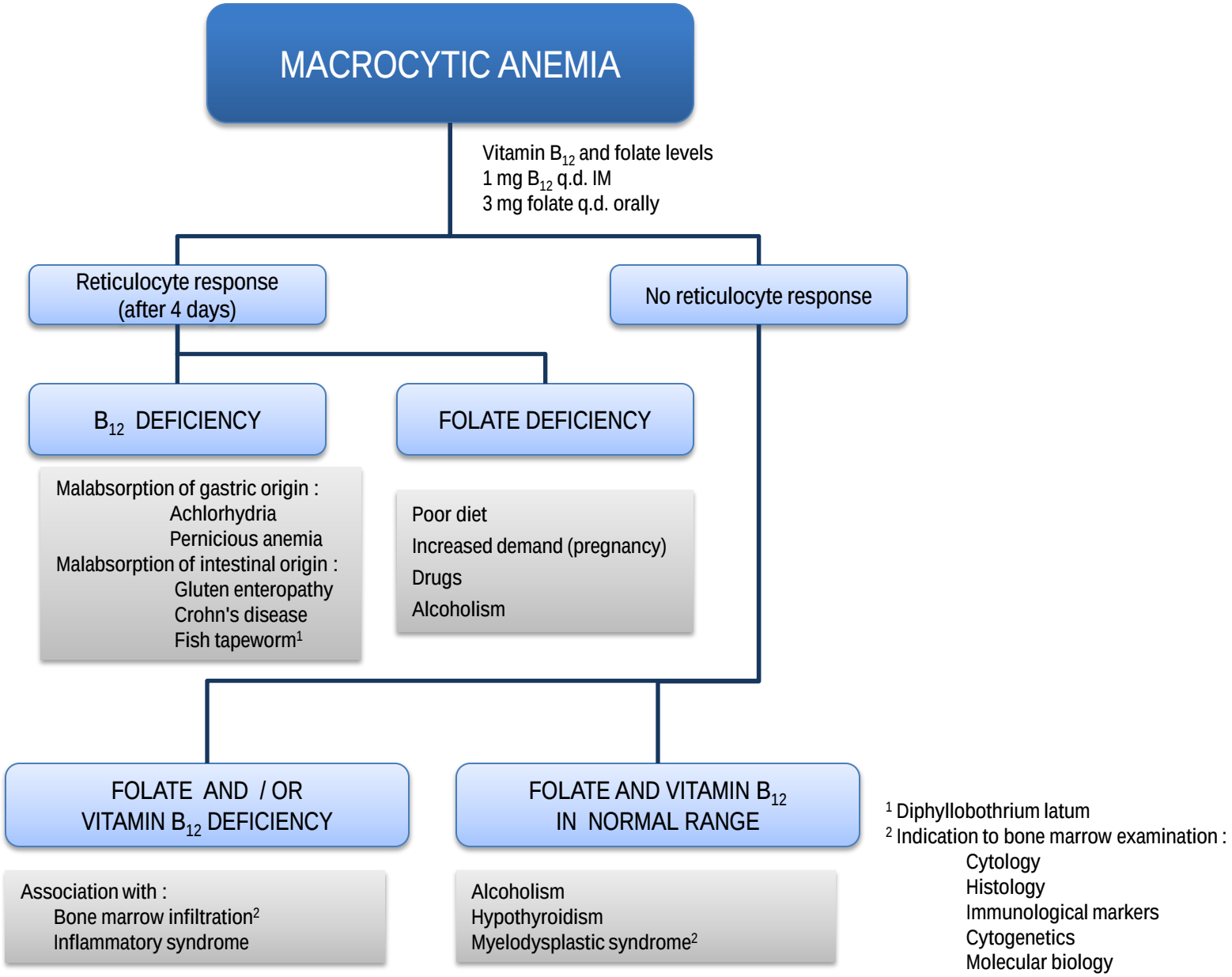
Part 4

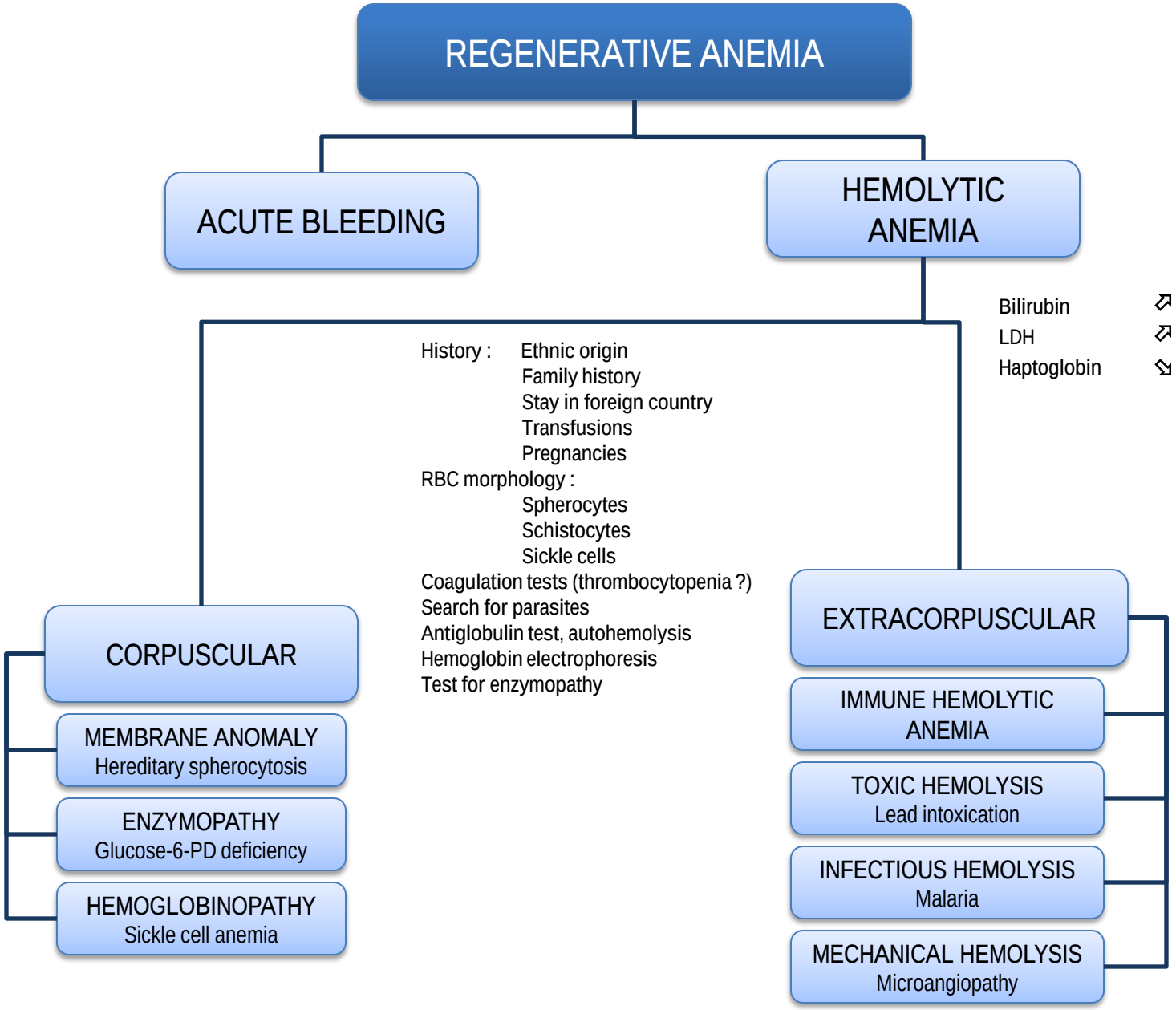
ALGORITHMS

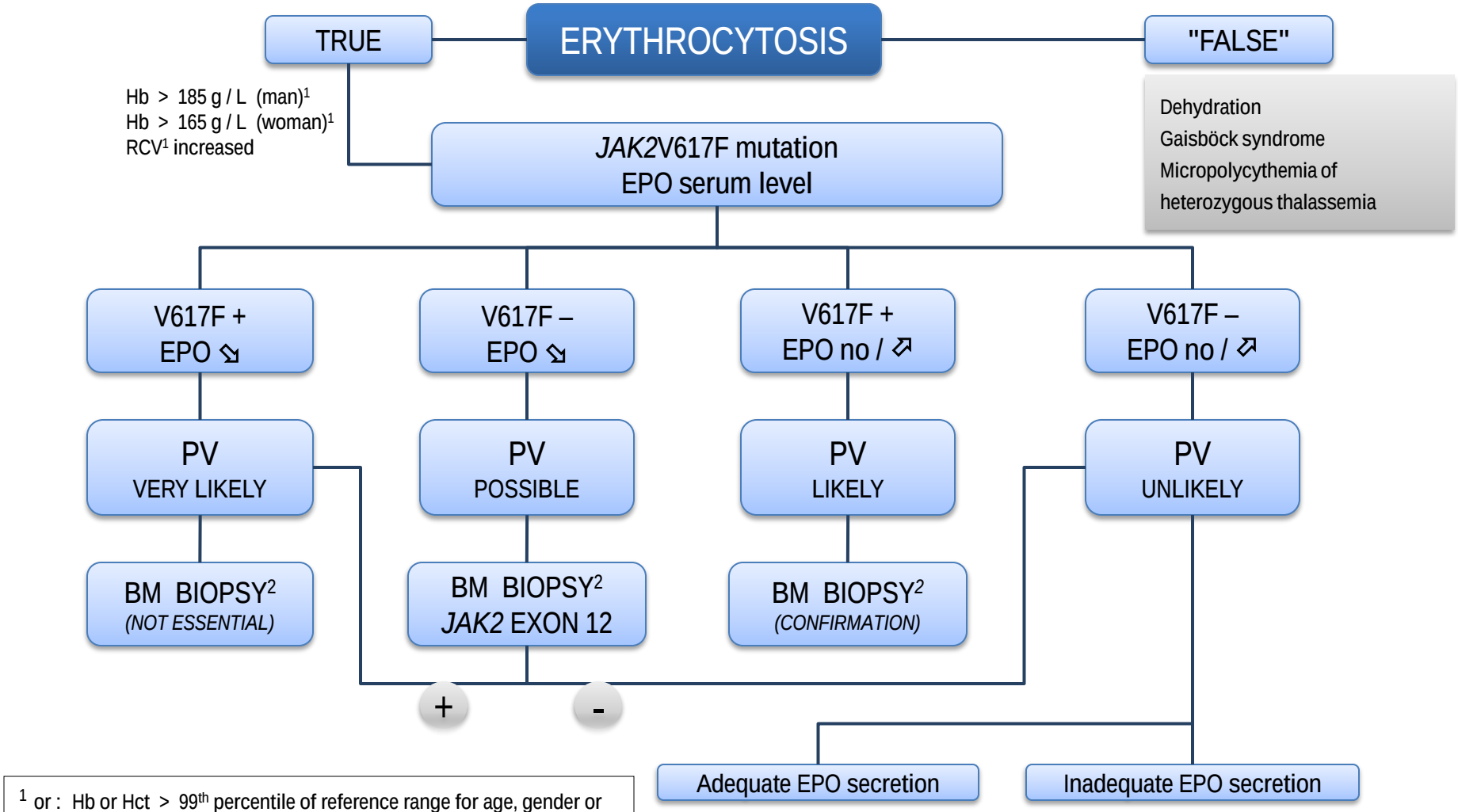

ANEMIA
Hb < 117 g / L : woman, child
Hb < 133 g / L : man
MCV
MCH
MCHC
HYPOREGENERATIVE
< 120 G / L
RETICULOCYTES
> 120 G / L
REGENERATIVE
SETTING OF PANCYTOPENIA
SOLITARY
Bone marrow aplasia
Bone marrow infiltration
Myelodysplastic syndrome
Bone marrow fibrosis
NORMOCYTIC NORMOCHROMIC
MICROCYTIC HYPOCHROMIC
MACROCYTIC NORMOCHROMIC
ACUTE BLOOD LOSS
HEMOLYTIC ANEMIA


NORMOCYTIC NORMOCHROMIC HYPOREGENERATIVE ANEMIA
WBC
Platelets
SOLITARY ANEMIA
PANCYTOPENIA
Bone marrow
HEMODILUTION
BONE MARROW APLASIA
MARROW INFILTRATION
MARROW FIBROSIS
Fluid retention
Pregnancy
Splenomegaly
Paraprotein
HYPERSPLENISM
Splenomegaly ?
CRP
Creatinin
Thyroid tests
INFLAMMATORY SYNDROME
RENAL FAILURE
Bone marrow
HYPOTHYROIDISM
PURE RED CELL APLASIA


MICROCYTIC HYPOCHROMIC ANEMIA
Serum iron ↘
Transferrin ↗
Ferritin ↘
Serum iron ↘
Transferrin ↘
Ferritin ↗
Serum iron ↗
Transferrin no / ↘
Ferritin ↗
CRP ↗
ESR ↗
Fibrinogen ↗
α2-globulins ↗
Hepcidin ↗
IRON DEFICIENCY
Chronic bleeding
Increased demand
Malabsorption
Poor diet
IRON UTILIZATION DISORDER
INFLAMMATORY ANEMIA
Acute and chronic infection
Cancer
Inflammatory arthritis
Bone marrow
(ring sideroblasts)
Hemoglobin electrophoresis
Molecular biology
SIDEROBLASTIC ANEMIA
HEMOGLOBINOPATHY
Lead intoxication
Drugs
Thalassemia
Hb E, C

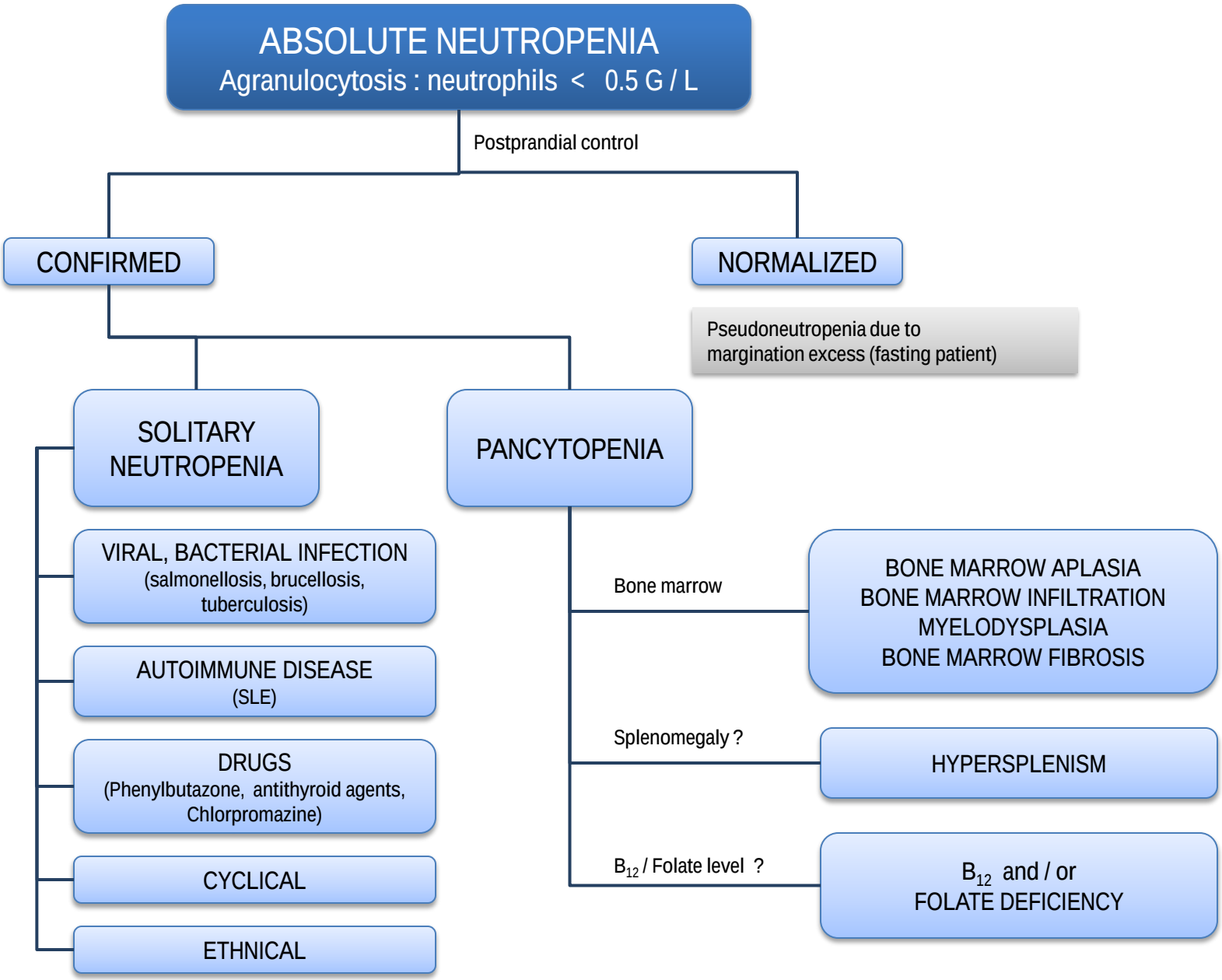

MACROCYTIC ANEMIA
Vitamin B_{12} and folate levels
1 mg B_{12} q.d. IM
3 mg folate q.d. orally
Reticulocyte response (after 4 days)
No reticulocyte response
B_{12} DEFICIENCY
FOLATE DEFICIENCY
Malabsorption of gastric origin :
Achlorhydria
Pernicious anemia
Malabsorption of intestinal origin :
Gluten enteropathy
Crohn's disease
Fish tapeworm[1]
Poor diet
Increased demand (pregnancy)
Drugs
Alcoholism
FOLATE AND / OR VITAMIN B_{12} DEFICIENCY
FOLATE AND VITAMIN B_{12} IN NORMAL RANGE
Association with :
Bone marrow infiltration[2]
Inflammatory syndrome
Alcoholism
Hypothyroidism
Myelodysplastic syndrome[2]
[1] Diphyllobothrium latum
[2] Indication to bone marrow examination :
Cytology
Histology
Immunological markers
Cytogenetics
Molecular biology

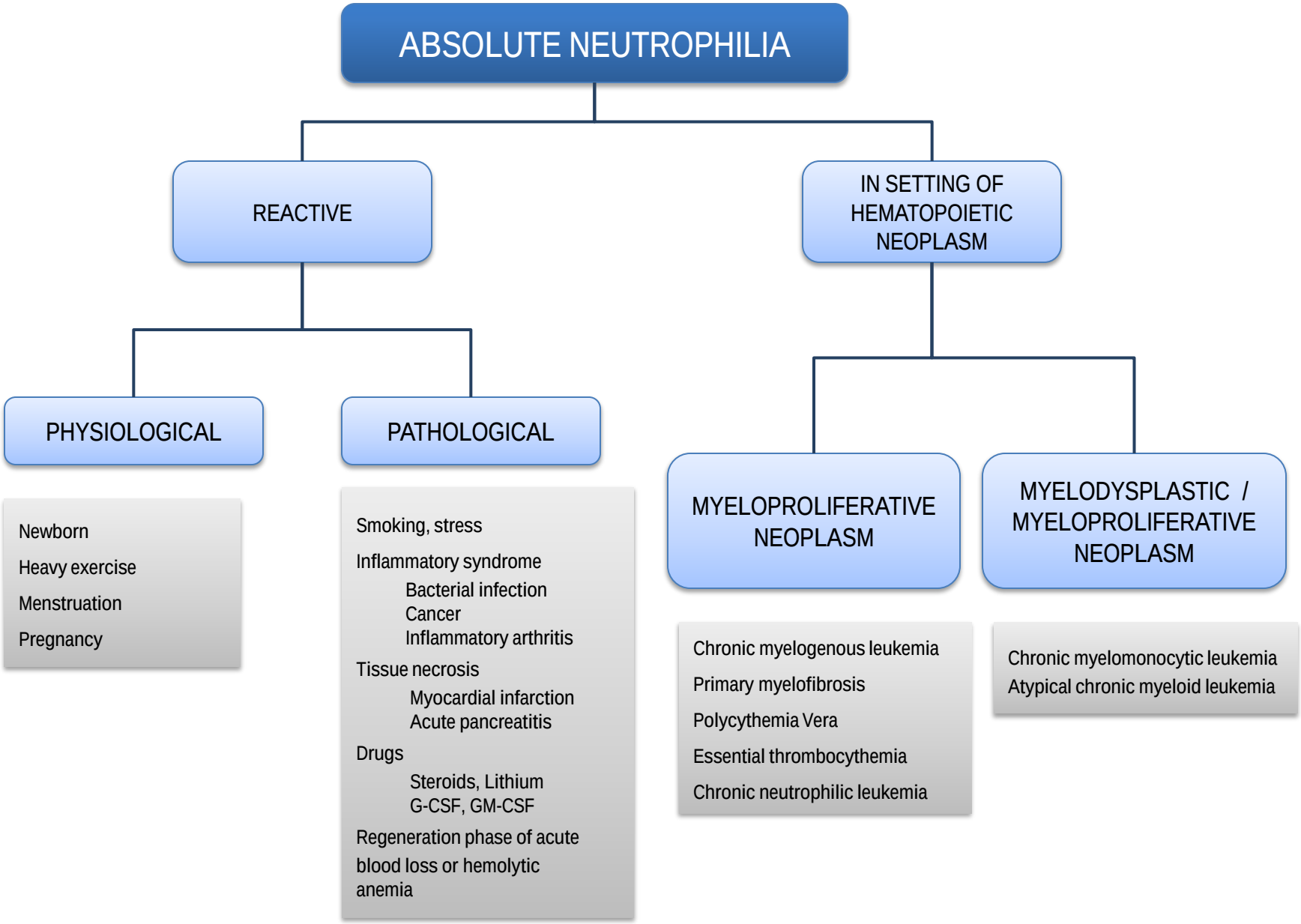

REGENERATIVE ANEMIA
ACUTE BLEEDING
HEMOLYTIC ANEMIA
Bilirubin ⇗
LDH ⇗
Haptoglobin ⇘
History : Ethnic origin
Family history
Stay in foreign country
Transfusions
Pregnancies
RBC morphology :
Spherocytes
Schistocytes
Sickle cells
Coagulation tests (thrombocytopenia ?)
Search for parasites
Antiglobulin test, autohemolysis
Hemoglobin electrophoresis
Test for enzymopathy
CORPUSCULAR
MEMBRANE ANOMALY
Hereditary spherocytosis
ENZYMOPATHY
Glucose-6-PD deficiency
HEMOGLOBINOPATHY
Sickle cell anemia
EXTRACORPUSCULAR
IMMUNE HEMOLYTIC ANEMIA
TOXIC HEMOLYSIS
Lead intoxication
INFECTIOUS HEMOLYSIS
Malaria
MECHANICAL HEMOLYSIS
Microangiopathy

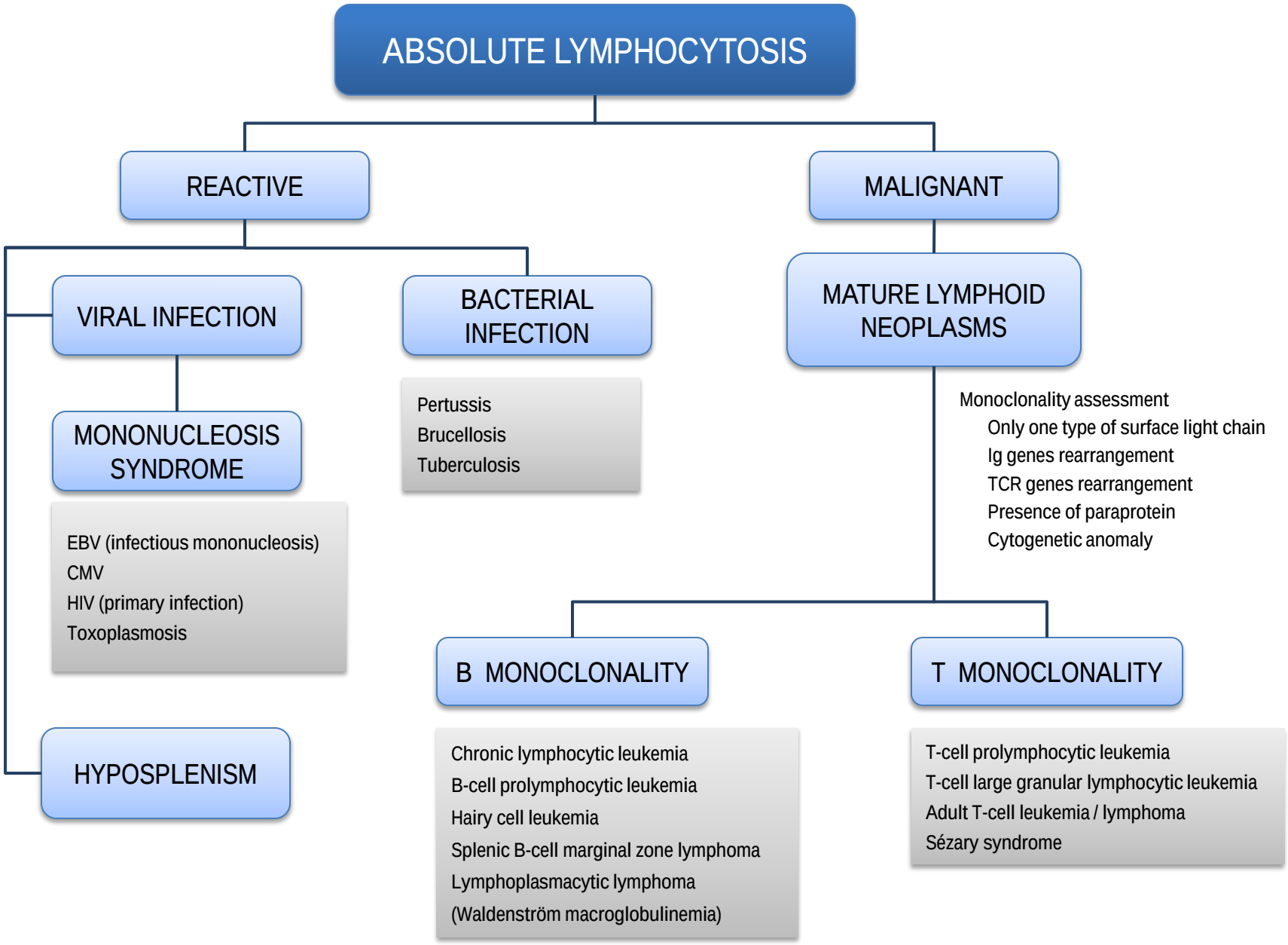


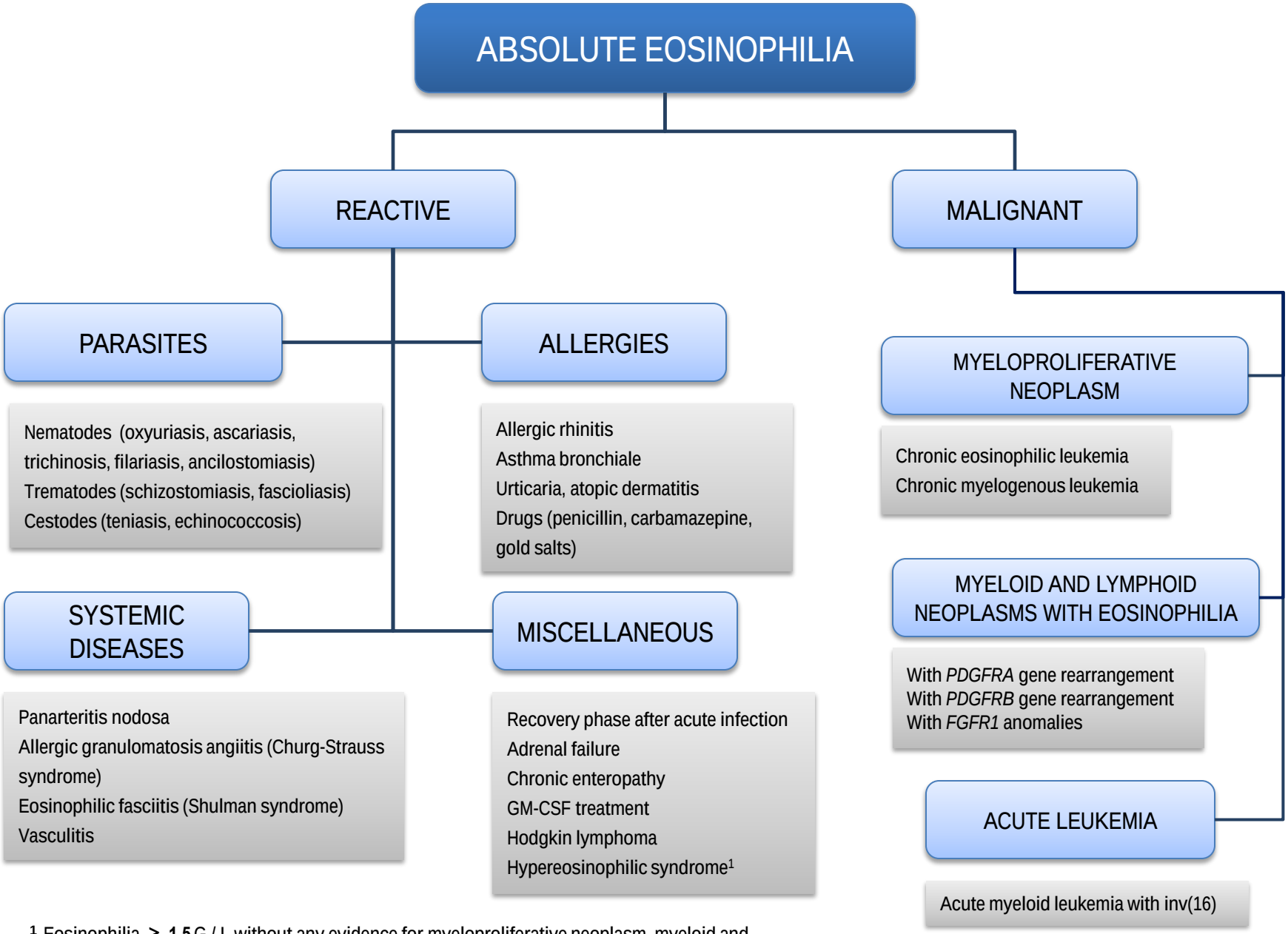
[1] or : Hb or Hct > 99th percentile of reference range for age, gender or altitude of residence
or : Hb > 170 g / L (men) or > 150 g / L (women) in case of sustained increase of ≥ 20 g / L from baseline that is not caused by correction of iron deficiency
or : RCV : Red Cell Volume > 25% over normal predicted value

[2] Hypercellularity, increased number of megakaryocytes with morphological anomalies, reticulin fibrosis

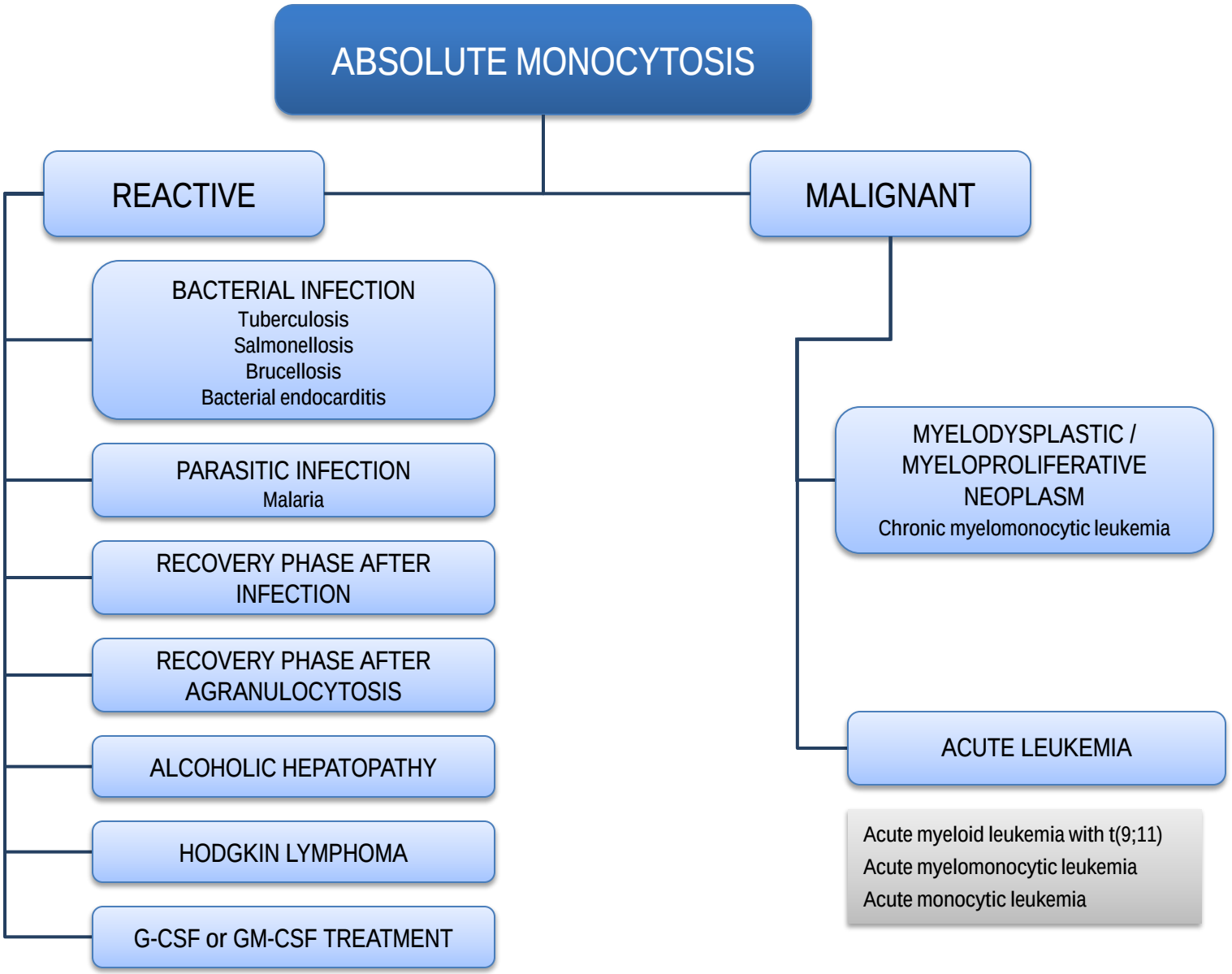

ABSOLUTE NEUTROPENIA
Agranulocytosis : neutrophils < 0.5 G / L
Postprandial control
CONFIRMED
NORMALIZED
Pseudoneutropenia due to margination excess (fasting patient)
SOLITARY NEUTROPENIA
PANCYTOPENIA
VIRAL, BACTERIAL INFECTION (salmonellosis, brucellosis, tuberculosis)
AUTOIMMUNE DISEASE (SLE)
DRUGS (Phenylbutazone, antithyroid agents, Chlorpromazine)
CYCLICAL
ETHNICAL
Bone marrow
BONE MARROW APLASIA
BONE MARROW INFILTRATION
MYELODYSPLASIA
BONE MARROW FIBROSIS
Splenomegaly ?
HYPERSPLENISM
B12 / Folate level ?
B12 and / or FOLATE DEFICIENCY

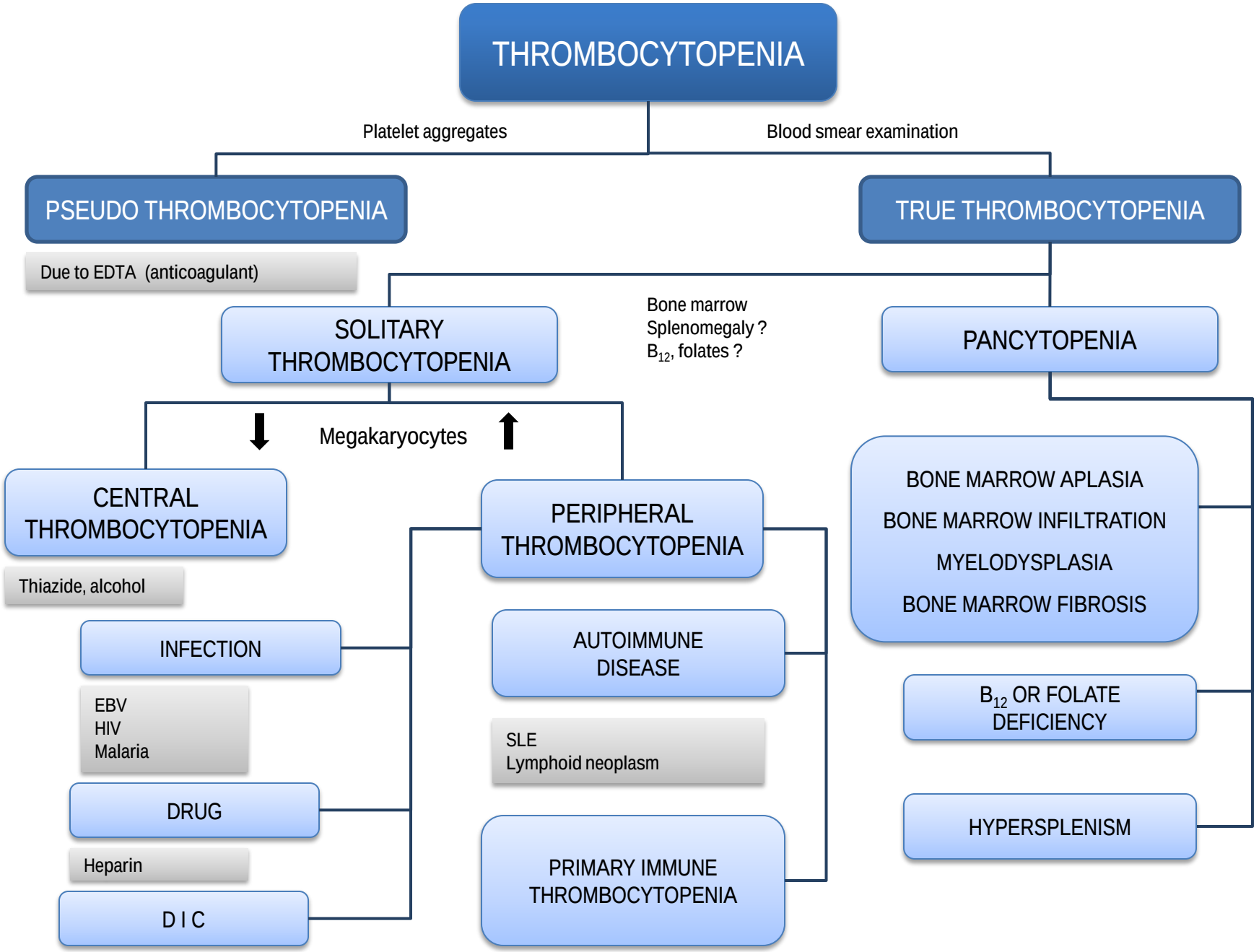

ABSOLUTE NEUTROPHILIA
REACTIVE
IN SETTING OF HEMATOPOIETIC NEOPLASM
PHYSIOLOGICAL
PATHOLOGICAL
MYELOPROLIFERATIVE NEOPLASM
MYELODYSPLASTIC / MYELOPROLIFERATIVE NEOPLASM
Newborn
Heavy exercise
Menstruation
Pregnancy
Smoking, stress
Inflammatory syndrome
Bacterial infection
Cancer
Inflammatory arthritis
Tissue necrosis
Myocardial infarction
Acute pancreatitis
Drugs
Steroids, Lithium
G-CSF, GM-CSF
Regeneration phase of acute blood loss or hemolytic anemia
Chronic myelogenous leukemia
Primary myelofibrosis
Polycythemia Vera
Essential thrombocythemia
Chronic neutrophilic leukemia
Chronic myelomonocytic leukemia
Atypical chronic myeloid leukemia

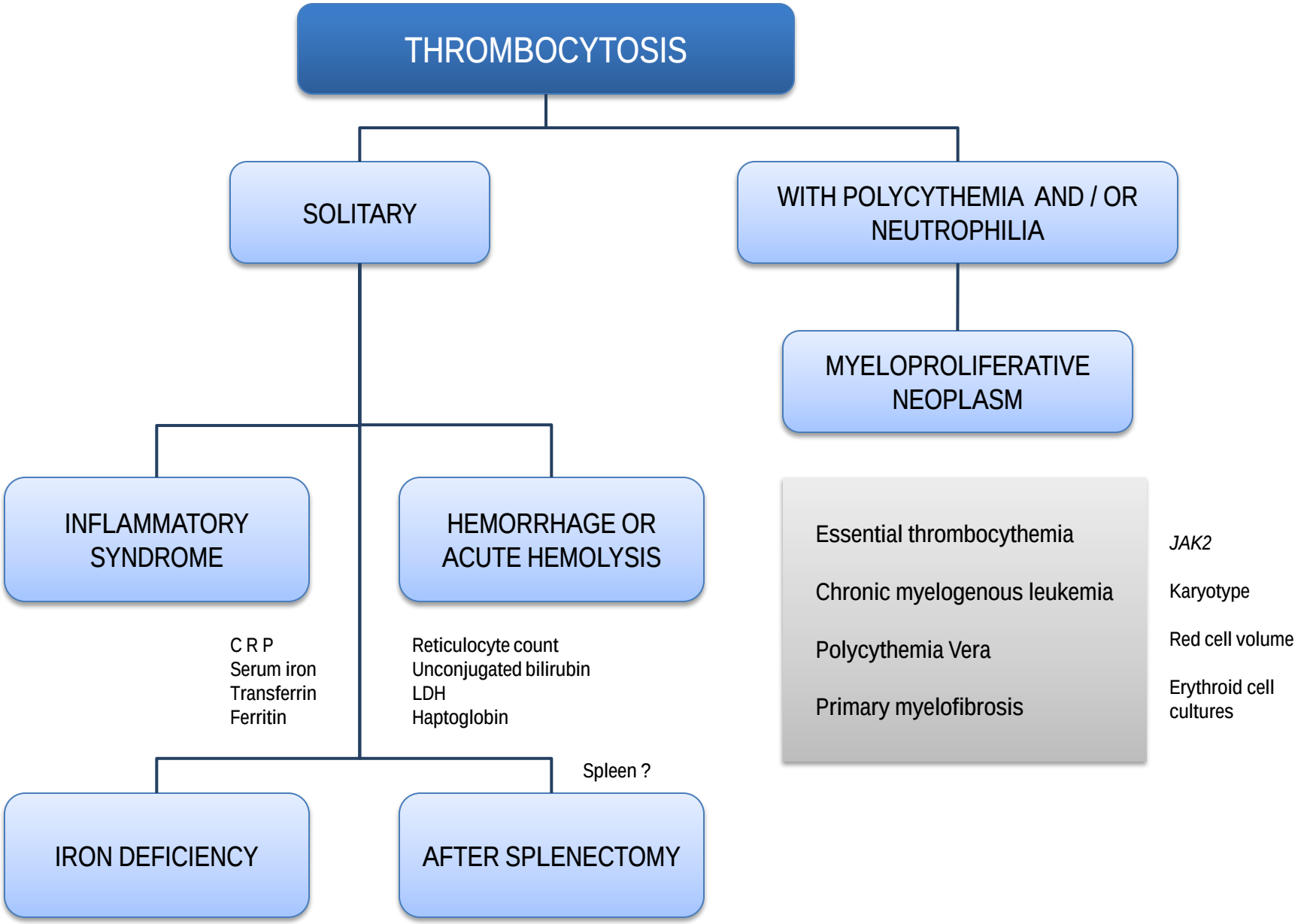

ABSOLUTE LYMPHOCYTOSIS
REACTIVE
MALIGNANT
VIRAL INFECTION
BACTERIAL INFECTION
MATURE LYMPHOID NEOPLASMS
MONONUCLEOSIS SYNDROME
Pertussis
Brucellosis
Tuberculosis
Monoclonality assessment
Only one type of surface light chain
Ig genes rearrangement
TCR genes rearrangement
Presence of paraprotein
Cytogenetic anomaly
EBV (infectious mononucleosis)
CMV
HIV (primary infection)
Toxoplasmosis
B MONOCLONALITY
T MONOCLONALITY
HYPOSPLENISM
Chronic lymphocytic leukemia
B-cell prolymphocytic leukemia
Hairy cell leukemia
Splenic B-cell marginal zone lymphoma
Lymphoplasmacytic lymphoma
(Waldenström macroglobulinemia)
T-cell prolymphocytic leukemia
T-cell large granular lymphocytic leukemia
Adult T-cell leukemia / lymphoma
Sézary syndrome

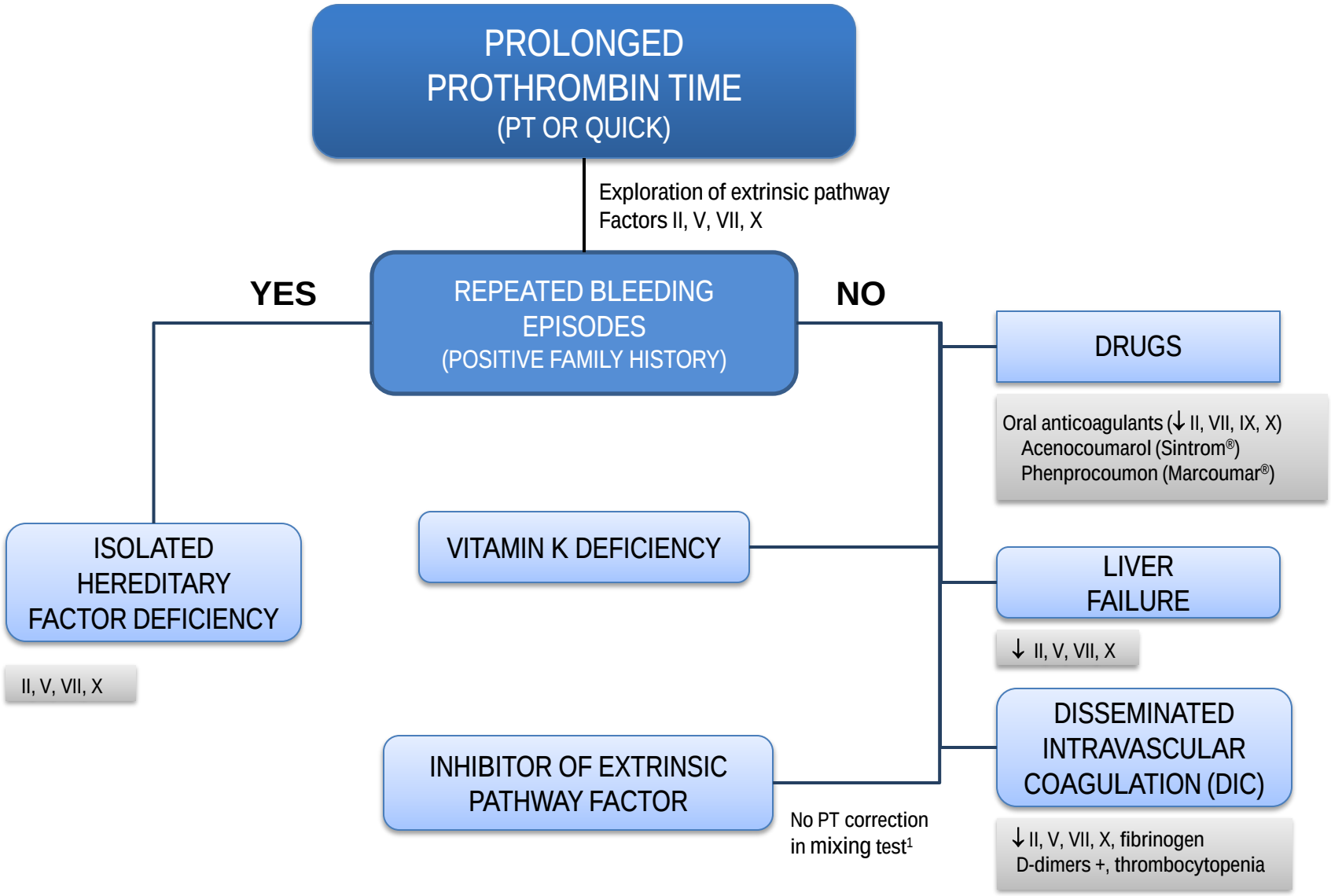


[1] Eosinophilia ≥ **1.5** G / L without any evidence for myeloproliferative neoplasm, myeloid and lymphoid neoplasm with eosinophilia and *PDGFRA*, *PDGFRB* or *FGFR1* anomaly, or AML

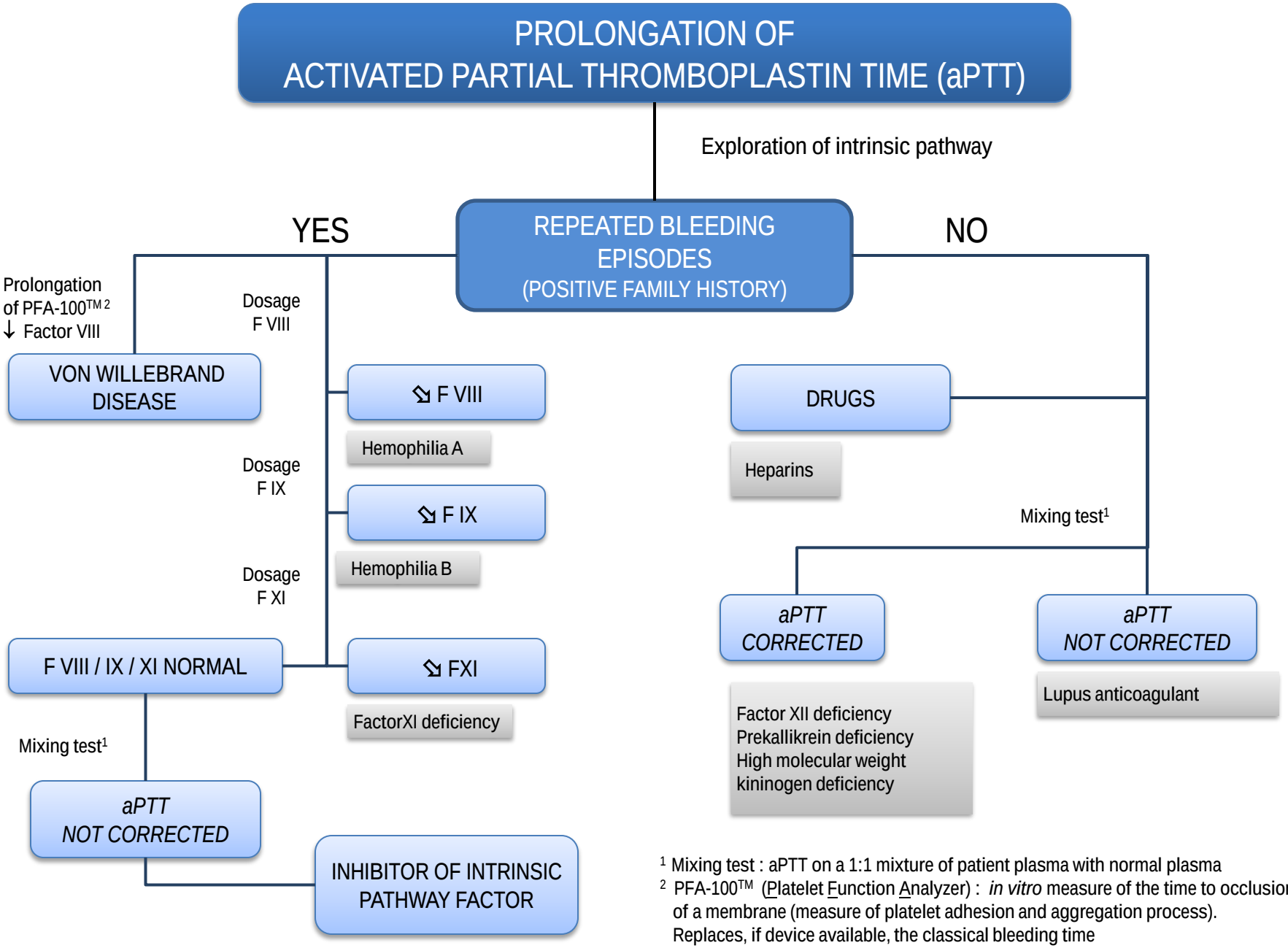

ABSOLUTE MONOCYTOSIS
REACTIVE
MALIGNANT
BACTERIAL INFECTION
Tuberculosis
Salmonellosis
Brucellosis
Bacterial endocarditis
PARASITIC INFECTION
Malaria
RECOVERY PHASE AFTER INFECTION
RECOVERY PHASE AFTER AGRANULOCYTOSIS
ALCOHOLIC HEPATOPATHY
HODGKIN LYMPHOMA
G-CSF or GM-CSF TREATMENT
MYELODYSPLASTIC / MYELOPROLIFERATIVE NEOPLASM
Chronic myelomonocytic leukemia
ACUTE LEUKEMIA
Acute myeloid leukemia with t(9;11)
Acute myelomonocytic leukemia
Acute monocytic leukemia


THROMBOCYTOPENIA
Platelet aggregates
Blood smear examination
PSEUDO THROMBOCYTOPENIA
Due to EDTA (anticoagulant)
TRUE THROMBOCYTOPENIA
Bone marrow
Splenomegaly ?
B12, folates ?
SOLITARY THROMBOCYTOPENIA
PANCYTOPENIA
Megakaryocytes
CENTRAL THROMBOCYTOPENIA
Thiazide, alcohol
PERIPHERAL THROMBOCYTOPENIA
BONE MARROW APLASIA
BONE MARROW INFILTRATION
MYELODYSPLASIA
BONE MARROW FIBROSIS
INFECTION
EBV
HIV
Malaria
AUTOIMMUNE DISEASE
SLE
Lymphoid neoplasm
B12 OR FOLATE DEFICIENCY
DRUG
Heparin
PRIMARY IMMUNE THROMBOCYTOPENIA
HYPERSPLENISM
D I C


THROMBOCYTOSIS
SOLITARY
WITH POLYCYTHEMIA AND / OR NEUTROPHILIA
MYELOPROLIFERATIVE NEOPLASM
INFLAMMATORY SYNDROME
HEMORRHAGE OR ACUTE HEMOLYSIS
C R P
Serum iron
Transferrin
Ferritin
Reticulocyte count
Unconjugated bilirubin
LDH
Haptoglobin
Spleen ?
IRON DEFICIENCY
AFTER SPLENECTOMY
Essential thrombocythemia
Chronic myelogenous leukemia
Polycythemia Vera
Primary myelofibrosis
JAK2
Karyotype
Red cell volume
Erythroid cell cultures



[1] Mixing test : PT / Quick on a 1:1 mixture of patient plasma with normal plasma



[1] Mixing test : aPTT on a 1:1 mixture of patient plasma with normal plasma

[2] PFA-100™ (Platelet Function Analyzer) : *in vitro* measure of the time to occlusion of a membrane (measure of platelet adhesion and aggregation process). Replaces, if device available, the classical bleeding time

BY WAY OF CONCLUSION

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Pieter Canham van Dijken, MD

Transfusion Medicine is presently not covered in this synopsis

Related morphological inconography may be found on :
http://ashimagebank.hematologylibrary.org

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