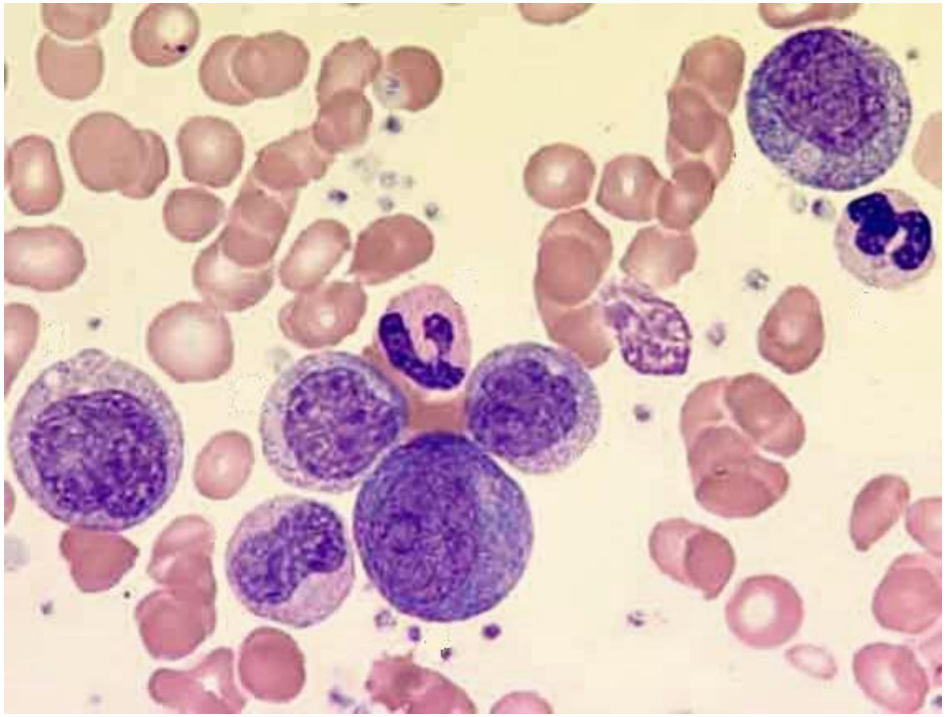
BASIC PHYSIOPATHOLOGY OF GENERAL HEMATOLOGY

A SYNOPSIS OF HEMATOLOGY



Pierre-Michel Schmidt
Pierre Cornu
Anne Angelillo-Scherrer
with the collaboration of :
Stéphane Quarroz
Valérie Parlier
Pieter Canham van Dijken

Version 14.0, 2012

SERVICE OF HEMATOLOGY CHUV - Lausanne

CONTENTS

CONTENTS (2)

CONTENTS (3)

CONTENTS (4)

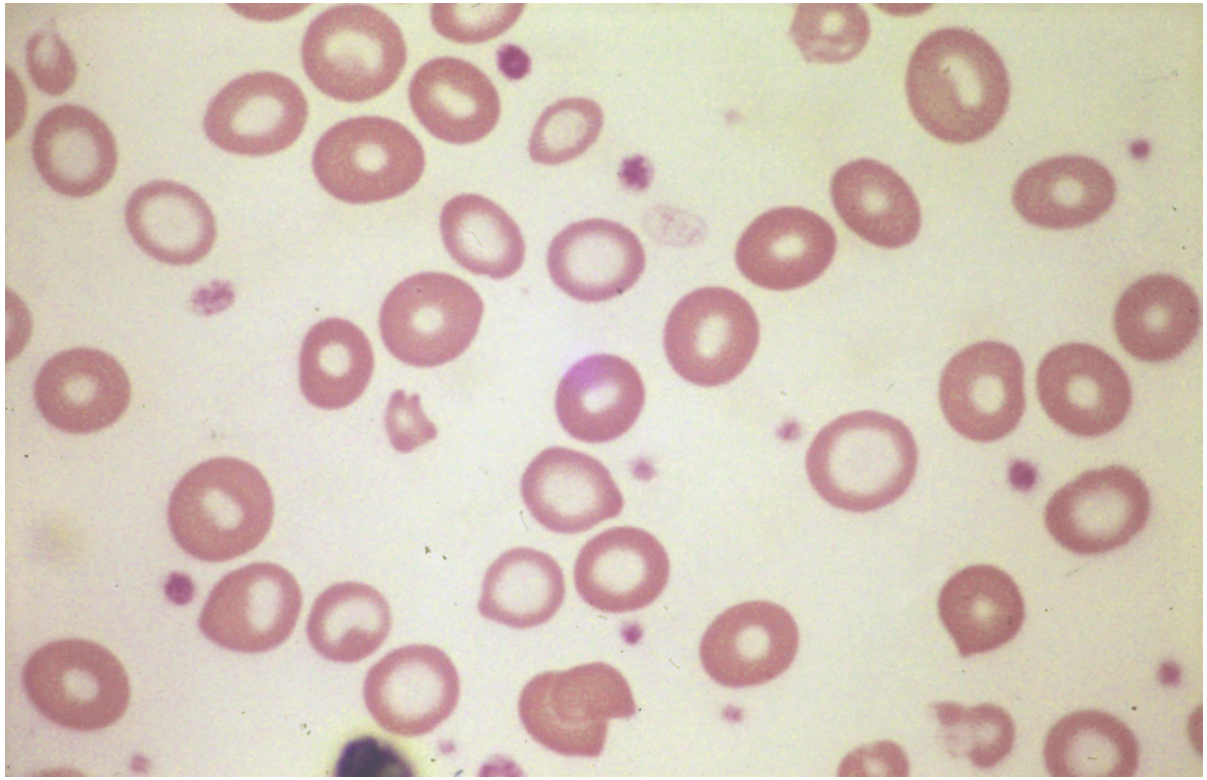
CONTENTS (5)

CONTENTS (6)

CONTENTS (7)

Part 1

RED BLOOD CELL PATHOLOGY



DIFFERENTIATION OF BLOOD CELLS

Early-acting hematopoietic growth factors

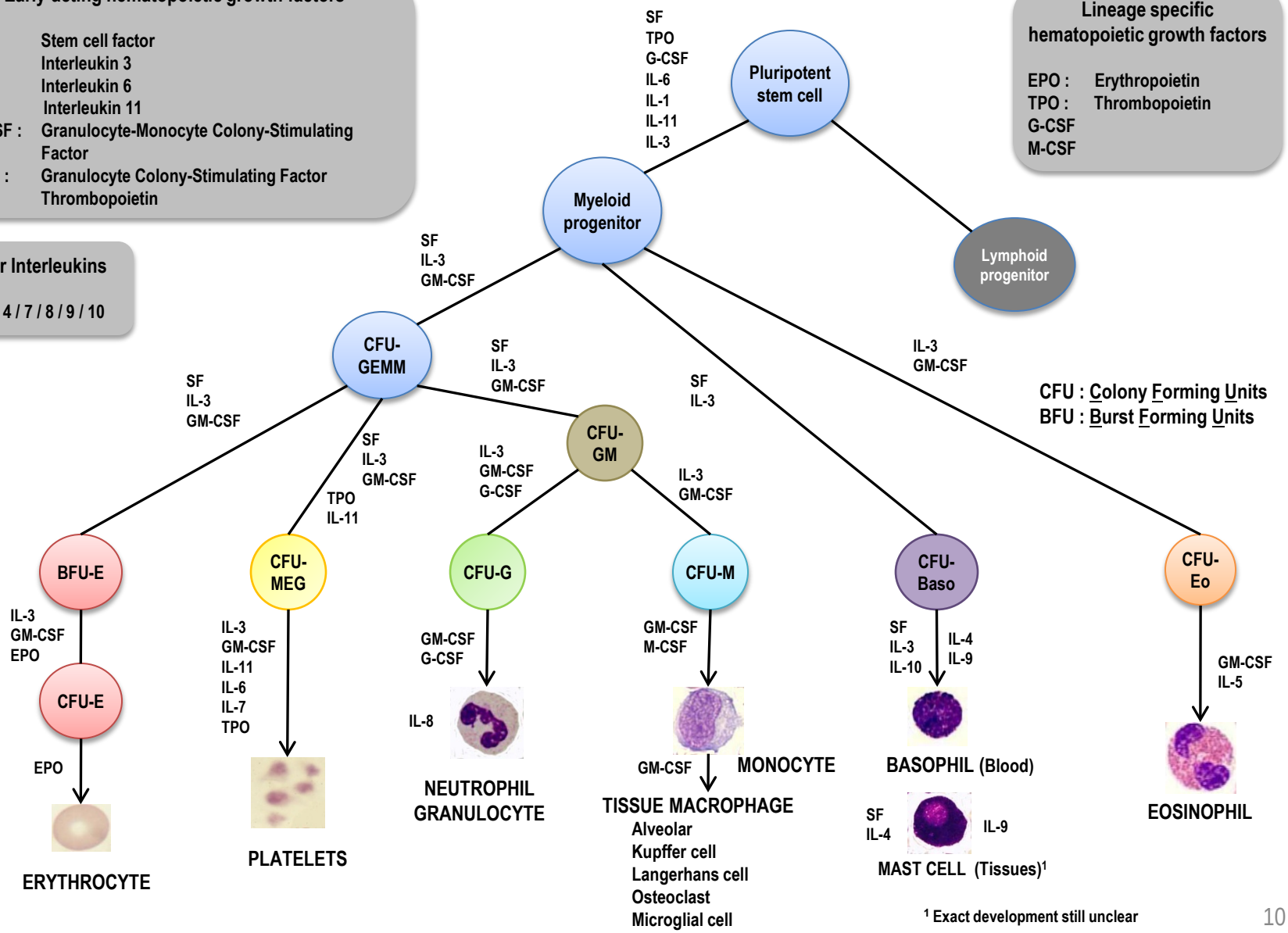
SF :	Stem cell factor
IL-3 :	Interleukin 3
IL-6 :	Interleukin 6
IL-11 :	Interleukin 11
GM-CSF :	Granulocyte-Monocyte Colony-Stimulating Factor
G-CSF :	Granulocyte Colony-Stimulating Factor
TPO :	Thrombopoietin

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

	UNITS	MEN	WOMEN
HEMOGLOBIN[1] *(Hb)*	g / L	133 – 177	117 – 157
HEMATOCRIT[1] *(Hct)*	%	40 – 52	35 – 47
ERYTHROCYTES[1] *(Ery)*	T / L	4,4 – 5,8	3,8 – 5,2
MCV	fL	81 – 99	
MCH	pg	27 – 34	
MCHC	g / L	310 – 360	
RDW[2] *(Anisocytosis index)*	%	< 15	
RETICULOCYTES *(relative value)*	‰	5 – 15	
RETICULOCYTES *(absolute value)*	G / L	20 – 120	
LEUKOCYTES	G / L	4 – 10	
THROMBOCYTES / PLATELETS	G / L	150 – 350	

[1] Increased values with prolonged stay at high altitude
[2] RDW : Red cell distribution width

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

LCH-CHUV, 2011

COMPLEMENTARY INDICES *

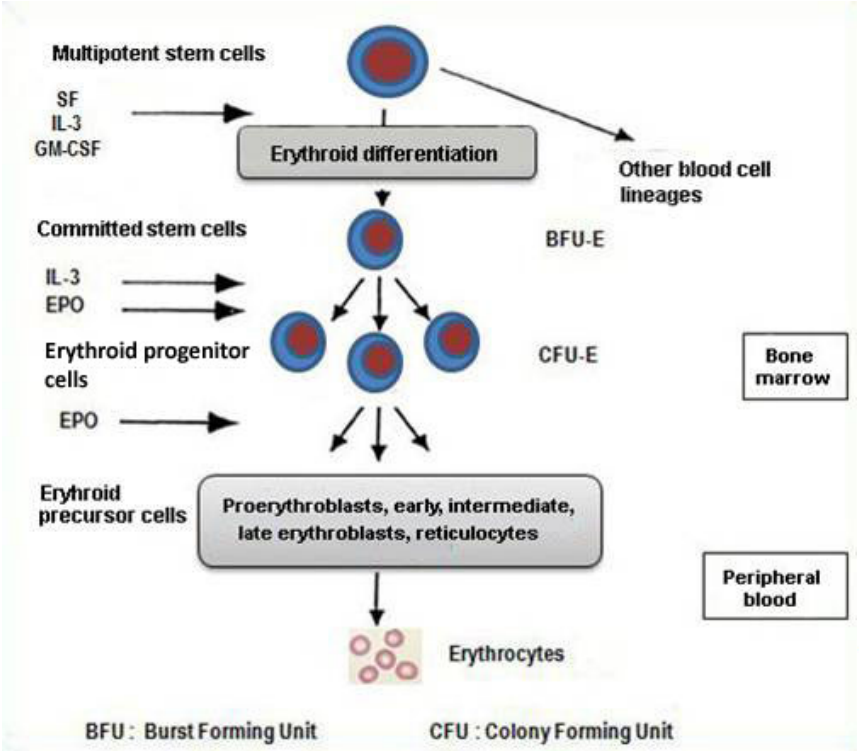
INDEX	UNIT	REFERENCE INTERVAL**
HYPO[3]	%	< 5.0
MCVr / MRV[4]	fL	104 - 120
CHr[5]	pg	28 - 33.5
IRF[6]	%	2,3 - 15,9
MPV[7]	fL	7 - 11,5
PDW[8]	%	9,0 - 13.0

* *Indices produced by hematological analyzers*

[3] HYPO : *Hypochromic RBC fraction*
[4] MCVr : *Mean Cellular Volume of reticulocytes* ** *or*
MRV : *Mean Reticulocyte Volume* **
[5] CHr : *Cellular Hemoglobin Content of reticulocytes* **
[6] IRF : *Immature Reticulocyte Fraction*
[7] MPV : *Mean Platelet Volume* **
[8] PDW : *Platelet Distribution Width* **

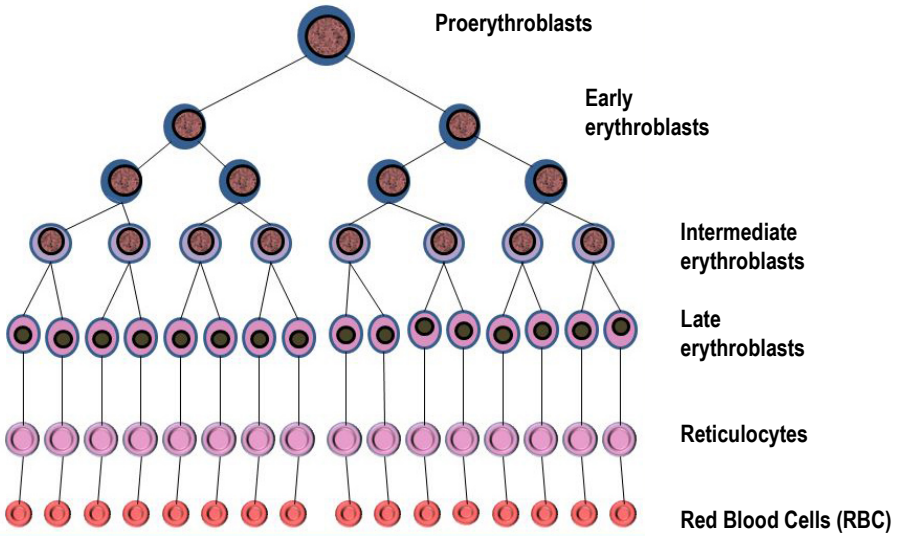
** These indices may vary depending on the type of analyzer and of preanalytic condittions

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 : p. 60.*



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

Modified from Hoffbrand A.V., Moss P.A.H., Pettit J.E. : Essential Haematology, 5h edition 2006; Blackwell Publishing : p.14.

EVALUATION OF ANEMIA

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 *(WHO 1997)*

Child (< 5 years)	< 110 g / L
Child (5-11 years)	< 115 g / L
Child (12-14 years)	< 120 g / L
Adult man	< 130 g / L
Adult woman	< 120 g / L
Pregnant woman	< 110 g / L

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	no	no	no
Microcytic hypochromic	⇩	⇩	⇩
Macrocytic normochromic	⇗	⇗	no

EVALUATION OF ANEMIA (4)
RETICULOCYTES

Absolute reticulocyte count :

< 120 G / L : Hyporegenerative anemia

> 120 G / L : Regenerative anemia

Reticulocyte production index (RPI)

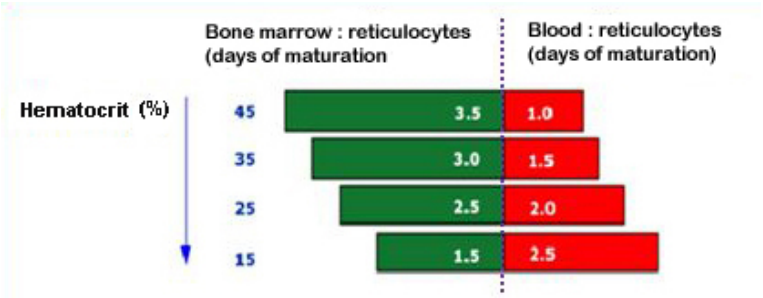
$$RPI = \frac{\text{Reticulocytes (‰)}}{10 \times \text{reticulocyte maturation time in blood (days)}^{1}} \times \frac{\text{Hematocrit}}{45}$$

Normal : 1.0 - 2.0

Hyporegenerative anemia : < 2.0

Regenerative anemia : > 2.0

Reticulocyte maturation related to anemia severity[1]



[1] Reticulocytes 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])*

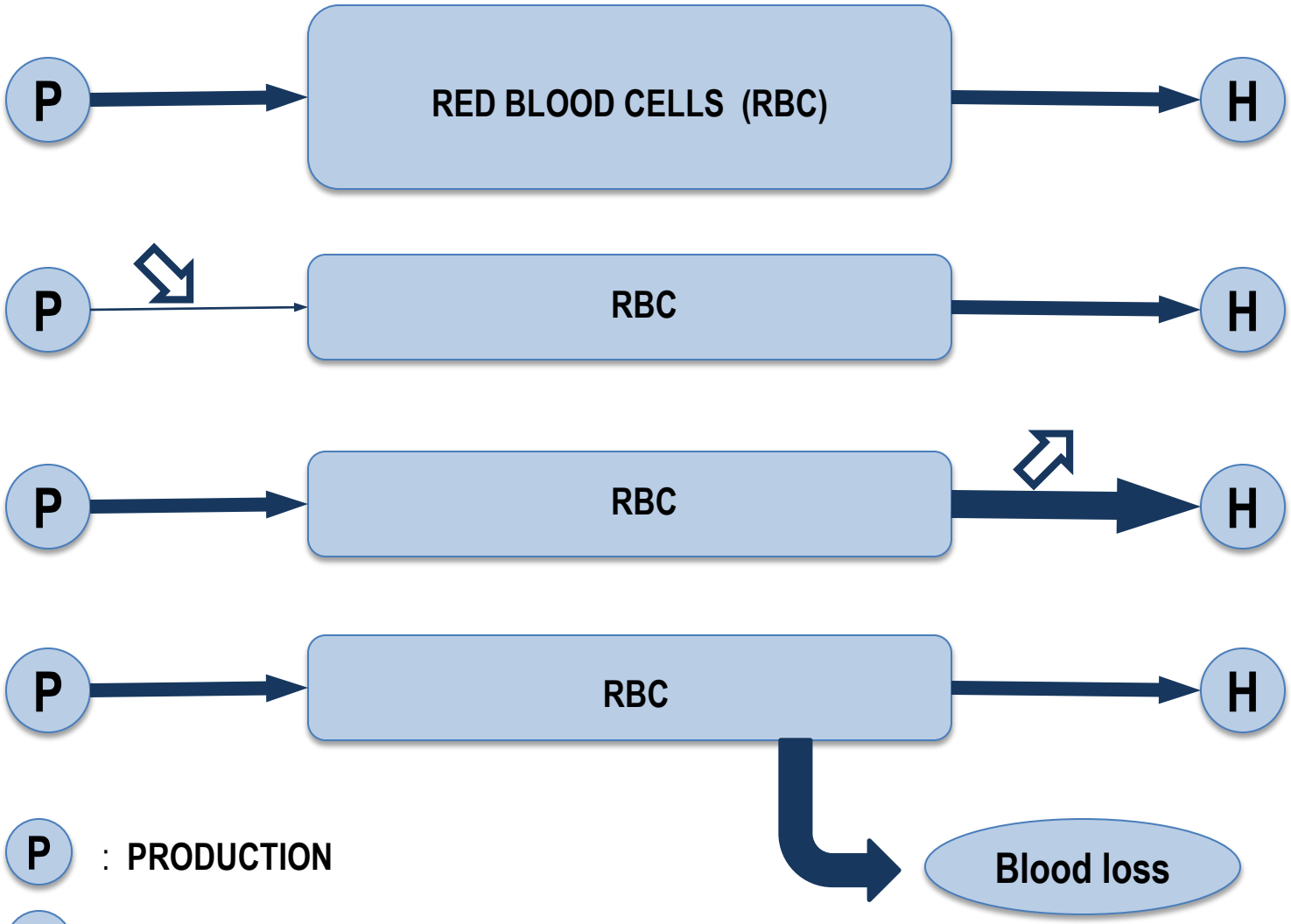
MFR (Medium-Fluorescence Reticulocytes) : medium

LFR (Low-Fluorescence Reticulocytes) : low → Mature reticulocytes

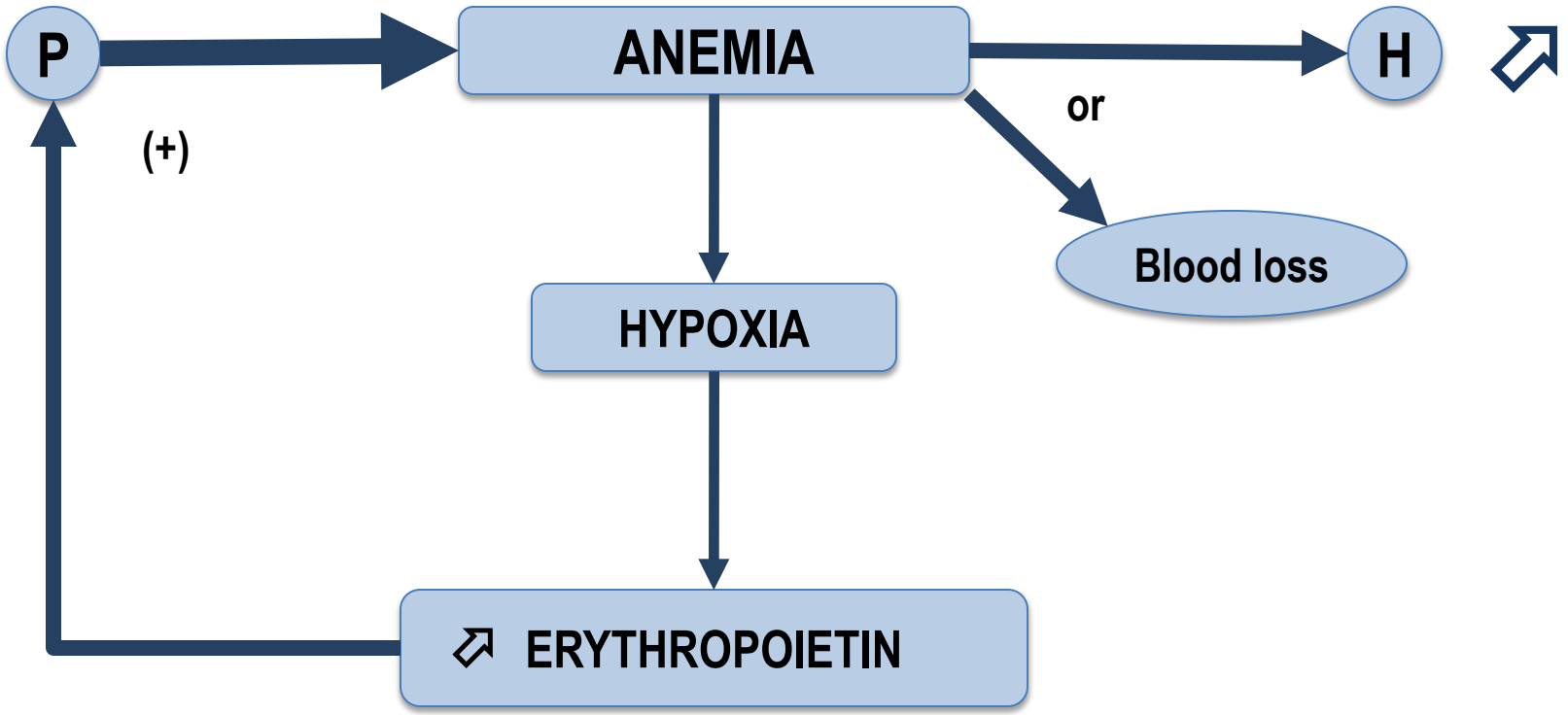
[2] By flow cytometry

[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



MECHANISMS OF ANEMIA (2)

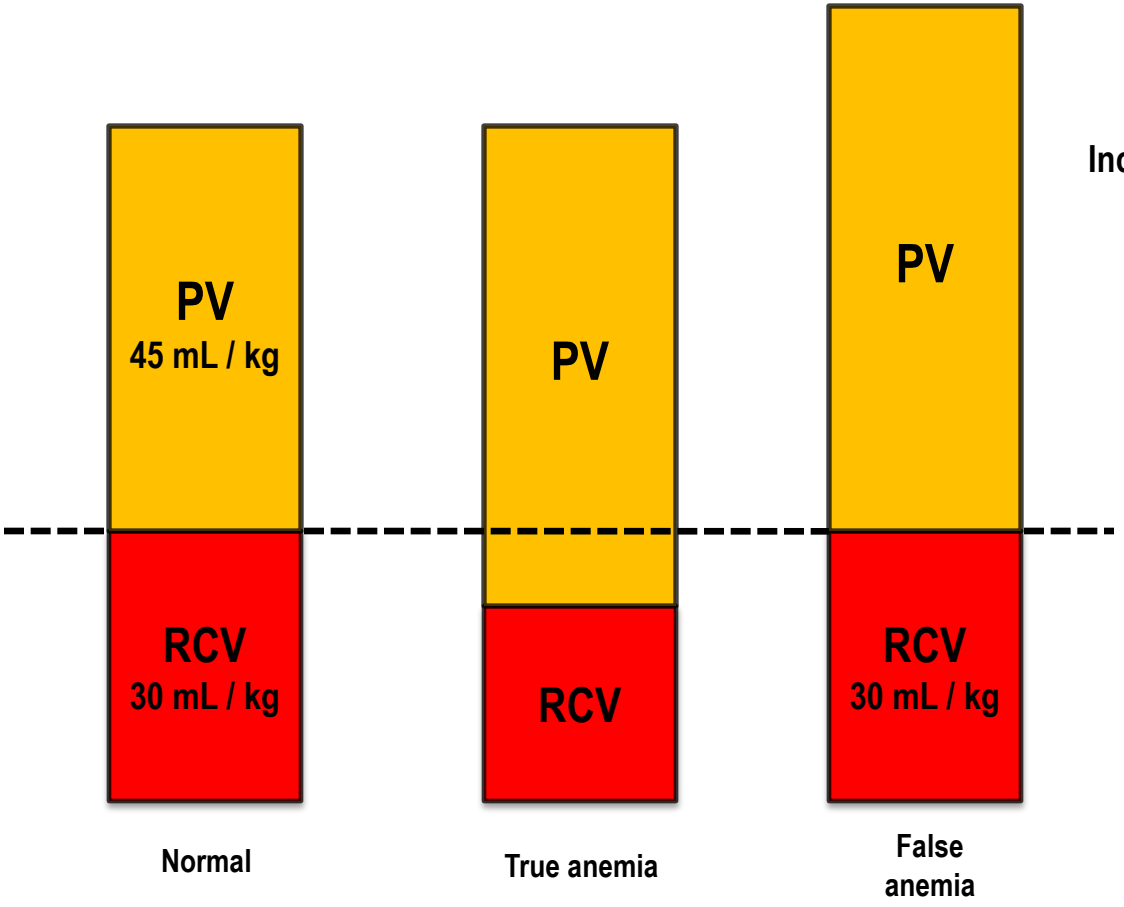


P : PRODUCTION

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[1] < 2.0)

NORMOCYTIC NORMOCHROMIC
- Renal failure
- Pure Red Cell Aplasia (Erythroblastopenia)
- 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 disease, hypothyroidism
- Myelodysplastic syndrome
- Bone marrow aplasia

REGENERATIVE ANEMIA
(Reticulocyte count > 120 G / L / RPI[1] > 2.0 / IRF[2] ⇗)

NORMOCYTIC NORMOCHROMIC
- Acute blood loss
- Hemolytic anemia

[1] RPI : Reticulocyte Production Index
[2] IRF : Immature Reticulocyte Fraction

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 (ERYTHROBLASTOPENIA)

HYPOTHYROIDISM[1]

IN THE CONTEXT OF PANCYTOPENIA *("CENTRAL" ORIGIN)*

BONE MARROW APLASIA[1]

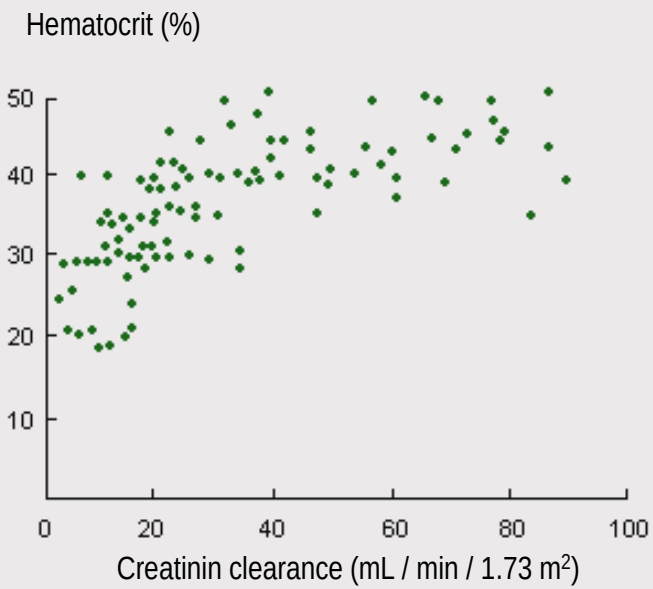
BONE MARROW INFILTRATION *(Acute leukemia, lymphoid neoplasm, metastatic cancer)*

BONE MARROW FIBROSIS

HEMOPHAGOCYTOSIS

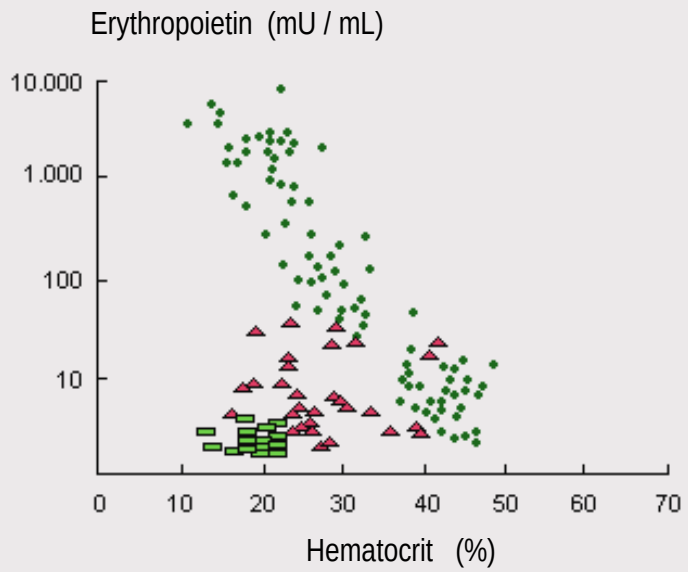
[1] **Normocytic or slightly macrocytic anemia**

ANEMIA OF RENAL FAILURE



Relation between hematocrit and creatinin clearance

Radtke H.W., 1979.



Relation between hematocrit and endogenous erythropoietin

Renal anemia : Absence of kidney

Presence of kidneys

Non renal anemia :

Modified from 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.

PURE RED CELL APLASIA - ERYTHROBLASTOPENIA

HEREDITARY

BLACKFAN-DIAMOND ANEMIA

ACQUIRED

PRIMARY

SECONDARY

THYMOMA (~ 5% thymomas are associated with red cell aplasia)
LYMPHOID NEOPLASM
CANCER *(lung, breast, stomach, thyroid, biliary tract, skin)*
COLLAGEN VASCULAR DISEASE
PARVOVIRUS B19
PREGNANCY
DRUG INDUCED :
- **Anticonvulsants**
- **Azathioprine**
- **Chloramphenicol**
- **Sulfonamides**
- **Isoniazid**
- **Procainamide**

BONE MARROW APLASIA
ETIOLOGY

HEREDITARY BONE MARROW APLASIA

FANCONI ANEMIA
DYSKERATOSIS CONGENITA

ACQUIRED BONE MARROW APLASIA / APLASTIC ANEMIA

IDIOPATHIC (> 2/3 of cases)

SECONDARY

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

APLASTIC ANEMIA (AA)
GENERAL DATA

Stem cell failure, leading to pancytopenia without splenomegaly

Immune mechanisms play an etiologic role in idiopathic AA

FEATURES :

Severe bone marrow hypocellularity with a decrease in all cell lines with remaining fat and marrow stroma

Normal residual hematopoietic cells. Absence of fibrosis or infiltration by abnormal (malignant) cells

Non megaloblastic hematopoiesis *(light RBC macrocytosis in peripheral blood is frequent)*

Symptoms of pancytopenia : bleeding, relapsing infections depending upon severity of the disease

CLASSIFICATION :

MODERATE AA	SEVERE AA (SAA)	VERY SEVERE AA (VSAA)
Marrow cellularity < 30% of normal ↘ of at least 2 of 3 cell lines below normal. ANC[2] > 0.5 G / L	Marrow cellularity < 20% of normal and at least 2 of following criteria : ARC[1] < 40 G / L / ANC[2] < 0.5 G / L / platelets < 20 G / L	Similar to SAA but with : ANC[2] < 0.2 G / L and / or infection(s)

[1] ARC : Absolute Reticulocyte Count

[2] ANC : Absolute Neutrophil Count

PROGNOSIS :

Related to severity of the disease

Without treatment less than 30% of patients with SAA or VSAA survive at 1 year

Response to treatment depends on the type of therapy, on patient age which limits indication to bone marrow transplantation

No age related limitation for immunosuppressive therapy

APLASTIC ANEMIA (2)

DRUG INDUCED BONE MARROW TOXICITY

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

CHLORAMPHENICOL INDUCED APLASTIC ANEMIA

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

APLASTIC ANEMIA (3)
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

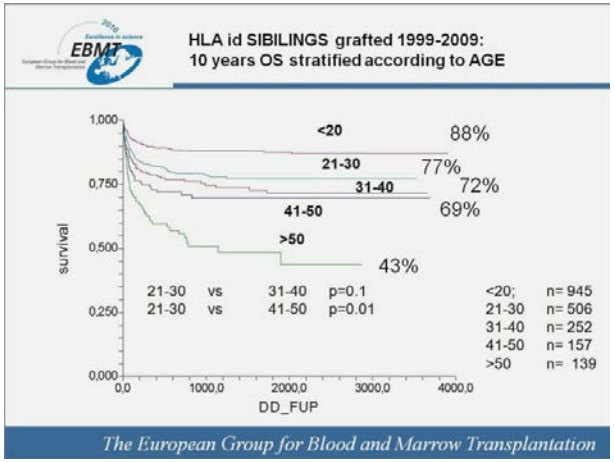
[1] Idiosyncrasy : occasional or uncommon bone marrow depression
[2] Patients collective recruited in USA, Europe and Asia
[3] Chloramphenicol, Phenylbutazone, anticonvulsants, gold salts, others
[4] Benzene : obligatory toxicity or idiosyncrasy
[5] On the basis of some studies, 40-70% of idiosyncratic 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.

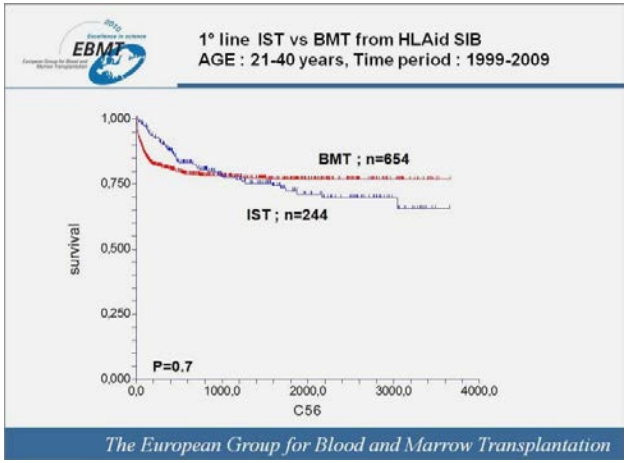
APLASTIC ANEMIA (4)
TREATMENT

BONE MARROW TRANSPLANTATION vs IMMUNOSUPRESSIVE TREATMENT

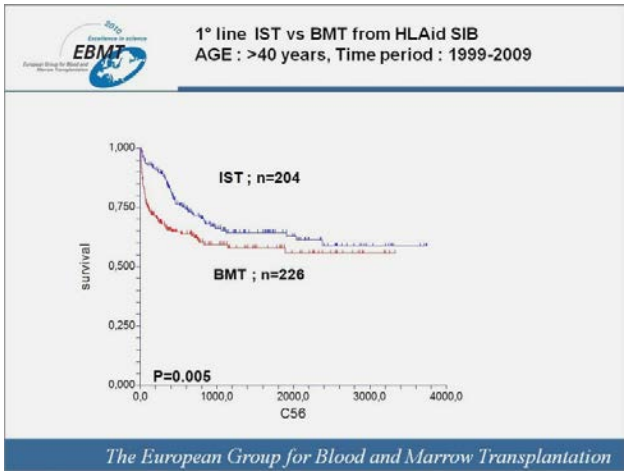
❶



❷



❸



❶ Survival of SAA patients treated by bone marrow transplantation (BMT)[1] is strongly age dependant. Increase of treatment related mortality proportional to age is the main cause

❷ For patients aged 21 to 40 years, bone marrow transplantation (BMT) appears equivalent to immunosuppressive treatment (IST), or slightly better at longer term

❸ Over 40 years of age, upfront IST is the treatment of choice

[1] In SAA and VSAA transplantation of bone marrow appears better than transplantation of peripheral blood stem cells

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

APLASTIC ANEMIA (5)
TREATMENT (2)

TREATMENT :

Withdrawal of potentially offending agents

Supportive care *(Blood and platelet transfusions to be used selectively in candidates to HST[1])*

Immunosuppressive treatment (IST) :

Anti-thymocyte globulin + Cyclosporin (± high dose steroids), mostly used

Hematopoietic stem cell transplantation (HST) :

Syngeneic, allogeneic in case of HLA-matched sibling / HLA-matched unrelated donor, reduced intensity conditioning transplant

MODERATE AA	SEVERE AA & VERY SEVERE AA		
ALL AGES	**< AGE 20**	**AGE 20 - 40**	**> AGE 40[2]**
Imunosuppression : *Anti-thymocyte globulin (ATG)* *+ Cyclosporin* *± steroids ± G-CSF*	**HST if HLA-matched sibling donor** **If not, immunosuppression :** *Anti-thymocyte globuline (ATG)* *+ Cyclosporin* *± steroids ± G-CSF* **Consider HST[1] from HLA-matched unrelated donor for a child or adolescent patient with VSAA**	**HST if HLA-matched sibling donor** **If not, immunosuppression :** *Anti-thymocyte globulin (ATG)* *+ Cyclosporin* *± steroids ± G-CSF* **Possibly HST from HLA-matched unrelated donor**	**Imunosuppression :** *Anti-thymocyte globulin (ATG)[3]* *+ Cyclosporin* *± steroids ± G-CSF*

[1] HST : Hematopoietic Stem cell Transplantation
For SAA and VSAA bone marrow transplantation appears superior to transplantation with peripheral blood hematopoietic stem cells

[2] Risk of transplant related mortality (e.g. GVHD) increasing with age

[3] For elderly patient with SAA or VSAA immunosuppressive treatment should omit ATG because of its toxicity

MICROCYTIC HYPOCHROMIC ANEMIA

DECREASED MCV, MCH AND MCHC

IRON DEFICIENCY

Chronic blood loss
Increased demand
Malabsorption
Poor diet

ANEMIA OF CHRONIC DISEASE

Acute and chronic infection
Inflammatory disorder
Cancer
Rheumatoid arthritis

IRON UTILIZATION DISORDER

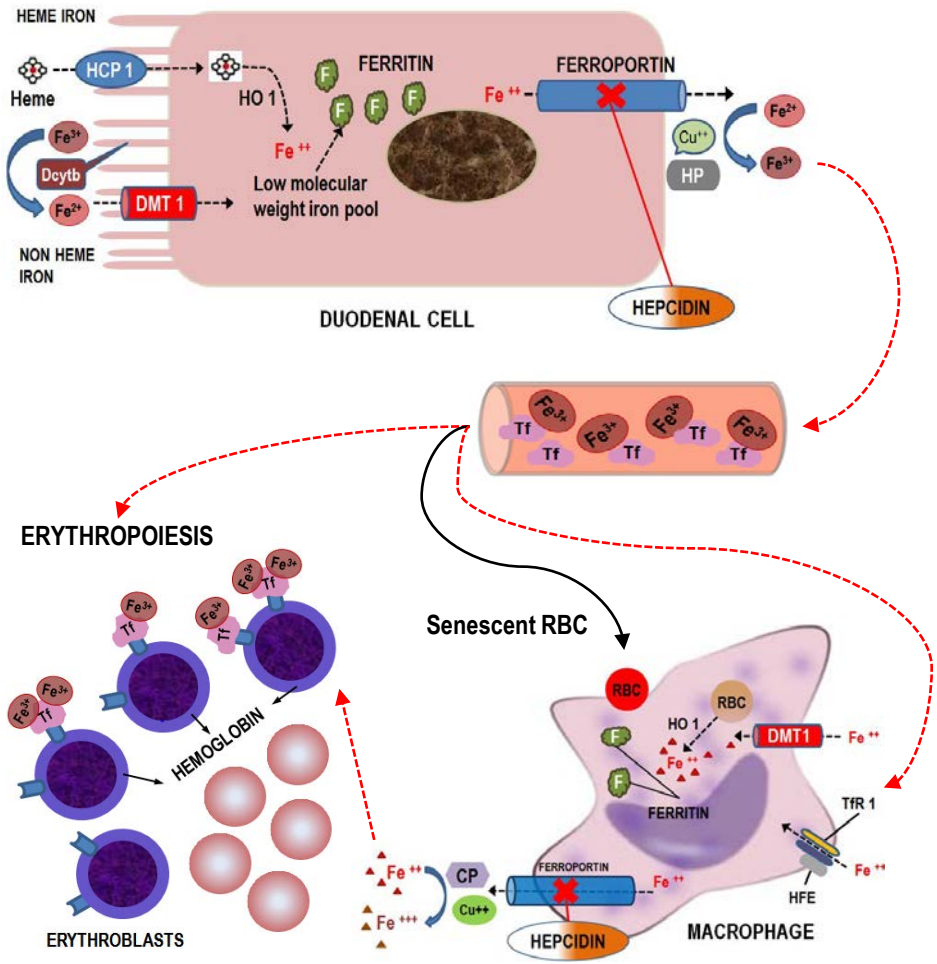
HEMOGLOBINOPATHY

β-Thalassemia
α-Thalassemia
Hemoglobinopathies E, C

SIDEROBLASTIC ANEMIA

Hereditary
Acquired : Primary
Secondary
Lead poisoning
Drugs
Alcohol

IRON METABOLISM



[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 : Hephaestin
[6] HO 1 : Heme Oxygenase 1
[7] CP : Ceruloplasmin
HFE : High Fe (Human hemochromatosis protein)

IRON ABSORPTION :

- Heme iron : by a specific pathway, probably **HCP 1**[1]. Then heme degradation through **Heme Oxygenase (HO 1**[6]**)** with iron recycling (duodenal cell)
- 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 which may store up to 4'000 iron atoms

IRON CIRCULATION

Fe^{++} leaves the cell (duodenal cell or macrophage) through the **Ferroportin** pathway, which is inhibited by **Hepcidin.**
Iron is reoxidated to Fe^{+++} through **Hephaestin (Hp**[5]**)** (duodenal cell) or **Ceruloplasmin (CP**[7]**)** in presence of **Cu^{++}** (macrophage)

Iron then binds to **Transferrin (Tf)** a specific bivalent transporter protein to be transfered to iron dependent cells (i.e. bone marrow erythroblasts for heme synthesis) through binding to the **Transferrin Receptors (TfR**[4]**)**

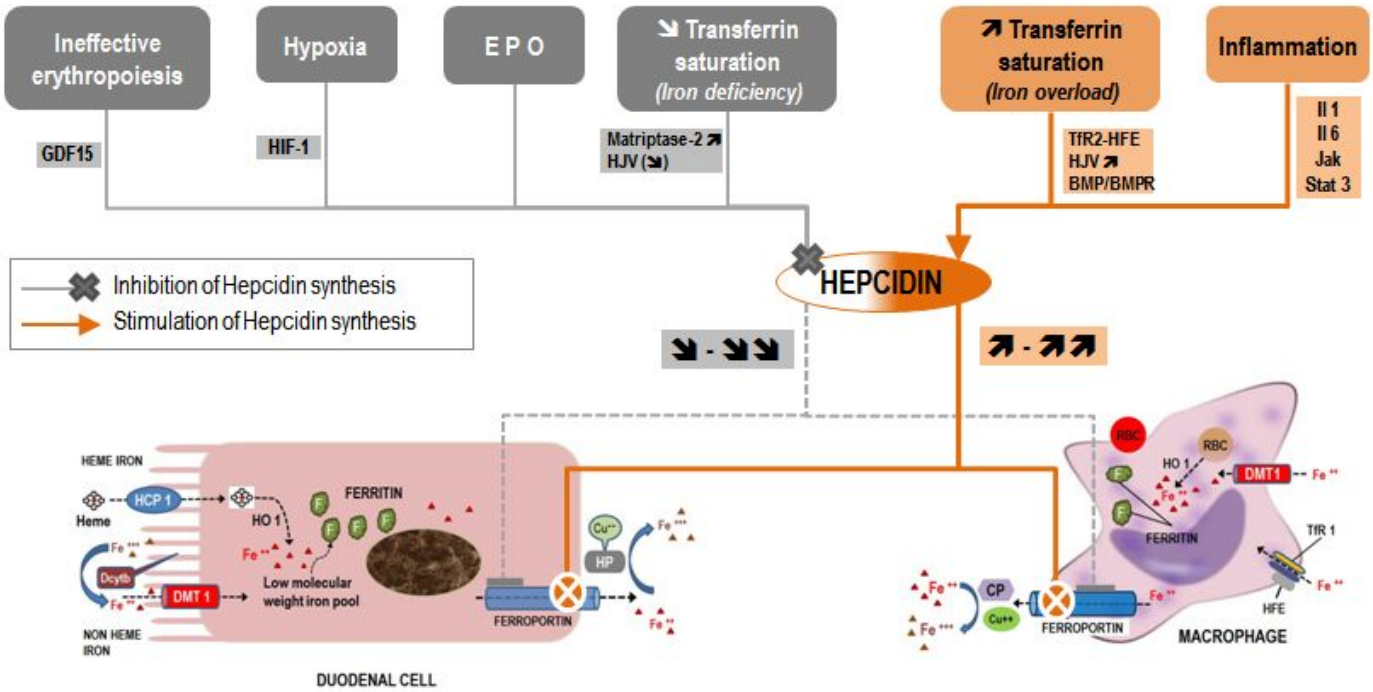
Iron is also stored in the macrophages which also "recycle" the senescent RBC with recuperation and storage of their Heme iron. Release of iron from the stores proceeds by the **Ferroportin** pathway, also inhibited by **Hepcidin**

⇗ **Hepcidin :** blocks Ferroportin by cellular internalization of the formed complex, stopping the process of iron release which remains in the cell. This may lead to iron overload in the macrophages 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*)

Mechanism cf. following page

IRON METABOLISM
REGULATION BY HEPCIDIN

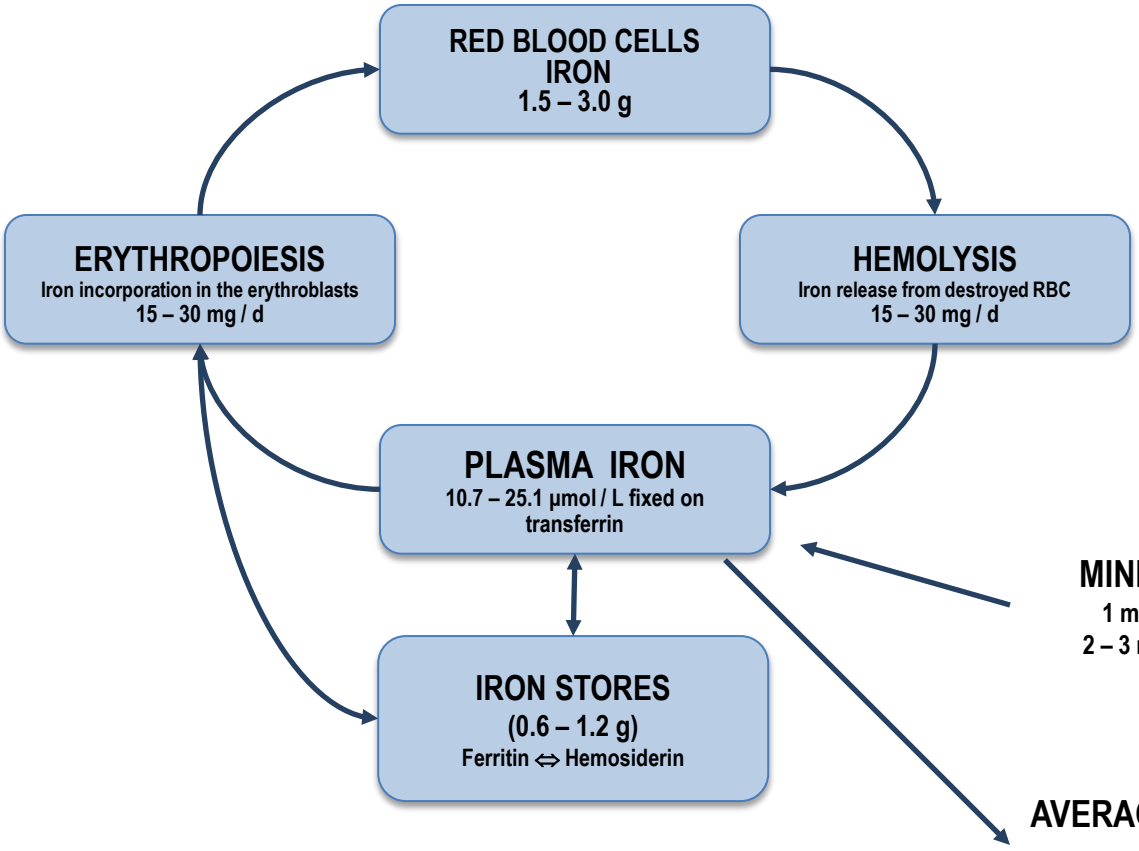


Hepcidin controls **Ferroportin** function and by this way regulates iron uptake and distribution. Mechanisms in **grey** color lead to **Hepcidin** decrease which results in normal or increased iron uptake and transfer
Causes of increased **Hepcidin** production are shown in **orange** color. Increased **Hepcidin** causes retention of iron in the duodenal cells and macrophages by turning down **Ferroportin** pathway (functional iron deficiency)

Rare mutations of **DMT1** or **Matriptase-2 genes** cause iron deficiency anemia, refractory to oral iron administration
(**IRIDA** : Iron-Refractory Iron Deficiency Anemia)

HCP 1 : *Heme Carrier Protein 1* / **DMT 1** : *Divalent Metal Transporter 1)* / **Dcytb** : *Duodenal Cytochrome B (Ferrireductase)*
HP : *Hephaestin* / **CP** : *Ceruloplasmin* / **HO 1** : *Heme Oxygenase 1* / **HFE** : *High Fe (Hemochromatosis protein)* / **TfR** : *Transferrin Receptor*
HIF-1 : *Hypoxia Induced Factor 1* / **HJV** : *Hemojuvelin* / **BMP / BMPR :** *Bone Morphogenetic Protein* / **GDF15** : *Growth Differentiation Factor 15*
Matriptase 2 : *Membrane protein (Gene : TMPRSS6) causing Hemojuvelin lysis*

IRON CYCLE



MINIMAL INTAKE
1 mg / day (Male)
2 – 3 mg / day (Female)

AVERAGE NORMAL LOSSES
1 mg / day (Male)
2 – 3 mg / day (Female)

Normal range[1] :	Iron (serum)	12.5 – 25.1 µmol / L (M[2])	10.7 – 21.4 µmol / L (F[3])
	Transferrin		24.7 – 44.4 µmol / L
	Ferritin (serum)	6 months - 2 years	15 – 120 µg / L
		M : > 2 years	30 – 300 µg / L
		F : 2 - 50 years	10 – 160 µg / L
		F : > 50 years	30 – 300 µg / L

[1] *LCC-CHUV, 2011* [2] *M : Male* [3] *F : Female*

PHYSIOLOGICAL IRON LOSSES

MAN : **1 mg / day : basal losses** (cellular desquamation, integuments, urine, feces, sweat)

WOMAN : **1 mg / day : basal losses**

+ menstruations : **2 – 3 mg / day**

– 50% if oral contraception

+ 100% if intrauterine device

IRON BIOAVAILABILITY

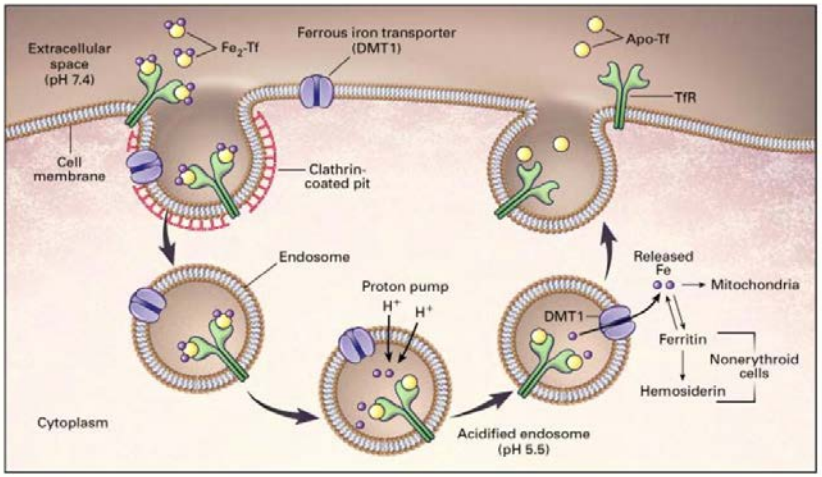
ABSORPTION :

Heme iron **25 – 30%**

Non heme iron **1 – 7%**

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

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.

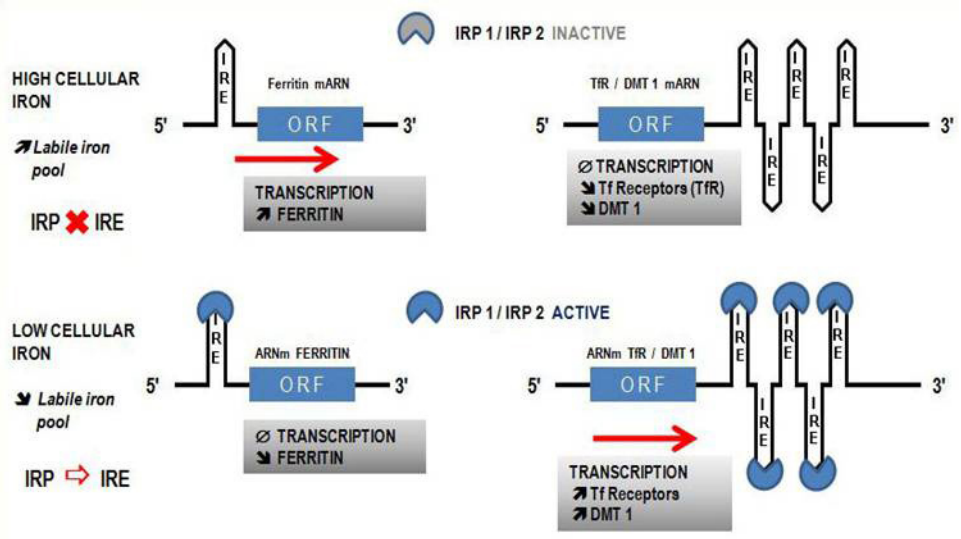
REGULATION OF FERRITIN, TRANSFERRIN RECEPTOR AND DMT 1

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 l**ow** 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

	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	↘

MICROCYTIC HYPOCHROMIC ANEMIA

SERUM IRON - TRANSFERRIN - FERRITIN

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

SOLUBLE TRANSFERRIN RECEPTORS :

Increased in isolated iron deficiency but also when combined with inflammatory processes
Normal in isolated inflammatory anemia

RBC ZINC PROTOPORPHYRIN (low specificity) :

Increased in severe iron deficiency, but also in inflammatory anemia and lead poisoning

RING SIDEROBLASTS :

Increased in sideroblastic anemia (indication to bone marrow examination), *cf.p. 45*

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.p. 38-39)*

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

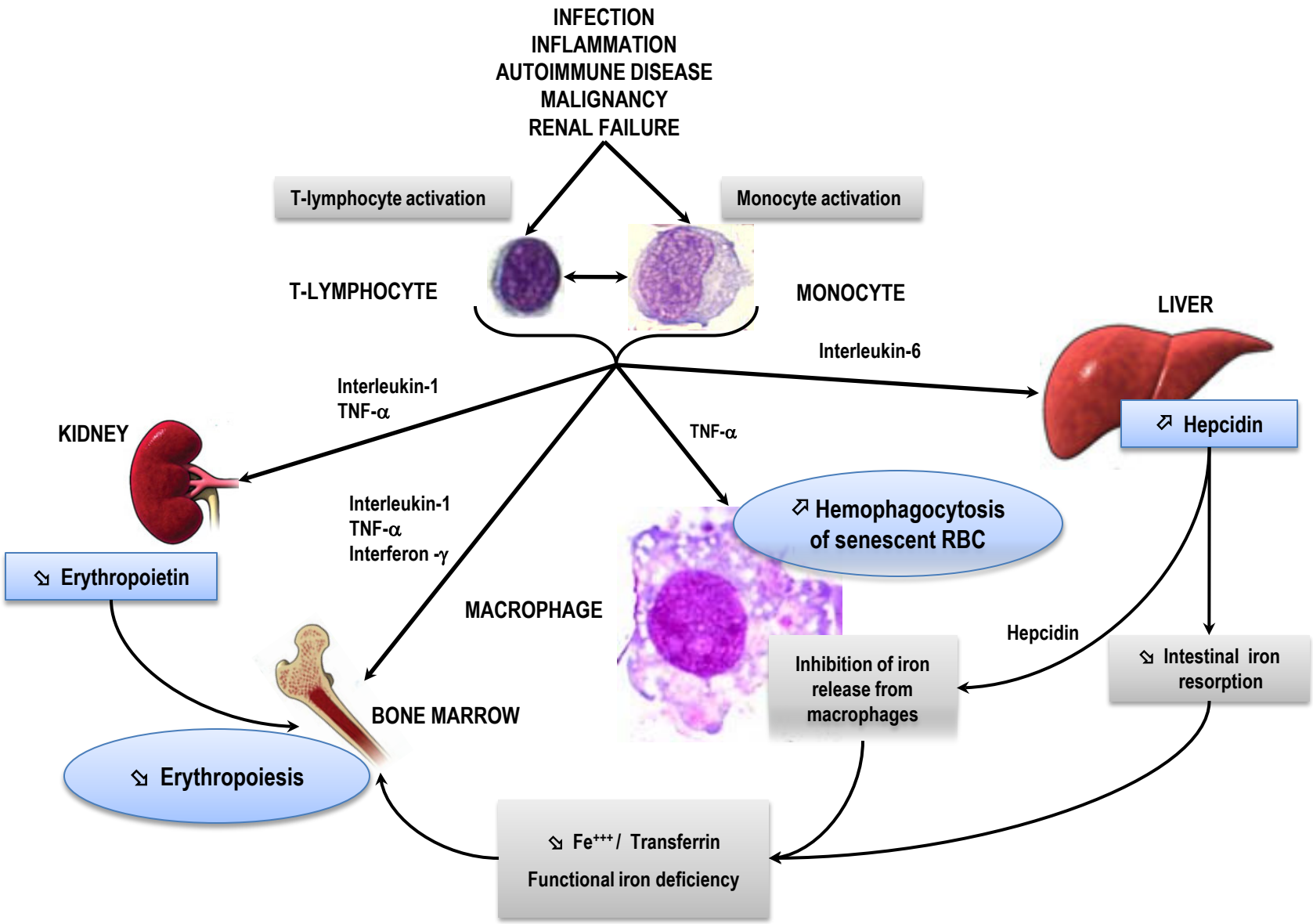
Indications :

Functional iron deficiency (Hb content in reticulocytes (CHr[1]) < 28 pg; hypochromic RBC fraction (HYPO[1]) : > 5%)
Malabsorption syndrome
Digestive oral iron intolerance
Poor patient compliance
Important chronic, persisting hemorrhage
Rare mutations of DMT 1 genes (vegetarians[2]) or of Matriptase-2 : IRIDA (cf.p. 32)

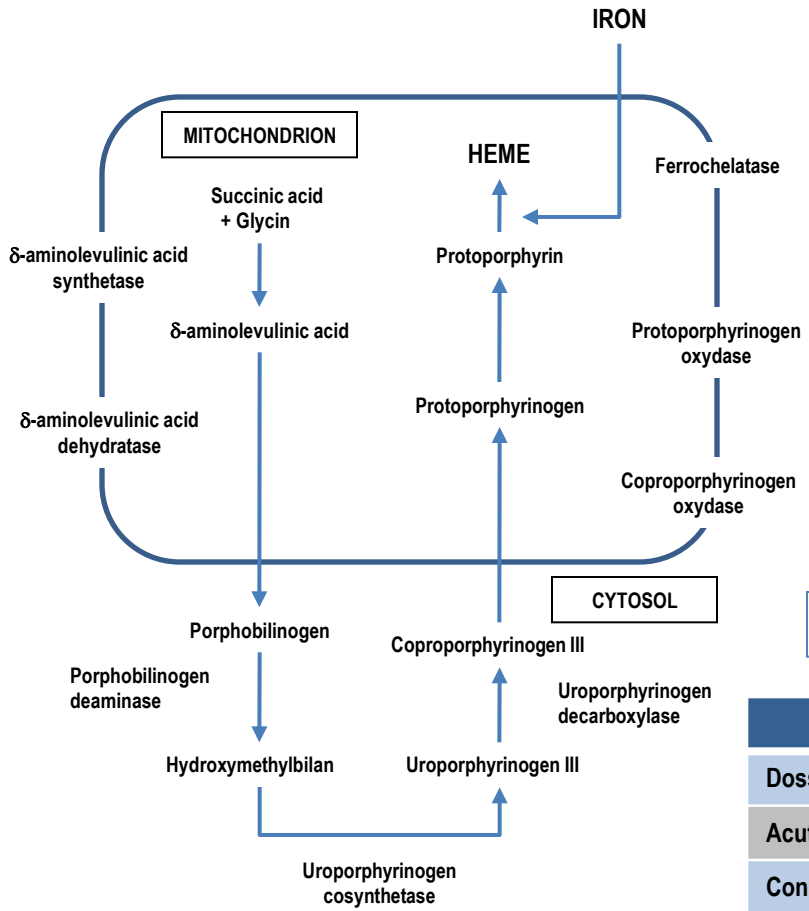
[1] These 2 parameters can only be measured by certain hematological analyzers

[2] In case of normal balanced diet, DMT 1 mutations have no consequence, due to normal absorption of heme iron through HCP 1 pathway

ANEMIA OF CHRONIC DISORDERS / INFLAMMATORY ANEMIA

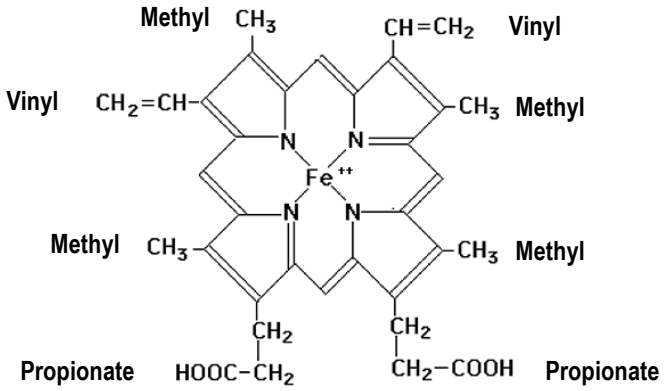


HEME SYNTHESIS



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

Porphyric nucleus + iron

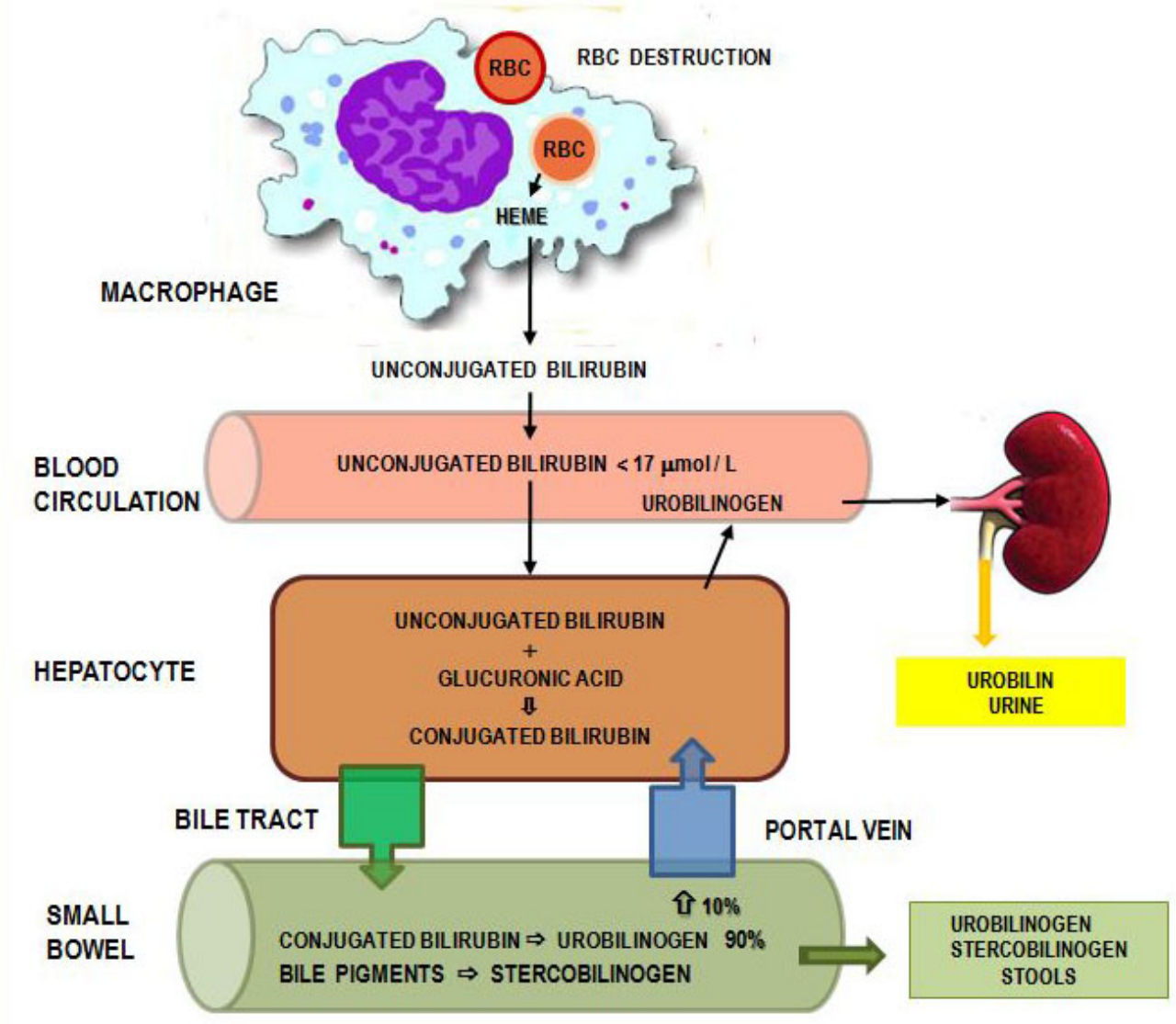


The heme molecule

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

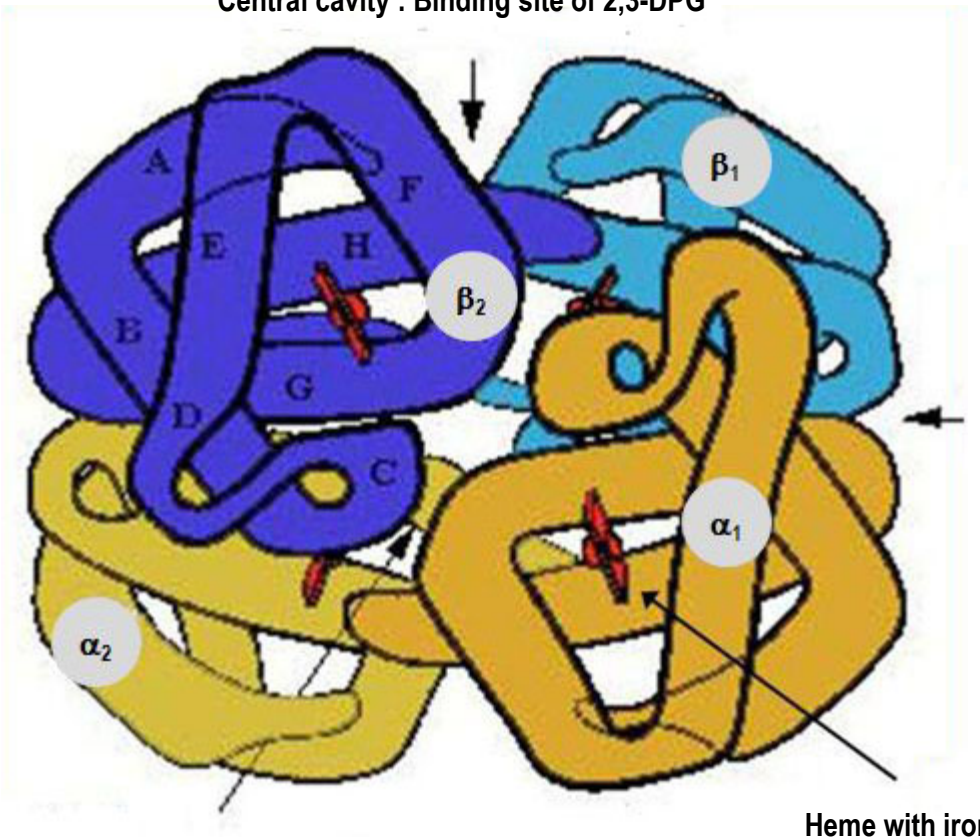
HEMOGLOBIN DEGRADATION



HEMOGLOBIN STRUCTURE

Central cavity : Binding site of 2,3-DPG

$\alpha_2\beta_2$ dimer



$\alpha_1\beta_1$ dimer

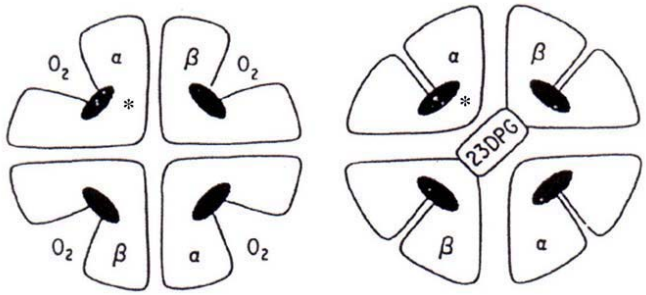
$\alpha_1\beta_1$ contact area

Heme with iron atom

$\alpha_1\beta_2$ contact area

Hemoglobin tetramer with contact areas

HEMOGLOBIN / INTERACTION O_2 AND 2,3-DPG



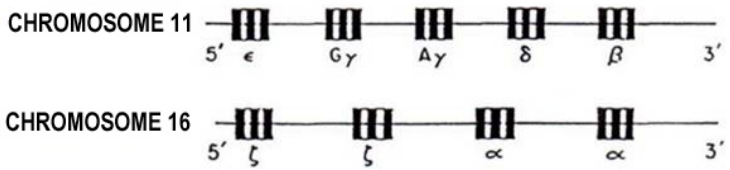
OXYHEMOGLOBIN DEOXYHEMOGLOBIN

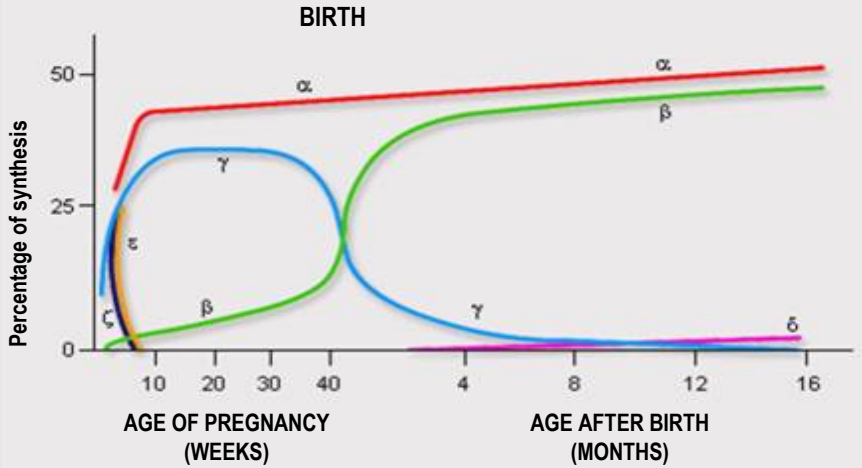
*** Heme**

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

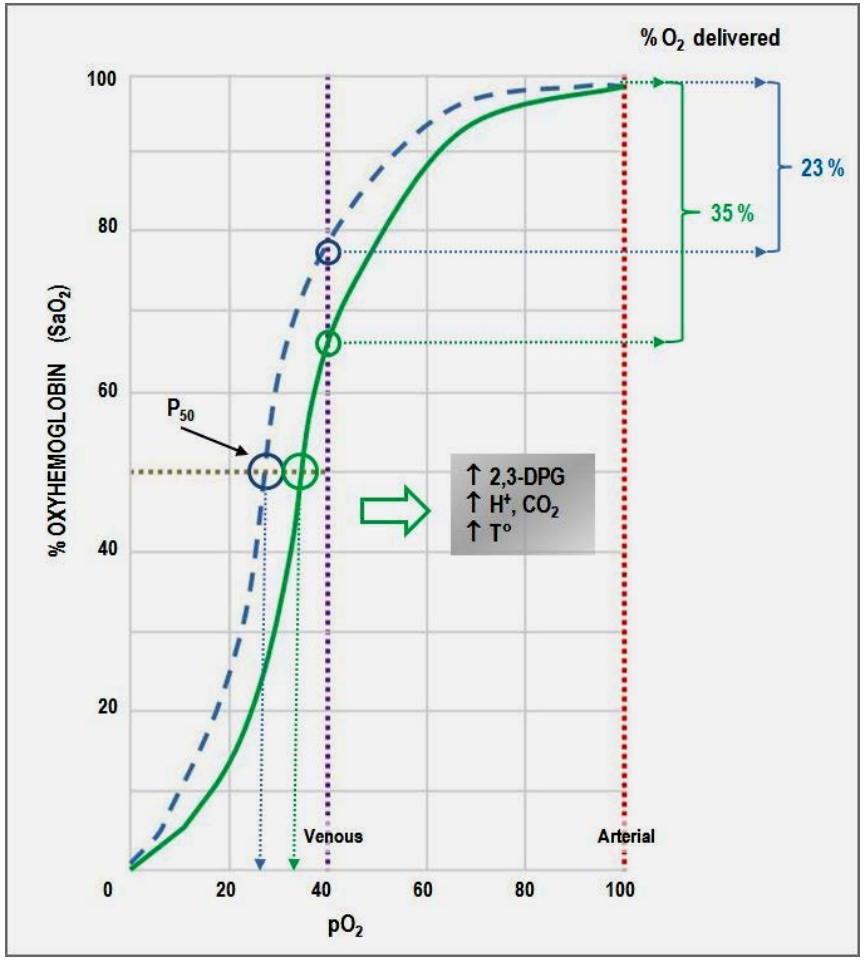




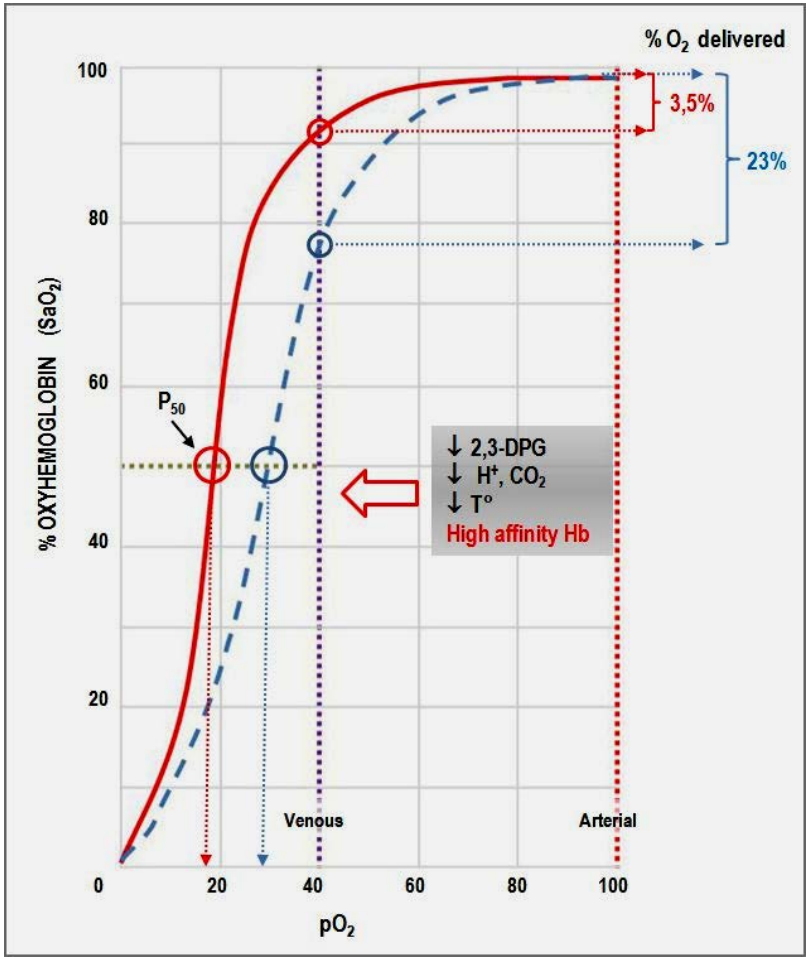
Synthesis of the different globin chains during ontogenesis

Modified from 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***

Normal curve : – – – –

ANEMIA WITH IRON UTILIZATION DISORDER
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 poisoning*
  - *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 globin α-chain synthesis**

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

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 or β / β^0 (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 : normal β gene

β^0 : mutation with no β chain synthesis

β^+ : mutation with residual low β chain synthesis

β-THALASSEMIA INTERMEDIA

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

β^+-thal / β^+ -thal (homozygocity) → marked ⇘ of β-chain synthesis (β^+ gene)

Anemia of variable severity (70 – 100 g / L) depending on the amount of residual β-chain synthesis (gene β^+)

Transfusion requirements less important than in β-thalassemia major

β-THALASSEMIA MAJOR

β^0-thal / β^0-thal (homozygocity) → absence of β chains synthesis

β^0-thal / β^+-thal (double heterozygocity) → severe ⇘ of β-chain synthesis

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

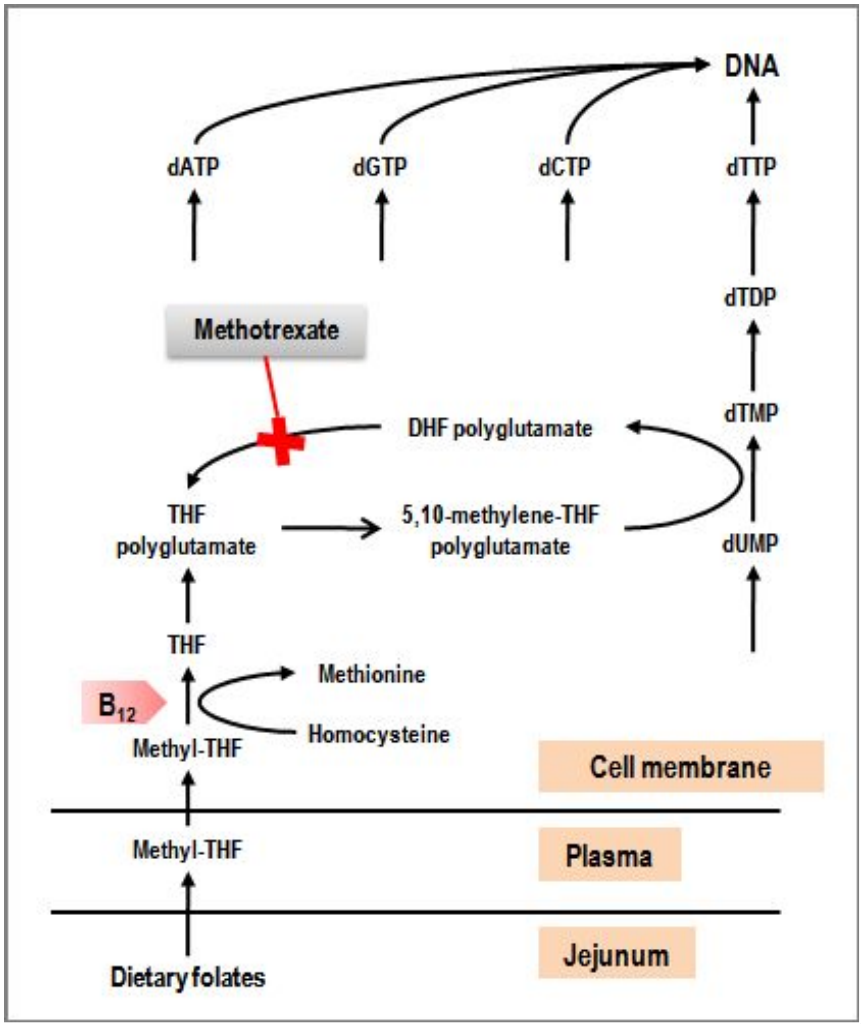
MEGALOBLASTIC MACROCYTIC ANEMIA

Vitamin B_{12} deficiency
Folate deficiency
Cytotoxic drugs
- *6-mercaptopurin*
- *5-fluorouracil*
- *Cytarabin*
- *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

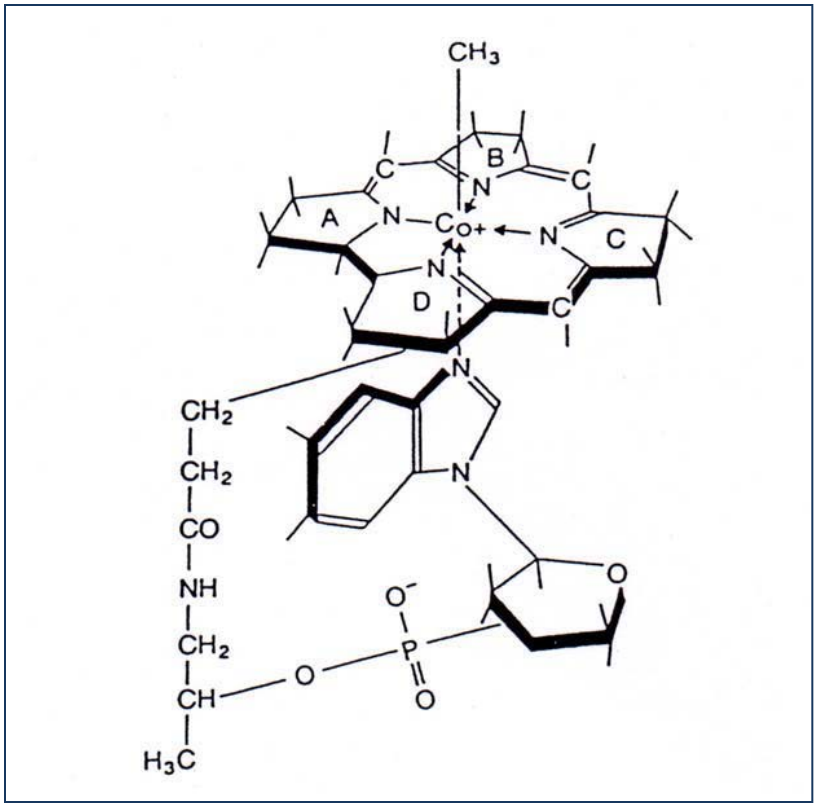
Other function of vitamin B_{12}

Propionyl-CoA $\xrightarrow{B_{12}}$ Methylmalonyl-CoA $\longrightarrow$ Succinyl-CoA

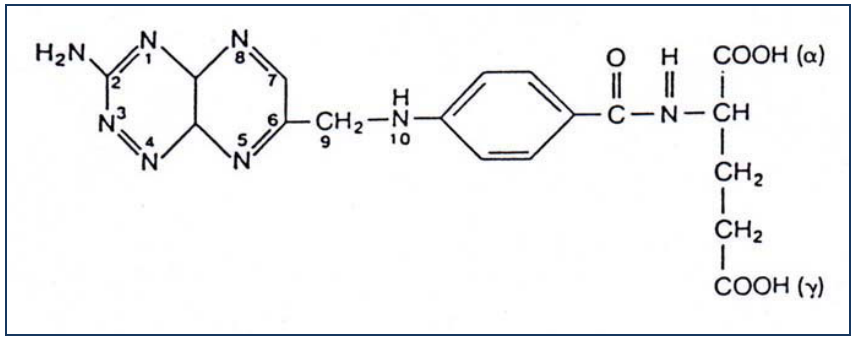
Vitamin B_{12} deficiency is responsible of homocysteine increase (cf. fig.) as of methylmalonic acid

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)**

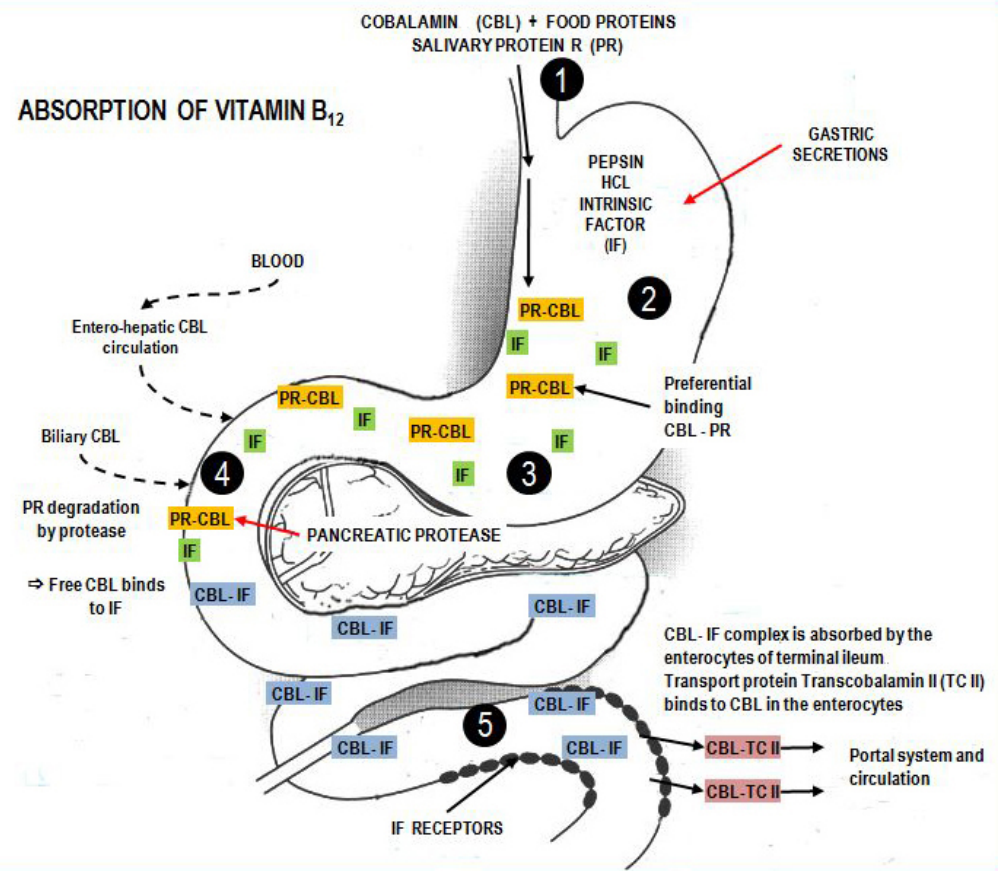
Hoffbrand A.V., Pettit J.E. : Essential Haematology, 3th edition 1993; Blackwell Science : p. 54 & 57.

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
Stores	2 – 3 mg	10 – 12 mg
Exhaustion of stores	2-4 years	3-4 months
Absorption		
Site	Ileum	Jejunum
Mechanism	Intrinsic factor	Conversion to methyltetrahydrofolate
Transport	Transcobalamins (TC) TC I and III or haptocorrins or R proteins : Binding to food proteins then cobalamins transport TC II : transport and intracellular cobalamins transfer	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[1]	7.0 – 45.1 nmol / L[1]

[1] *LCC-CHUV, 2012*

ABSORPTION OF VITAMIN B_{12}



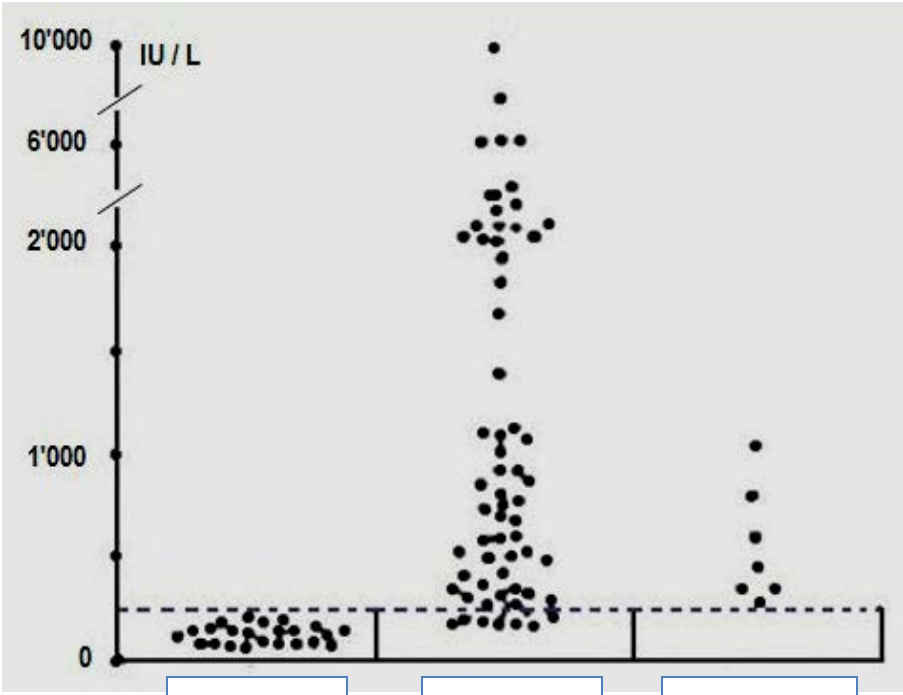
Cobalamins of dietary origin are bound unspecifically to the **food proteins.** In the stomach **peptic digestion at low pH** splits proteins from cobalamins which then bind to **R proteins (***or haptocorrins)* of salivary origin. In the duodenum R proteins are degradated by pancreatic proteases which allows the **binding of cobalamins to the intrinsic factor of gastric origin.** The **ileal receptor** of the vitamin B_{12} / IF complex is the **cubulin**

TC I and TC III are abundant in the secondary granules of neutrophils

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

1. **Cobalamin dietary deficiency**
2. **Anomaly of cobalamin - 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)

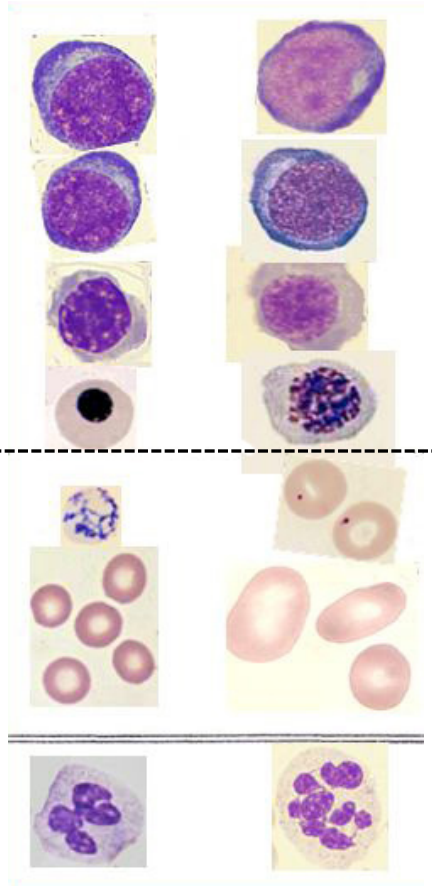
	Urinary excretion of radiolabeled vitamin B_{12} (%)	
	B_{12} alone	B_{12} + IF
Normal subject	18 (9 – 36)	–
Pernicious anemia	0.5 (0 – 1.2)	13 (6 – 31)
Malabsorption (gluten enteropathy)	3.6 (0 – 19)	3.3 (0 – 10)

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
PROERYTHROBLASTS		MEGALOBLASTS (Asynchronism of nucleocytoplasmic maturation)
EARLY ERYTHROBLASTS		
INTERMEDIATE ERYTHROBLASTS		NORMAL HEMOGLOBIN SYNTHESIS
LATE ERYTHROBLASTS		
BLOOD: RETICULOCYTES		HOWELL-JOLLY BODIES
BLOOD: RED BLOOD CELLS		LOW OR ABSENT RETICULOCYTE COUNT
WHITE BLOOD CELLS		MACROCYTES MEGALOCYTES
NEUTROPHILS		HYPERSEGMENTED NEUTROPHILS



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

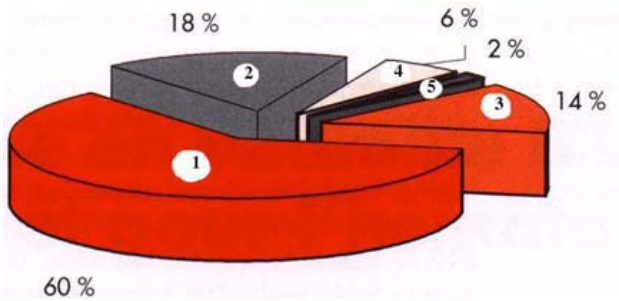
CAUSES OF VITAMIN B_{12} DEFICIENCY

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

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

LABORATORY TESTS

- **Methylmalonic acid *(plasma).* Normal range : < 0.28 µmol / L[1]**
- **Homocysteine *(plasma).* Normal range : 5 − 15 µmol / L[1]**

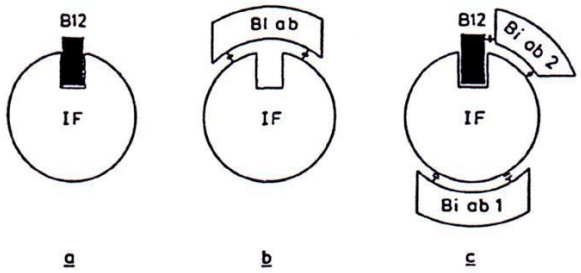
SCHILLING TEST

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%)

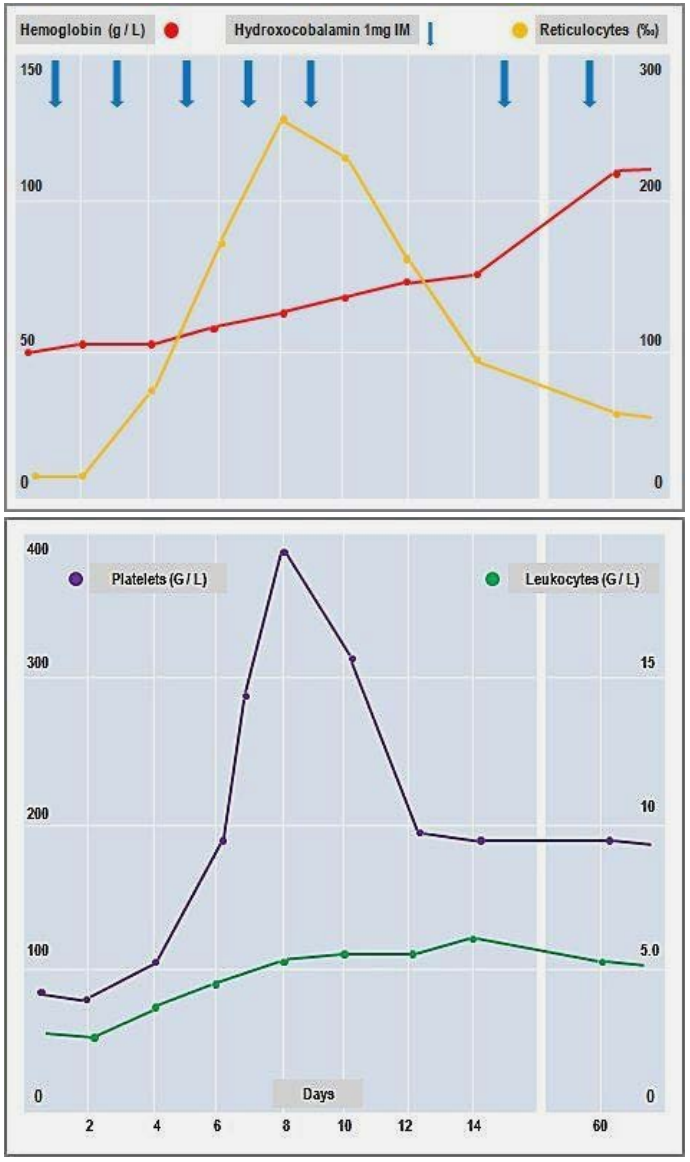


Schematic presentation of intrinsic factor (IF), vitamin B_{12} and of antibody directed against intrinsic factor :
a) Normal binding between IF and vitamin B_{12}
b) Blocking antibody
c) Coupling antibody

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

[1] LCC-CHUV, 2011

PERNICIOUS ANEMIA (3)
RESPONSE TO HYDROXOCOBALAMIN SUBSTITUTION



Modified from Hoffbrand A.V., Moss P.H.A., Pettit J.E. : Essential Haematology 5th edition 2006; Blackwell Publishing : p 55.

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

BLOOD LOSS	% BLOOD VOLUME	SYMPTOMS
0.5 – 1.0 L	10 – 20	Possible vaso-vagal reaction
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

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. next page

EXTRAVASCULAR HEMOLYSIS

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

INTRAVASCULAR HEMOLYSIS

- ⇗ plasmatic Hb (> 50 mg / L)

Hemoglobinuria

Hemosiderinuria

HEMOLYSIS DUE TO CORPUSCULAR ANOMALY

Hereditary *(except PNH[1])*

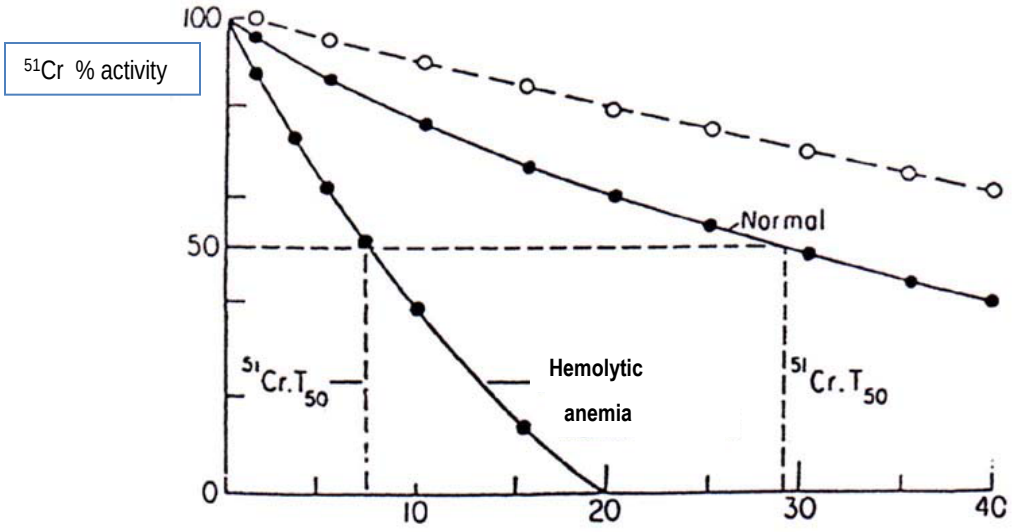
Homozygous or heterozygous

HEMOLYSIS DUE TO EXTRACORPUSCULAR ANOMALY

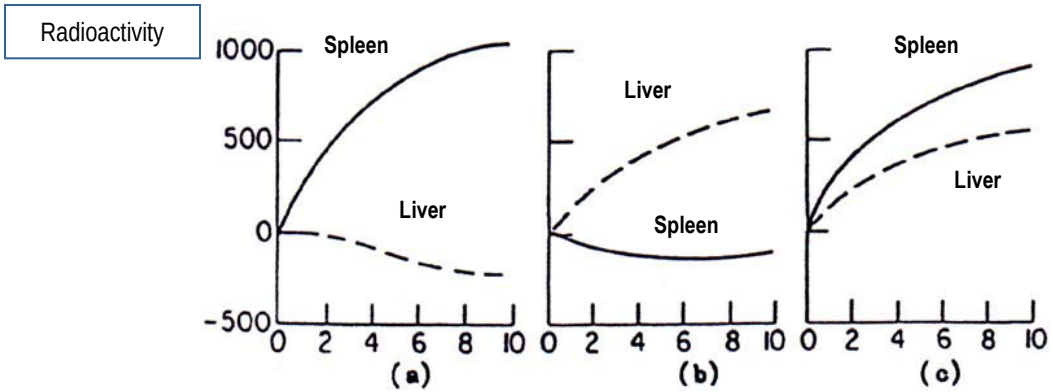
Acquired

[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.p. 46-48)

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

SICKLE CELL DISEASE

HEMOGLOBINS E, C

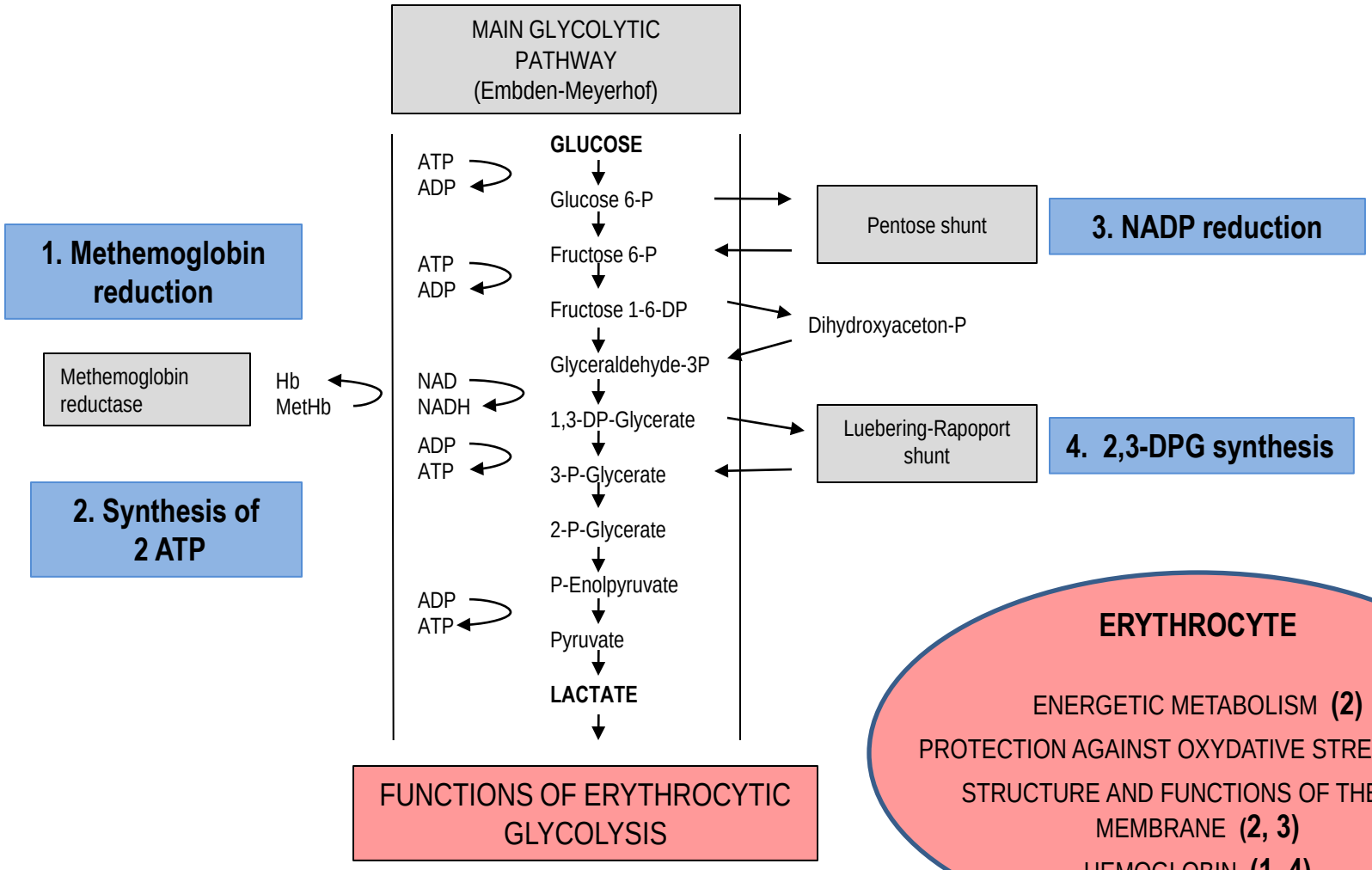
UNSTABLE HEMOGLOBINS

HEMOGLOBINS M[1]

HEMOGLOBINS WITH INCREASED OR REDUCED OXYGEN AFFINITY

[1] M : Methemoglobin

GLYCOLYSIS OF RED BLOOD CELLS



ERYTHROCYTE

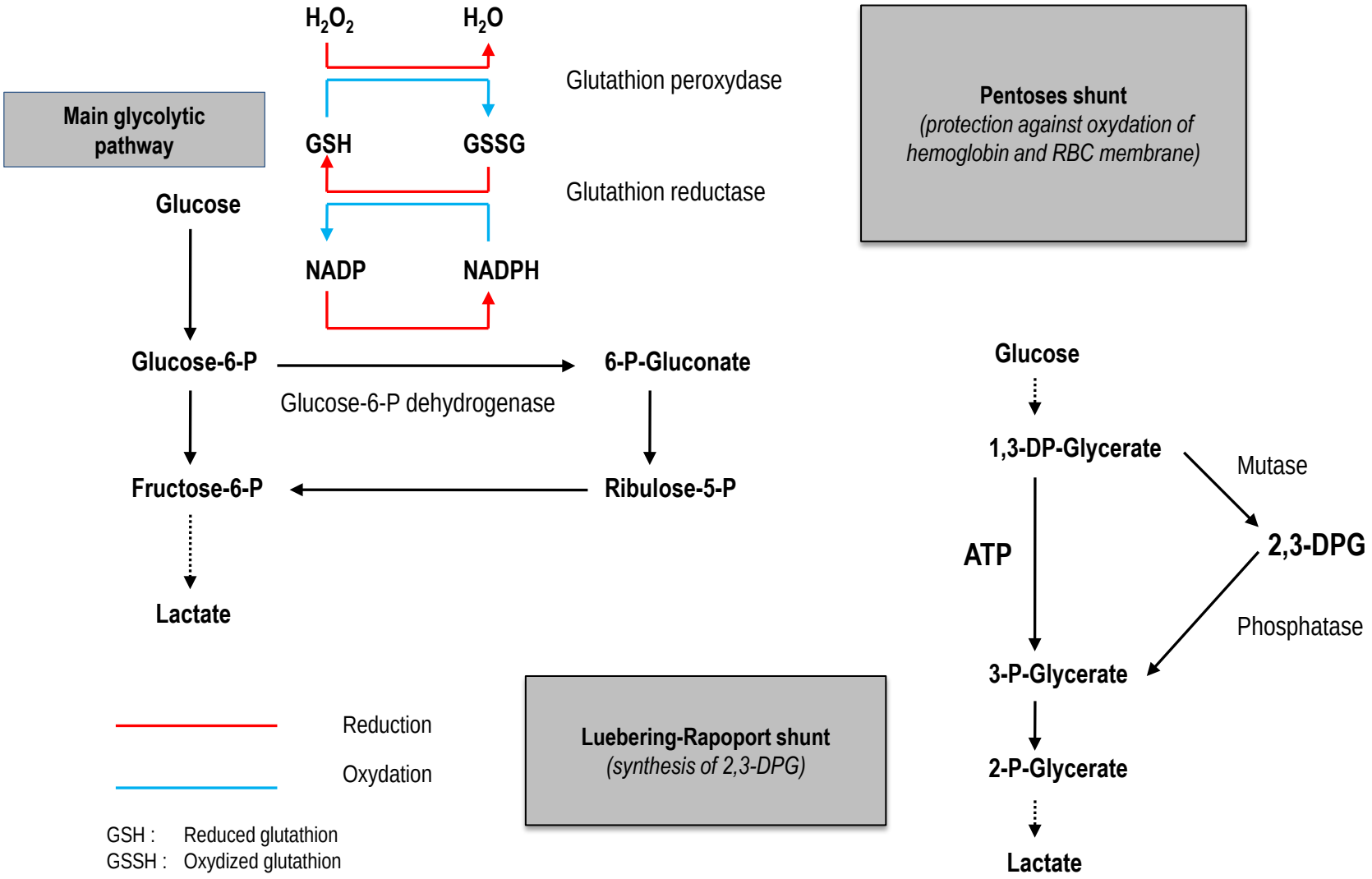
ENERGETIC METABOLISM **(2)**

PROTECTION AGAINST OXYDATIVE STRESS **(1, 3)**

STRUCTURE AND FUNCTIONS OF THE RBC MEMBRANE **(2, 3)**

HEMOGLOBIN **(1, 4)**

GLYCOLYSIS OF RED BLOOD CELLS (2)



GSH : Reduced glutathion
GSSH : Oxydized glutathion

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)

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 (+) : ***Physiological form, predominant***

A (+) : ***Physiological form, 30% African colored***

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

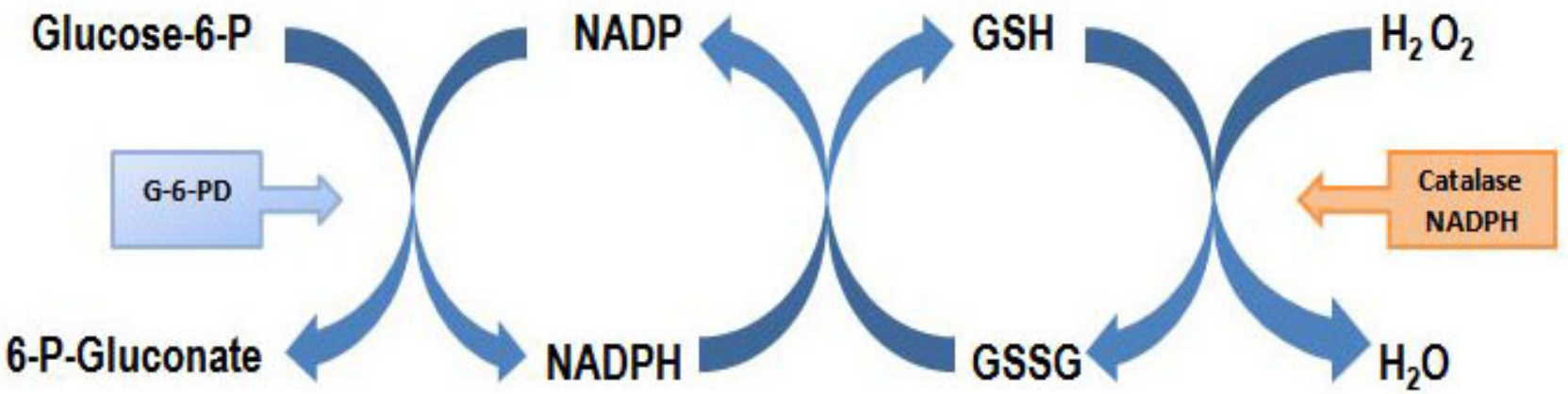
Mediterranean *[formerly B (-)] :* ***Activity < 1%***

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

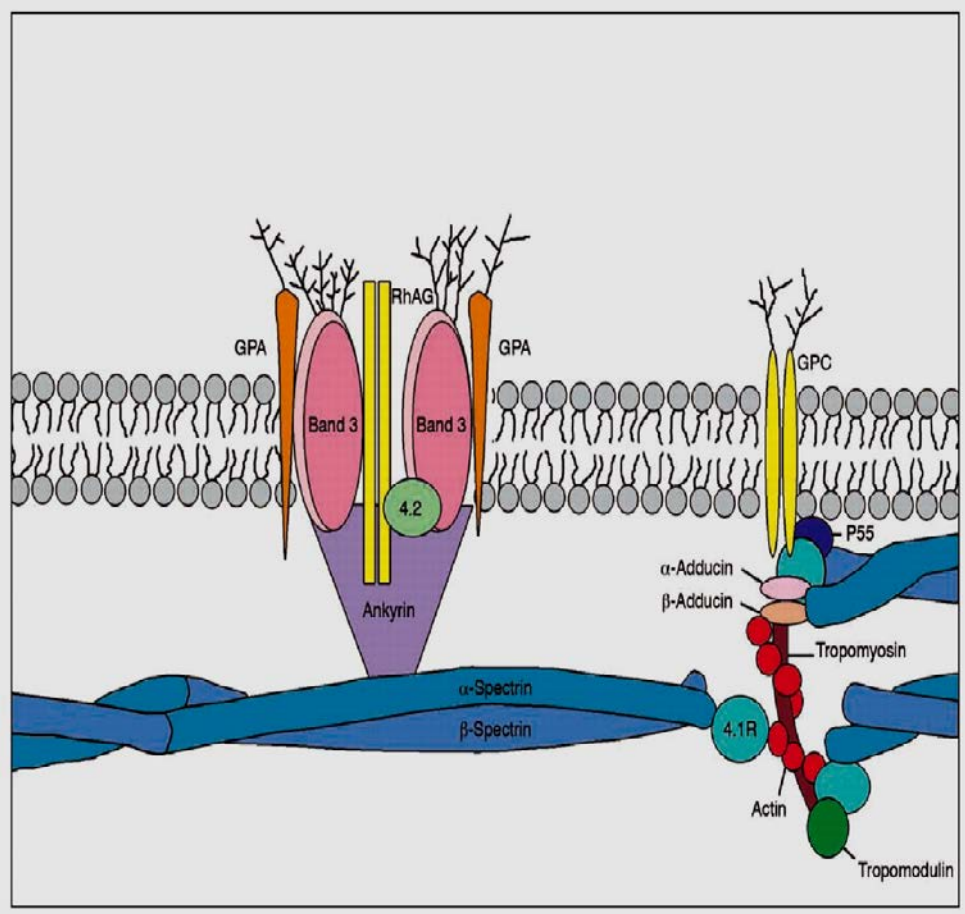
FOOD

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.

STRUCTURE OF RED BLOOD CELL MEMBRANE



Composite structure with double layer lipidic membrane anchored to a two-dimensional elastic network **(cytoskeleton)** with tethering sites (transmembrane proteins)

Vertical fixation involves the cytoplasmic domain of **Band 3** protein**, Ankyrin, Protein 4.2** and **Spectrin**

Horizontal interaction involves **Spectrin** (α- and β-chains), with **Protein 4.1R, Actin, Tropomodulin, Tropomyosin** and **Adducins**

Protein 4.1R interacts also with the transmembrane **Glycophorin C (GPC)** and protein P55 in a triangular mode

GPA : Glycophorin A
RhAG : Rhesus Antigen

ANOMALY OF RED BLOOD CELL MEMBRANE

HEREDITARY SPHEROCYTOSIS

AUTOSOMAL DOMINANT *(cf. next 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

PATHOPHYSIOLOGY

Anomalies of spectrin, ankyrin, band 3, which may be combined

Spherocytes with loss of plasticity and splenic trapping *(sequestration)*

Volume usually 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., Schröter W., J Pediatr 1990; 117 : 409-416, & Pekrun A., Eber S.W., Kuhlmey A., Schröter W., Ann Hematol 1993; 67 : 89-93.

PAROXYSMAL NOCTURNAL HEMOGLOBINURIA (PNH)
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) / Homologous Restriction Factor (HRF)**

Clonal anomaly of hematopoietic stem cell

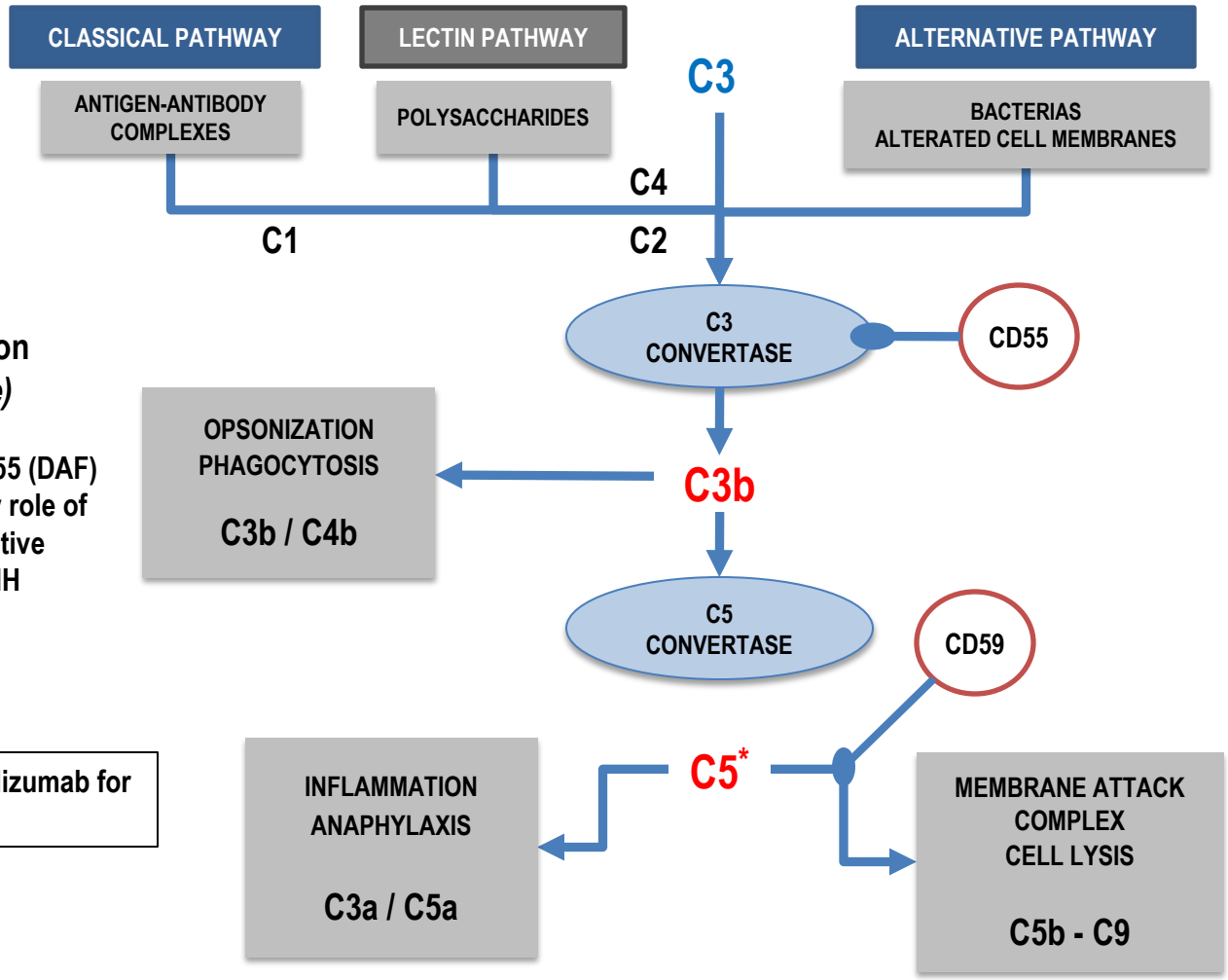
Lysis affects also neutrophils and platelets which also present functional anomalies

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 (MIRL / 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

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 : 167-177 and Am J Clin Pathol 2009; 132 : 564-572.)*

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)*

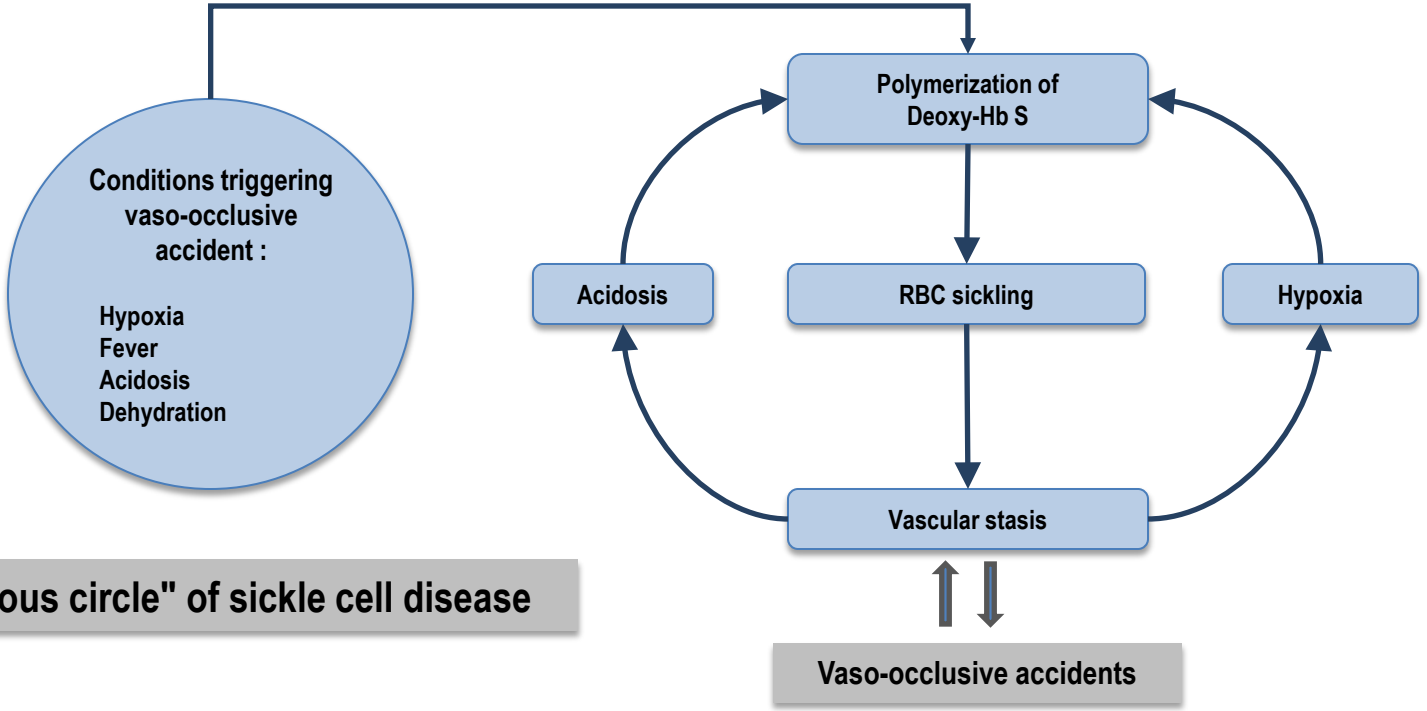
HEMOGLOBINS M

Cyanosis due to methemoglobinemia

HEMOGLOBINS WITH INCREASED OR REDUCED OXYGEN AFFINITY

SICKLE CELL DISEASE
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, fungal and parasitic infections in immunodeficient patients

TOXIC HEMOLYTIC ANEMIA
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 ABLE 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
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)

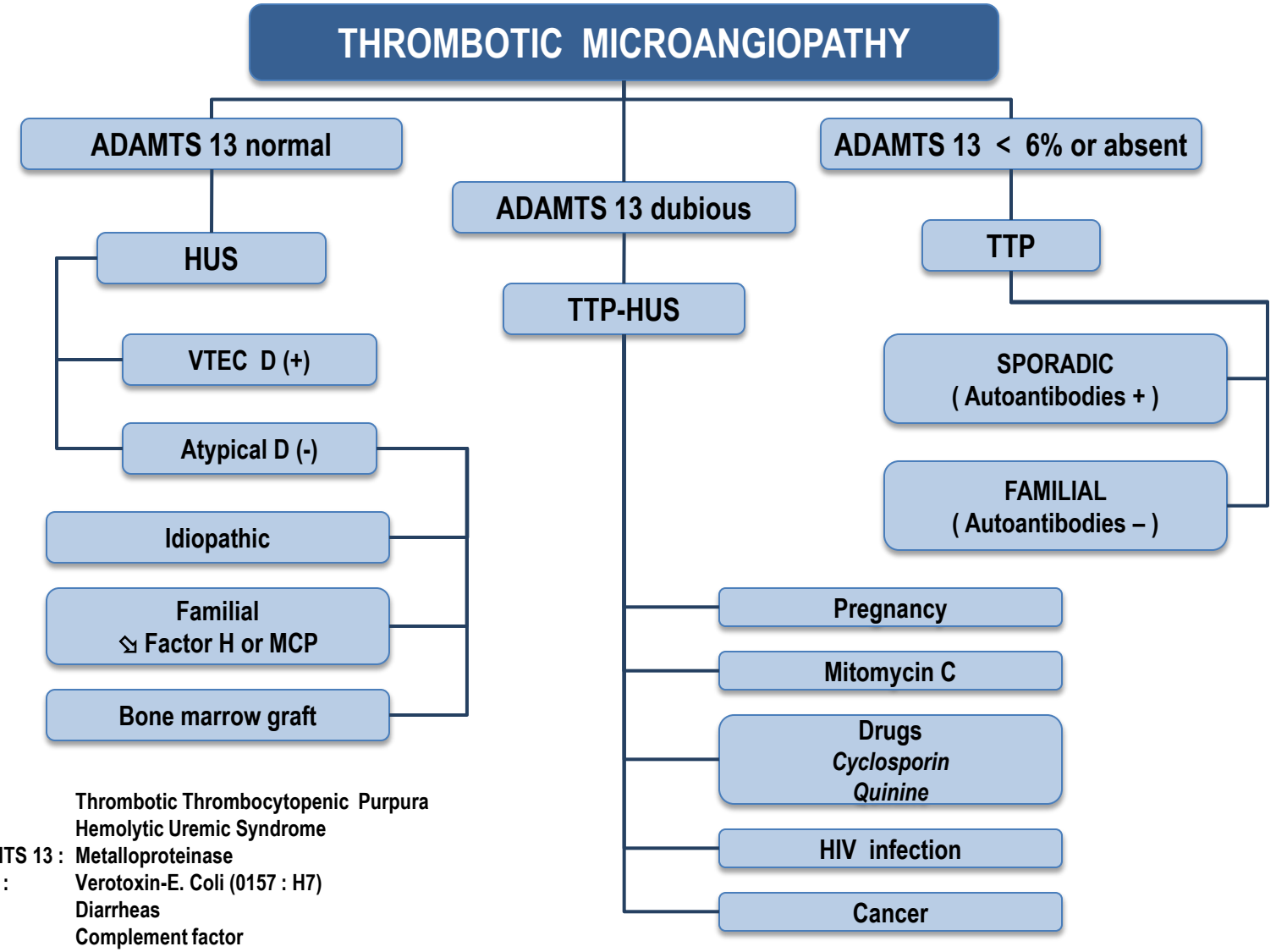
Treatment : *Dialysis*

* ***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*)

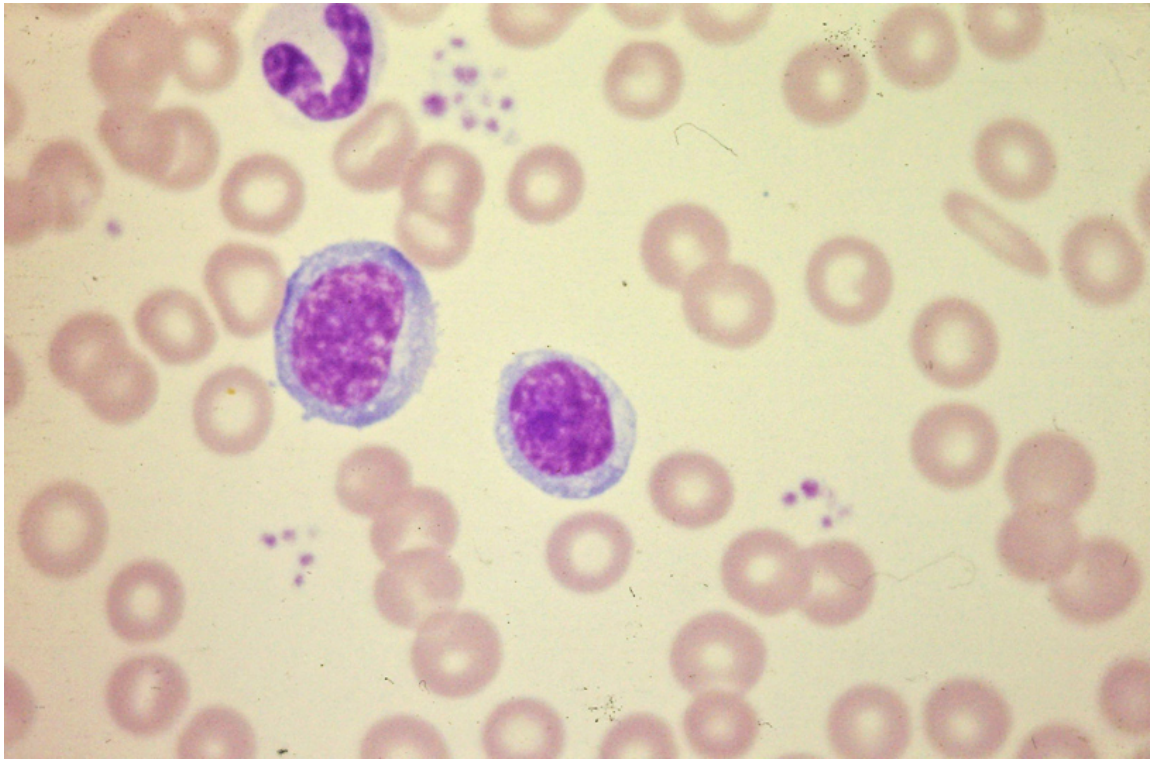


TTP : Thrombotic Thrombocytopenic Purpura
HUS : Hemolytic Uremic Syndrome
ADAMTS 13 : Metalloproteinase
VTEC : Verotoxin-E. Coli (0157 : H7)
D : Diarrheas
H : Complement factor
MCP : Membrane Cofactor Protein

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

LEUKOCYTES : 4.0 – 10.0 G / L		
	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, 2011

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 counts :

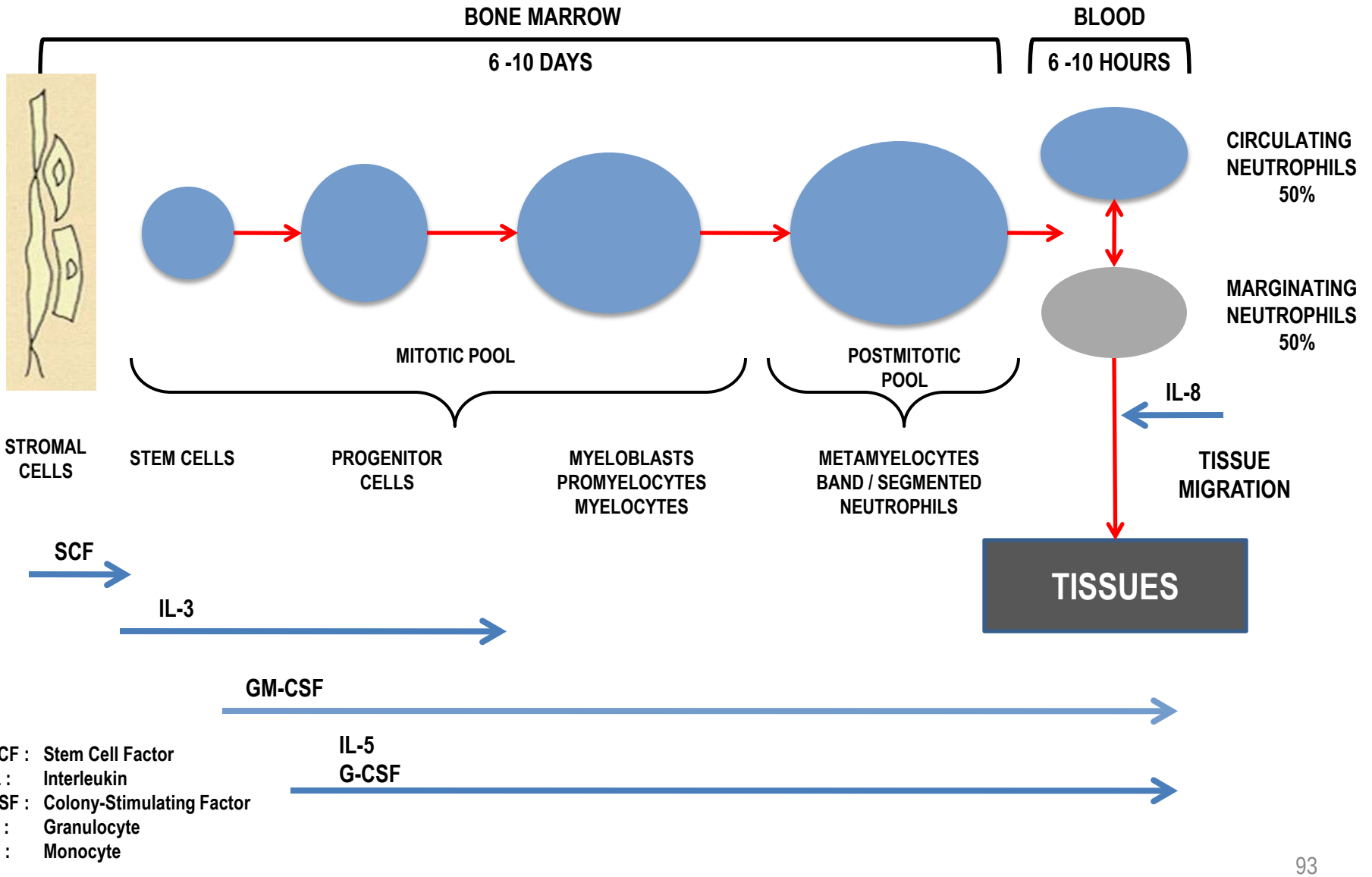
e.g. : chronic lymphocytic leukemia

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

→ ***Neutropenia relative but non absolute***

→ ***Lymphocytosis relative and 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 > 10.0 G / L)*

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

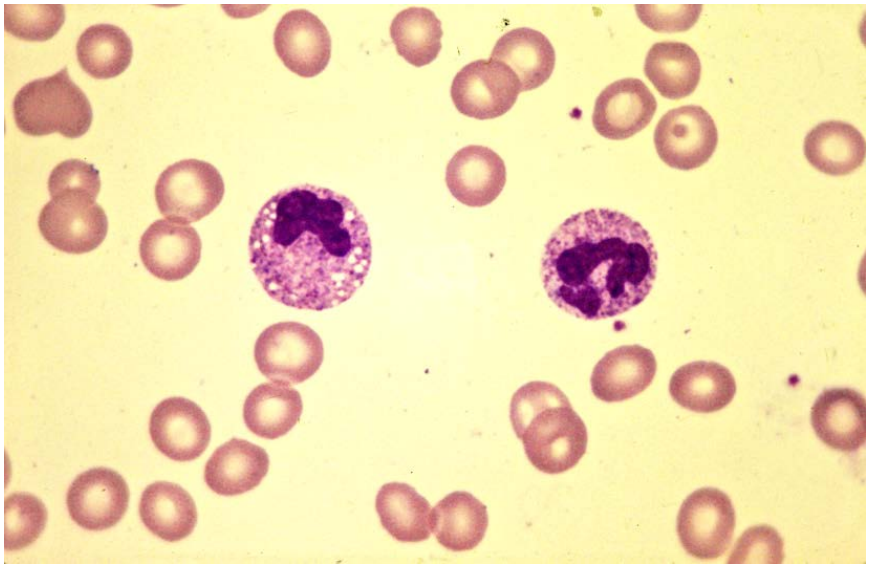
Neutrophil left shift : band neutrophil count > 1.0 G / L (or > 25% if leukocyte count ≤ 4.0 G / L)

Coarse granules of 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
REDUCED PRODUCTION

QUANTITATIVE

- **Bone marrow aplasia**
- **Bone marrow infiltration**
- **Bone marrow fibrosis**
- **T-cell large granular lymphocytic leukemia (T-LGLL)**
- **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

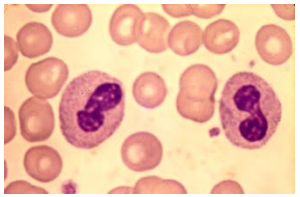
Drugs : **Mianserin** ***(antidepressant),*** **sulfasalazine, phenylbutazone** ***(antiinflammatory agents),*** **cotrimoxazole** ***(antiinfective)*****, metamizole** ***(analgesic)*****, carbamazepine** ***(anticonvulsant),*** ***carbimazole (antithyroid drug)***

[1] **Immune pathogenic mechanism possible**

HEREDITARY MORPHOLOGICAL NEUTROPHIL ANOMALIES

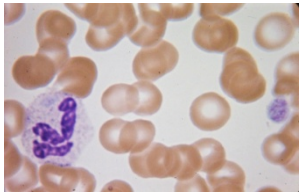
PELGER-HUET ANOMALY

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



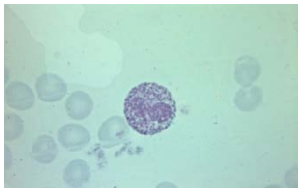
MAY-HEGGLIN ANOMALY

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



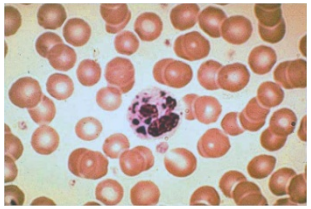
ALDER-REILLY ANOMALY

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.p. 137)

MONOCYTES / MACROPHAGES
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 : Lymph node

(B and T) 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

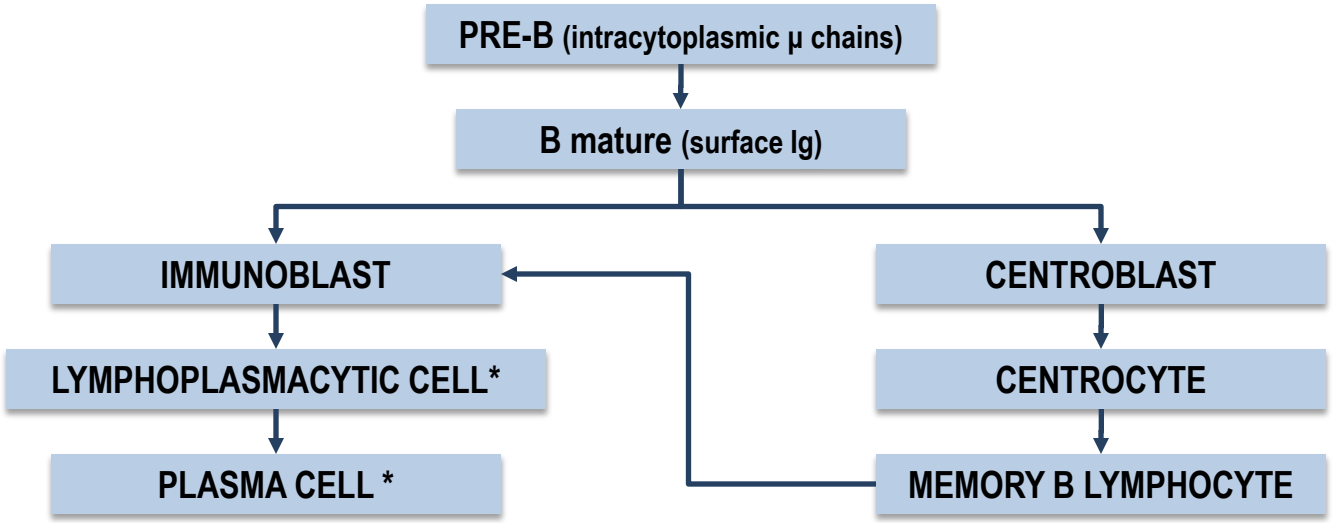
BONE MARROW

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

MIGRATION TO BLOOD AND SECONDARY LYMPHOID ORGANS

→ **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)**

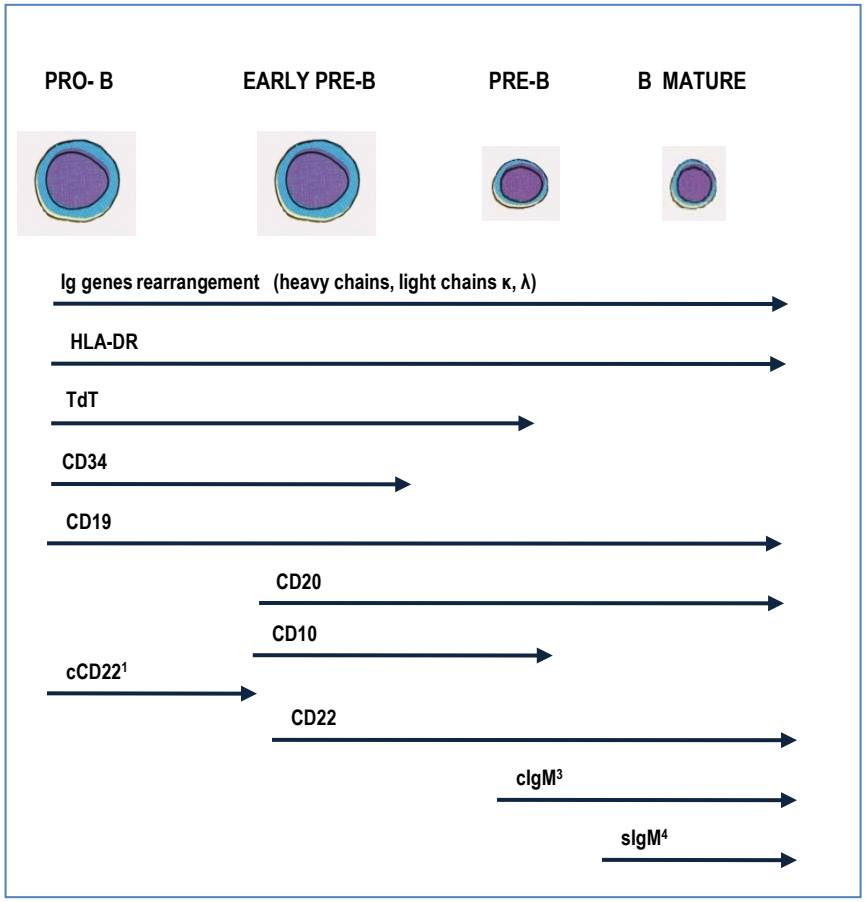
Expression of CD2, CD3, *CD4 + CD8 -* or *CD4 - CD8 +*

MIGRATION TO PERIPHERAL BLOOD AND SECONDARY LYMPHOID ORGANS

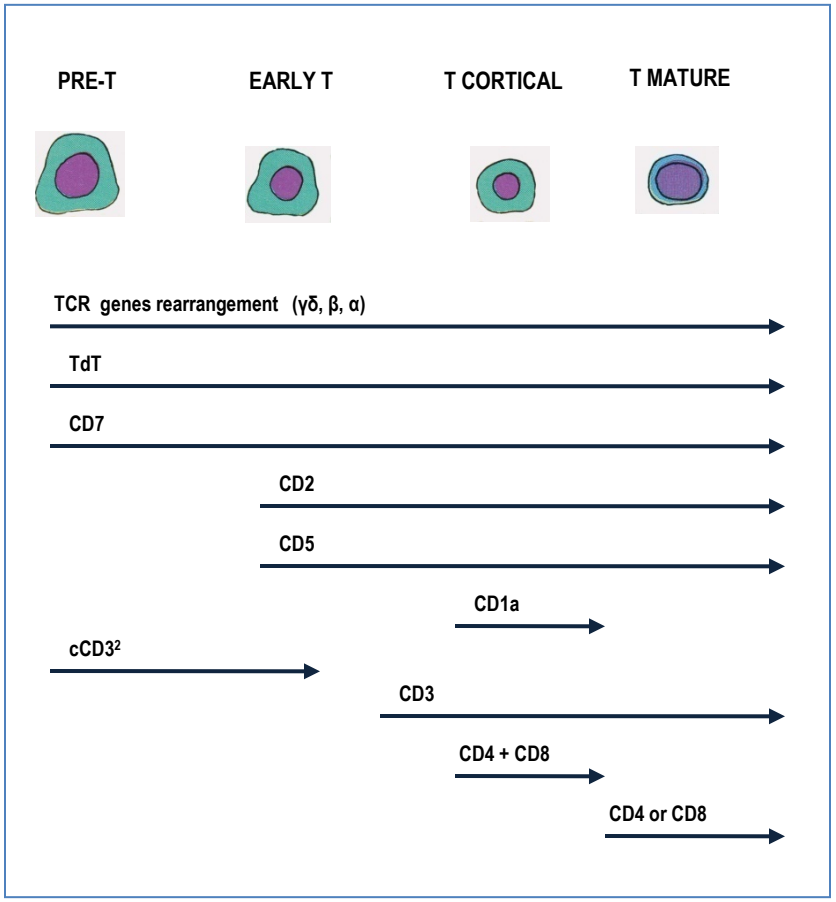
[1] **During positive and negative selections approximately 90% of T-lymphocytes (thymocytes) are eliminated through apoptosis (cell death)**

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 - , CD2 +, 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

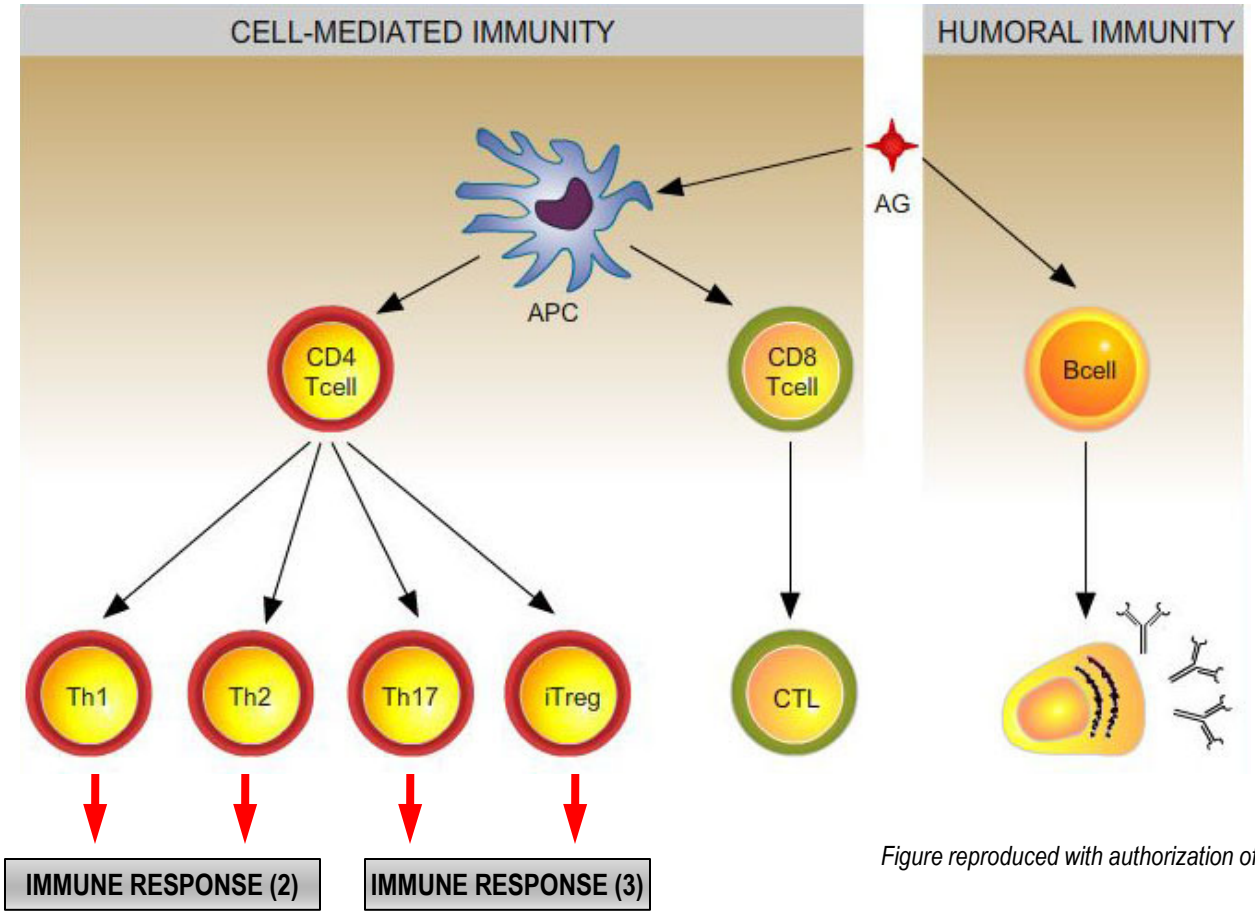
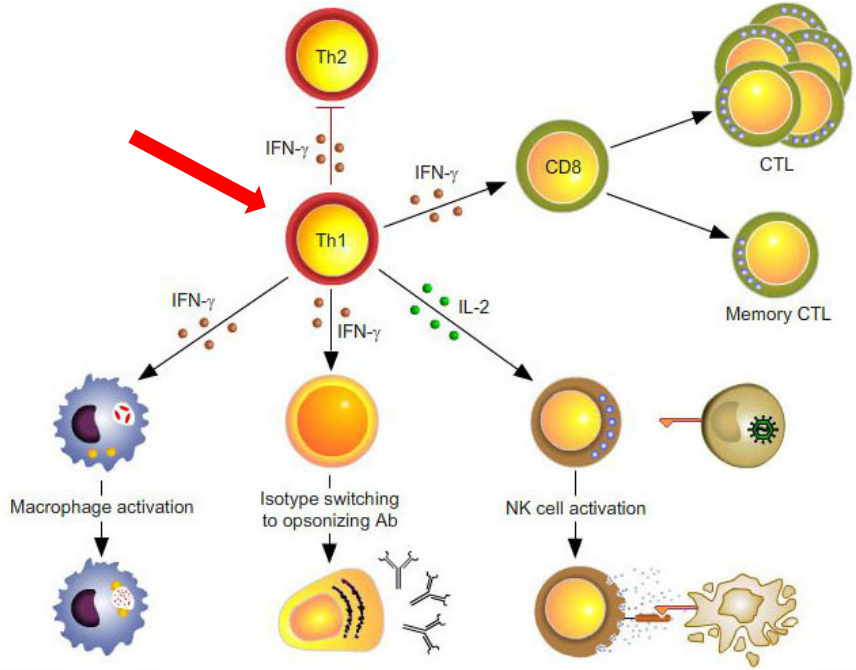


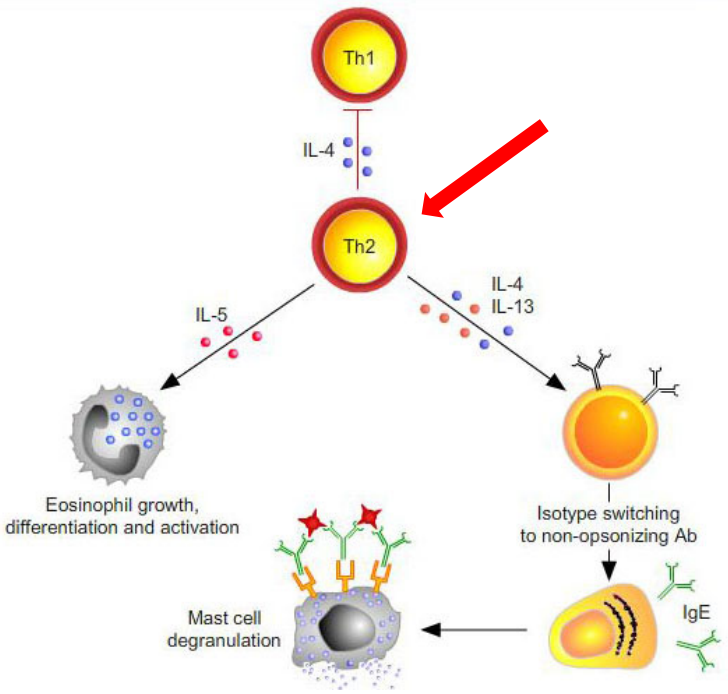
Figure reproduced with authorization of HSeT

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)





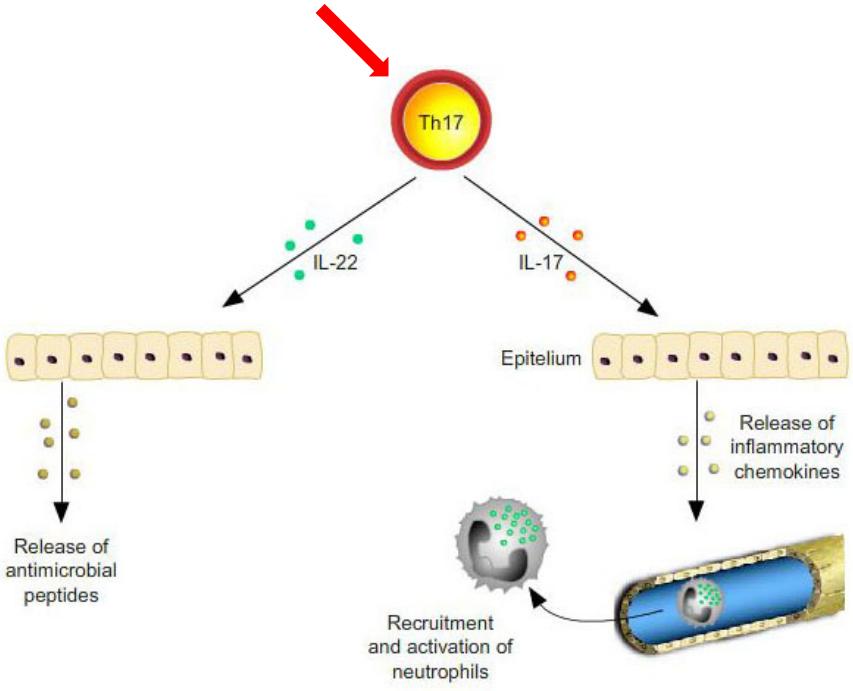
Th1 cells are required for defense against intracellular pathogens. They are characterized by the production of **IFN-γ** and **IL-2**. IFN-γ activates the microbicidal activity of macrophages, stimulates B cells to produce antibodies that are involved in the opsonization and phagocytosis of particulate 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

Figures reproduced with authorization of HSeT

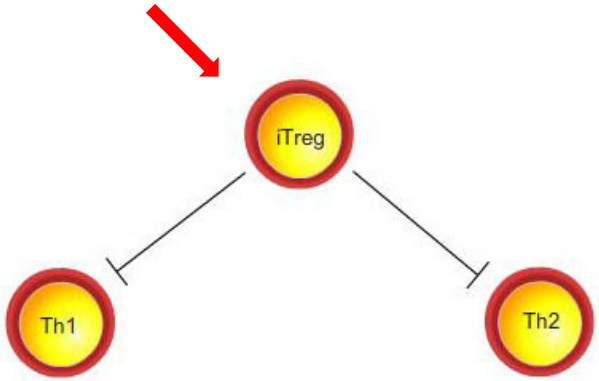
LYMPHOCYTES / IMMUNE RESPONSE (3)

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

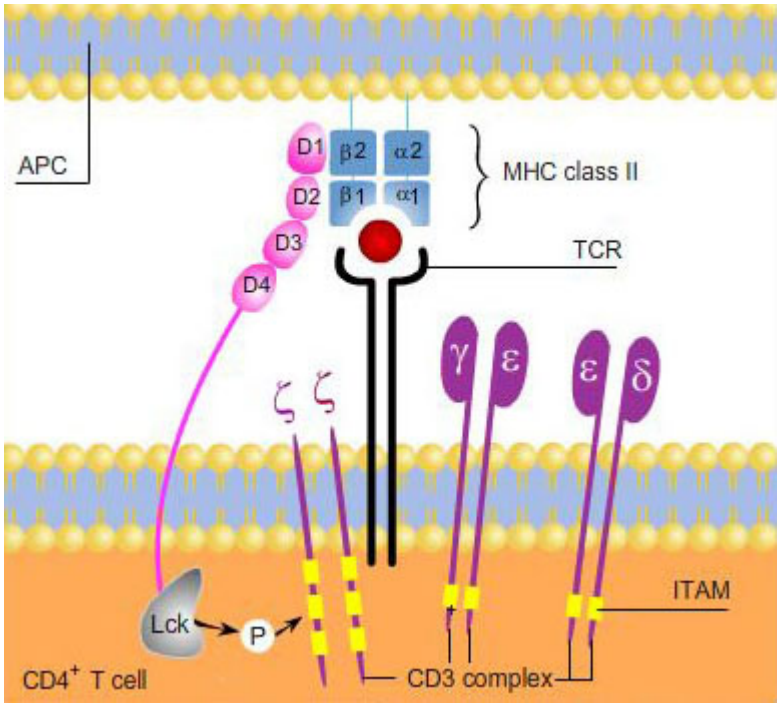
LYMPHOCYTES iTreg

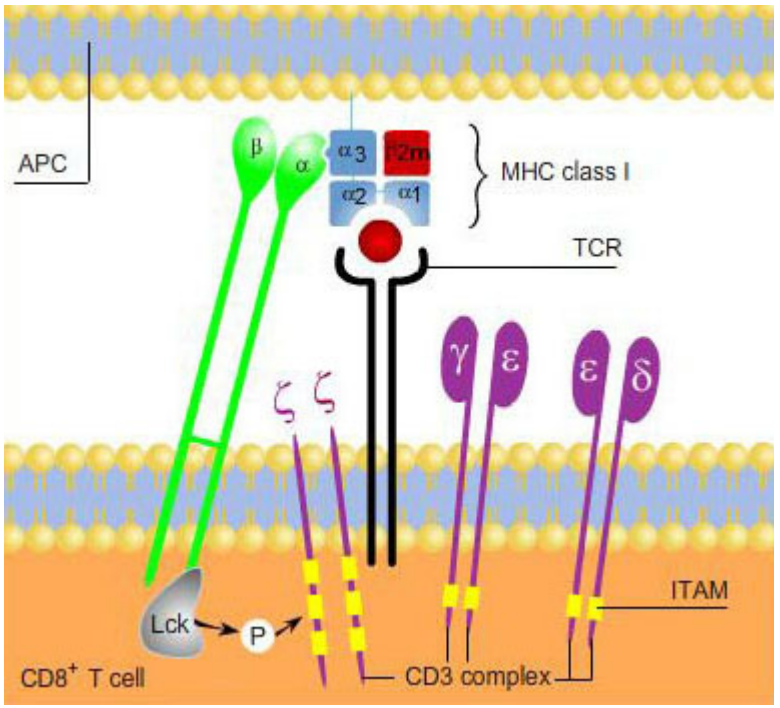


Induced **Treg cells** have functions in the suppression of Th1 and Th2 cell immune responses. Whether Treg cells also suppress Th17 cell responses is less clear

Figures reproduced with authorization of HSeT

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 β2 domain of MHC class II

CD8 is a dimer (either homodimer α or heterodimer αβ) that interacts via its α chain with the α3 domain of MHC class I

APC : Antigen Presenting Cell

Figures reproduced with authorization of HSeT

LYMPHOCYTOSIS / LYMPHOPENIA

LYMPHOCYTOSIS

RELATIVE : **> 40%**

ABSOLUTE : **> 4.0 G / L**

REACTIVE

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

Thyrotoxicosis

Hyposplenism

MALIGNANT

Lymphoid neoplasm

ABSOLUTE LYMPHOPENIA < 1.5 G / L

ACQUIRED

HIV, Hodgkin lymphoma, chemotherapy, radiotherapy, steroids, ATG (Anti-thymocyte 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

MYELOID NEOPLASMS (cf.p. 120-162)

LYMPHOID NEOPLASMS (cf.p. 163-204)

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
Waldenström Macroglobulinemia
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]
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 leukemia / lymphoma
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. 201-204)

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 FEATURES

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 (PV)
Chronic myelogenous leukemia (CML) *BCR-ABL 1* +
Essential thrombocythemia (ET)
Primary myelofibrosis
Chronic neutrophilic leukemia
Chronic eosinophilic leukemia, NOS[1]
Mastocytosis (cf.p. 137)
Myeloproliferative neoplasm, unclassifiable

[1] NOS : Not Otherwise Specified

POLYCYTHEMIA VERA (PV)

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[2] or other functionally similar mutation such as *JAK2* exon 12 mutation[3]
MINOR	B1	Bone marrow biopsy showing hypercellularity for age with trilineage growth (panmyelosis) with prominent erythroid, granulocytic and megakaryocytic hyperplasia
	B2	Endogenous erythropoietin serum level below the reference range for normal
	B3	Spontaneous 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

[2] ***JAK2*V617F exon 14 : 95-97%**
[3] ***JAK2* exon 12 : about 3%**

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.p. 132)
- Transformation in myelodysplastic syndrome or acute leukemia (> 10% after treatment with cytotoxic drugs)

PROGNOSIS

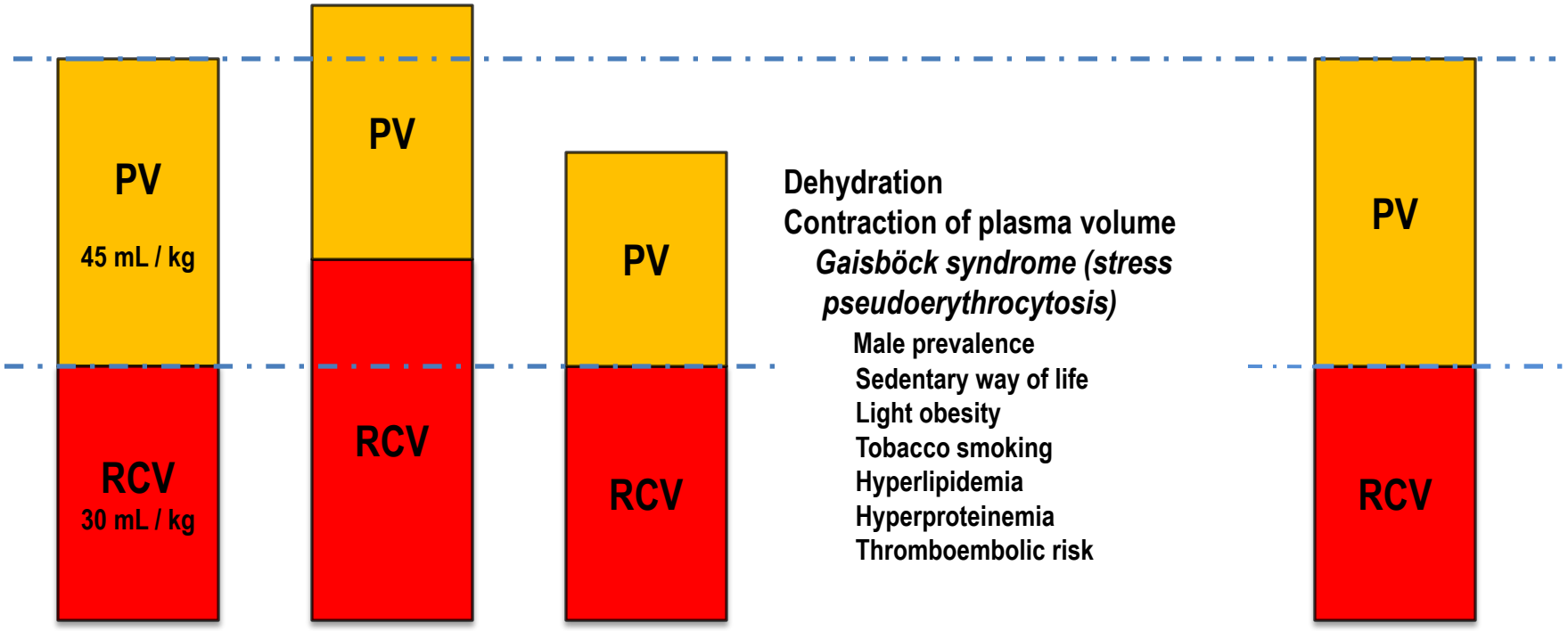
- Median survival : > 10 years

TREATMENT *(Targets : hematocrit < 45%; platelets < 450 G / L)*

- Phlebotomies
- Hydroxyurea, Pipobroman, α-Interferon, pegylated α-Interferon
- Aspirin
- ^{32}P : age > 70 years in case of insufficient compliance of the patient *(increased risk of leukemic transformation !)*
- *Investigational : JAK2 ± specific tyrosine kinase inhibitors (TKI)*

DIFFERENTIAL DIAGNOSIS OF ERYTHROCYTOSIS

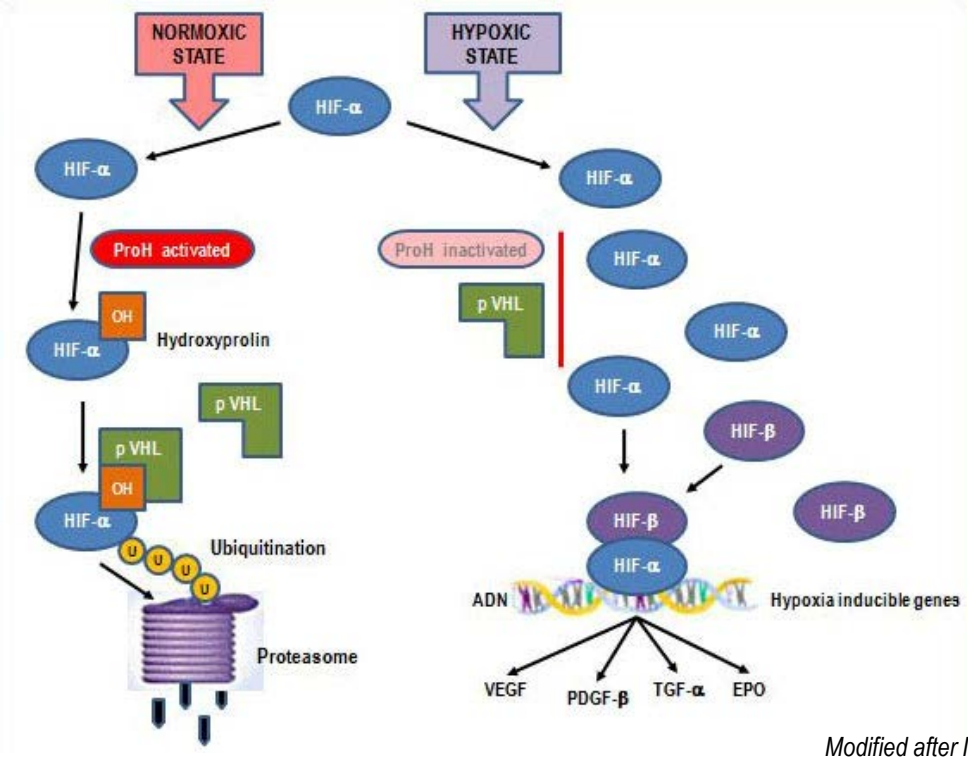
RBC VOLUME AND PLASMA VOLUME



PV : PLASMA VOLUME
RCV : RED CELL VOLUME

DIFFERENTIAL DIAGNOSIS OF TRUE ERYTHROCYTOSIS

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 and 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)

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)

PROGNOSTIC SCORES

The efficacy of TK inhibitors, as primary treatment of choice, has reduced the interest for the prognostic Sokal[1] (1984) or Hasford[1] (1998) scores, validated for chemotherapy treatment
A new score (EUTOS[2]) might be a prognostic tool to assess the probability of reaching complete cytogenetic remission. Its validation needs confirmation

[1] See : www.leukemia-net.org/content/leukemias/cml/cml_score
[2] See : www.leukemia-net.org/content/leukemias/cml/eutos_score

CYTOGENETICS

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

[2] *Hasford J. et al.. : Predicting complete cytogenetic response and subsequent progression-free survival in 2060 patients with CML on imatinib treatment : The EUTOS score. Blood 2011; 118 (3) : 686-692.*

CHRONIC MYELOGENOUS LEUKEMIA (CML) (2)

COURSE IN 3 PHASES

CHRONIC **4-5 years**

ACCELERATION[1] **< 6-8 months**

- **Blasts** 10-19% *(blood and / or nucleated bone marrow cells)*
- **Basophils** ≥ 20% *(blood)*
- **Thrombopenia** < 100 G / L *(treatment independant)*
- **Clonal genetic evolution**
- **Thrombocytosis** > 1'000 G / L *(unresponsive to treatment)*
- **Increasing splenomegaly and leukocytosis** *(unresponsive to treatment)*

TRANSFORMATION

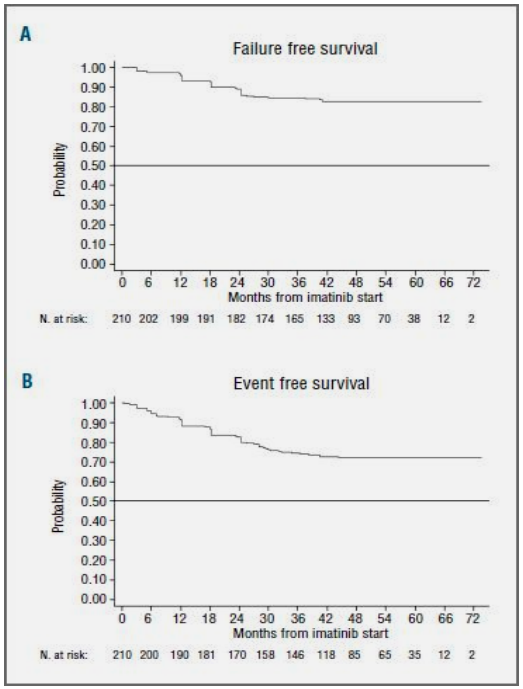
- **Blasts :** ≥ 20% *(blood and / or nucleated bone marrow cells)*
- **Extramedullary blast cell proliferation**

[1]*Modified from Vardiman J.W., Harris N.L., Brunning R.D.: The World Health Organization (WHO) classification of the myeloid neoplasms. Blood 2002; 100 : 2292-2302.*

PROGNOSIS

Depends on :

- **Clinical stage**
- **Prognostic factors**
- **Response to tyrosine kinase inhibitors**



Actuarial curves of relapse free survival (A) and event free survival (B), including failure and withdrawal of Imatinib (all causes included)

Cervantes F & al., Haematologica 2010; 95 :1317-1324.

CHRONIC MYELOGENOUS LEUKEMIA (CML) (3)

TREATMENT

Tyrosine kinase inhibitors (TKI)

↘ proliferation and apoptosis induction of the *BCR-ABL 1* + cell lineages

Possible TKI resistance due to different mutations

Efficacy (+ / -) of TKI in presence of the main mutations

Mutation	Imatinib (*Glivec®*)	Dasatinib (*Sprycel®*)	Nilotinib (*Tasigna®*)
T315I	–	–	–
Y253H	–	+	–
F317L	±	±	+

Hydroxyurea (HU)

α-Interferon (α-IFN), pegylated α-Interferon

Allogeneic hemopoietic stem cell / bone marrow transplantation : only established curative treatment (in case of TKI resistance, in acceleration and transformation phases)

Investigational : farnesyltransferase inhibitors, Decitabine, Cladribine, Isotretinoid, Homoharringtonine, antisense oligonucleotides, immunotherapy

AGE BASED THERAPEUTIC SELECTION

< 60 years : in case of insufficient response to TK inhibitor allogeneic hemopoietic stem cell / bone marrow transplantation. 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 : Imatinib, α-Interferon (+ Cytarabine), Hydroxyurea

[1] GVL : Graft-Versus-Leukemia

ESSENTIAL THROMBOCYTHEMIA (ET)

SYMPTOMS AND CLINICAL FEATURES

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

DIAGNOSTIC CRITERIA

1	Sustained platelet count ≥ 450 G / L[1]
2	*JAK2*V617F[2] mutation present or other clonal marker[3]
3	Exclusion of : PV[4], primary myelofibrosis[5], *BCR-ABL1* positive CML[6], myelodysplastic syndrome[7] or other myeloid neoplasm
4	Exclusion of secondary thrombocytosis[8], normal iron stores
5	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

DIAGNOSIS REQUIRES CRITERIA 1 + 2 + 3 or 1 + 3 - 5

[1] Sustained during the work-up process

[2] Approximately 50% of cases

[3] i.e. *MPLW515L, W515K : 1-4%*

[4] 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

[5] 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)*

[6] Absence of *BCR-ABL 1*

[7] Absence of dyserythropoiesis and dysgranulopoiesis

[8] Exclusion of secondary thrombocytosis (cf.p. 133)
(The presence of a condition associated with secondary thrombocytosis may not exclude the diagnosis of ET if the first 3 criteria are met)

Beer P.A., Green A.R. : Hematology Am Soc Hematol Educ Program 2009, p. 621-628.

ESSENTIAL THROMBOCYTHEMIA (2)

POSSIBLE COURSE

Polycythemia Vera
Myelofibrosis (cf.p. 132)
Acute leukemia (3-10%)

TREATMENT

Hydroxyurea
Pipobroman
α-IFN, pegylated α-IFN
Anagrelide (could potentially favor evolution to myelofibrosis)
Aspirin (platelet antiaggregant)

MEDIAN SURVIVAL

Depending on the risk factors[1]

Age ≥ 60 years and leukocytes ≥ 15 G / L : 10 years

Age ≥ 60 years or leukocytes ≥ 15 G / L : 17 years

Age < 60 years and leukocytes < 15 G / L : 25 years

[1] *Wolanskyj A.P., Schwanger S.M., McClure R.F., Larson D.R. , Tefferi A.: Essential Thrombocythemia Beyond the First Decade : Life Expectancy, Long-term Complication Rates, and Prognostic Factors. Mayo Clin Proc 2006; 81 : 159-166.*

ESSENTIAL THROMBOCYTHEMIA (3)

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). cf.p. 135
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 > 5 cm 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 : weight loss > 10% in 6 months, night sweats, unexplained fever (> 37.5°C)

[1] Below 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.p. 121-137)*

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

Myelodysplastic syndrome *(cf.p. 139-147)*

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
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 *JAK2*V617F[7] mutation or other clonal marker *(e.g. MPL* W515K/L[8]*)* 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]**

DIAGNOSIS : ALL 3 MAJOR + 2 MINOR CRITERIA

[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

[7] Approximately 50% of cases

[8] < 5% of cases

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

[2] *Risk factors:*
1) *Hb < 100 g / L*
2) *Leukocytes < 4 G / L or > 30 G / L*

LILLE[1] PROGNOSTIC SCORE

Risk group	Factors[2] (n)	% of patients	Median survival (months)
Low	0	47	93
Intermediate	1	45	26
High	2	8	13

COMPLICATIONS

Splenic infarction
Infections (neutropenia)
Bleeding (thrombocytopenia and / or platelet anomalies)
Acute leukemia (5-30%)

TREATMENT

Wait and watch
Hydroxyurea
Transfusion support
Sectorial splenic radiotherapy
Splenectomy
Allogeneic bone marrow transplantation with non myeloablative conditioning
Investigational : *pegylated α-Interferon; Thalidomide (± prednisone), Lenalidomide (± Prednisone), Pomalidomide (immunomodulators); Etanecerpt (TNF-α inhibitor)*

[1] *Dupriez B. et coll. : Prognostic factors in agnogenic myeloid metaplasia : a report of 195 cases with a new scoring system. Blood 1996; 88 : 1013-1018.*

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

If these criteria are not met, the diagnosis may be reactive eosinophilia, idiopathic hypereosinophilia or idiopathic hypereosinophilic syndrome (HES) (cf.p. 101)

[1]NOS : Not Otherwise Specified

MASTOCYTOSIS

CLASSIFICATION

Cutaneous mastocytosis *(urticaria pigmentosa)*, diffuse cutaneous mastocytosis, solitary cutaneous mastocytosis
Systemic mastocytosis *(indolent or aggressive)*
Mastocytic leukemia
Mastocytic sarcoma
Extracutaneous mastocytoma

SYSTEMIC MASTOCYTOSIS

Clonal mastocyte proliferation (tissue basophils) with secretion of tissular mediators :
Histamine, heparin, leukotrienes, prostaglandins, PAF (Platelet Activating Factor), Cytokines (TNF)

Target organs :
- Bone marrow
- Lymph nodes
- Spleen, liver
- Heart
- Presence of cutaneous localisation or not
- Osteoblastic bone lesions, less frequently osteolytic

Biochemistry :	⇗ of serum tryptase
Immunophenotype :	CD9, CD33, CD45, CD68, CD117, CD2 or CD2/CD25
Cytogenetics :	Frequent KIT mutation (Asp816Val)

Symptoms :
- Cutaneous flash, pruritus
- Abdominal pain
- Bronchospasm

Evolution :
- Indolent forms
- Aggressive forms
  - Initially
  - Mastocytosis associated with myeloid or lymphoid neoplasia
  - Mastocytic leukemia

Treatment : Antihistamines, α-Interferon, tyrosine kinase inhibitors, anti-leukotrienes

Survival :
- Nearly normal for indolent forms
- Few months for aggressive forms

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)(q33;p13) or variant translocation. Demonstration of *ETV6-PDGFRB* fusion gene or of rearragement of *PDGFRB*
Hematological features : chronic myelomonocytic leukemia with / without eosinophilia, chronic eosinophilic leukemia, Ph-neg. chronic myelogenous leukemia with eosinophilia, primary myelofibrosis, juvenile myelomonocytic leukemia with eosinophilia, acute myelogenous leukemia, chronic basophilic leukemia	

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	**+**

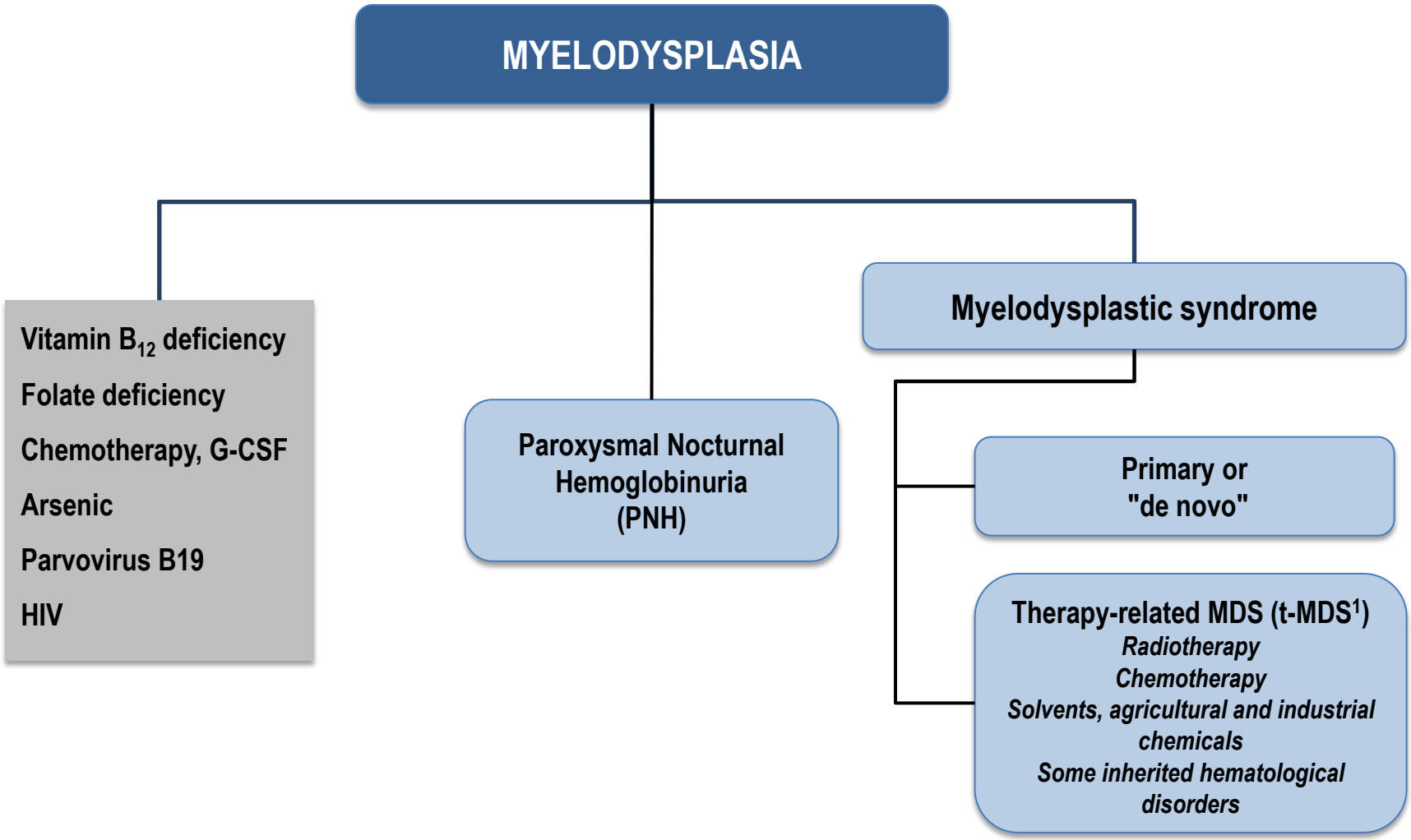
Peripheral blood with 1-3 cytopenia(s)

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 > 1.0 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	PERIPHERAL BLOOD	BONE MARROW
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		AML

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.

AML : Acute Myeloid Leukemia

MDS : Myelodysplastic Syndrome

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

ACQUIRED HEMOGLOBINOPATHY

α-Thalassemia Myelodysplastic Syndrome (ATMDS)

MYELODYSPLASTIC SYNDROME
PROGNOSTIC SCORES

Prognostic scores evaluate the risk of leukemic transformation

IPSS (International Prognostic Scoring System)

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		

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

[1] Blasts in bone marrow [2] This percentage is now considered 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

WPSS (WHO classification-based Prognostic Scoring System)

Variables	0	1	2	3
WHO category	RA, RARS, 5q-	RCMD, RCMD-RS	RAEB-1	RAEB-2
Karyotype	Favorable	Intermediate	Unfavorable	–
Transfusion requirement	∅	Regular[1]	–	–

Risk groups	Score
Very low	0
Low	1.0
Intermediate	2.0
High	3.0 - 4.0
Very high	5.0 - 6.0

[1] At least one RBC transfusion every 8 weeks over a 4 months period

MYELODYSPLASTIC SYNDROMES
UNFAVORABLE PROGNOSTIC FACTORS

Age > 60 years	⇗ Serum β_2-microglobulin
Performance status / comorbidities	Mutations of FLT3 gene
White blood cells > 20 G / L	Absence of TET2 mutation
Lymphocytes < 1.2 G / L	Monosomy 5 or del(5q) + other chromosomal anomaly
Severe anemia	⇗ TNF-α level
Refractory thrombocytopenia	Transfusion dependency
High percentage of CD 34 expressing precursor cells	Bone marrow fibrosis
MCV < 100 fL	Low level of circulating endothelial cells
Increased expression of WT1 (Wilms tumor gene)	Presence of ALIPs *(Abnormal Localization of Immature Precursors)* on BM histology

MYELODYSPLASTIC SYNDROMES
COMPLICATIONS / COURSE / SURVIVAL

COMPLICATIONS

Recurrent infection
Bleeding episodes
Immunologic disorders

5 YEAR CUMULATIVE RISK OF TRANSFORMATION IN ACUTE LEUKEMIA[1]

RA, RARS :	**< 2%**
RCMD, 5q- syndrome :	**~ 10%**
RAEB-1 :	**11%**
RAEB-2 :	**40%**

RA : Refractory anemia
RARS : Refractory Anemia with Ring Sideroblasts
RCMD : Refractory Cytopenia with Multilineage Dysplasia
RAEB : Refractory Anemia with Excess Blasts

SURVIVAL RELATED TO PROGNOSTIC SCORES

IPSS[2]	**Score 0 :**	**5.7 years**
	Score 0.5-1.0 :	**3.5 years**
	Score 1.5-2.0 :	**1.2 years**
	Score ≥ 2.5 :	**0.4 year**

WPSS[3]	**Score 0 :**	**8.5 years**
	Score 1.0 :	**6.0 years**
	Score 2.0 :	**3.5 years**
	Score 3.0-4.0 :	**1.7 years**
	Score 5.0-6.0 :	**0.1 year**

[1] *Germing U., Strupp C., Kuendgen A., Isa S., Knipp S., Hildebrandt B., Giaconidis A., Aul C., Gattermann N., Haas R.: Prospective validation of the WHO proposals for the classification of myelodysplastic syndromes. Haematologica 2006; 91 : 1596-1604.*

[2] *Estey E.H., Schrier S.L. : Prognosis of the Myelodysplastic Syndromes in adults; January 2012, UpToDate.*

[3] *Malcovati L. & al. : Time dependant Prognostic Scoring System for Predicting Survival and Leukemic Evolution in MDS. J Clin Oncol 2007; 25: 3503-3510.*

TREATMENT OF MYELODYSPLASTIC SYNDROME

SYMPTOMATIC TREATMENT

Transfusional supportive care (RBC, platelets)
Iron chelators (oral or parenteral application)
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, possibly with reduced intensity conditioning)

Investigational[1] : **TNF-α inhibitors (Etanercept)**
Clofarabine (nucleosidic analogue of adenosine)
Arsenic trioxide
Histone deacetylase inhibitors (valproic acid)
Farnesyltransferase inhibitors
Thrombopoietin analogues (Romiplostim)

[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
REFRACTORY ANEMIA WITH RING SIDEROBLASTS (RARS) ASSOCIATED WITH THROMBOCYTOSIS
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 (cf.p. 104)

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

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, monoblastic or monocytic leukemia

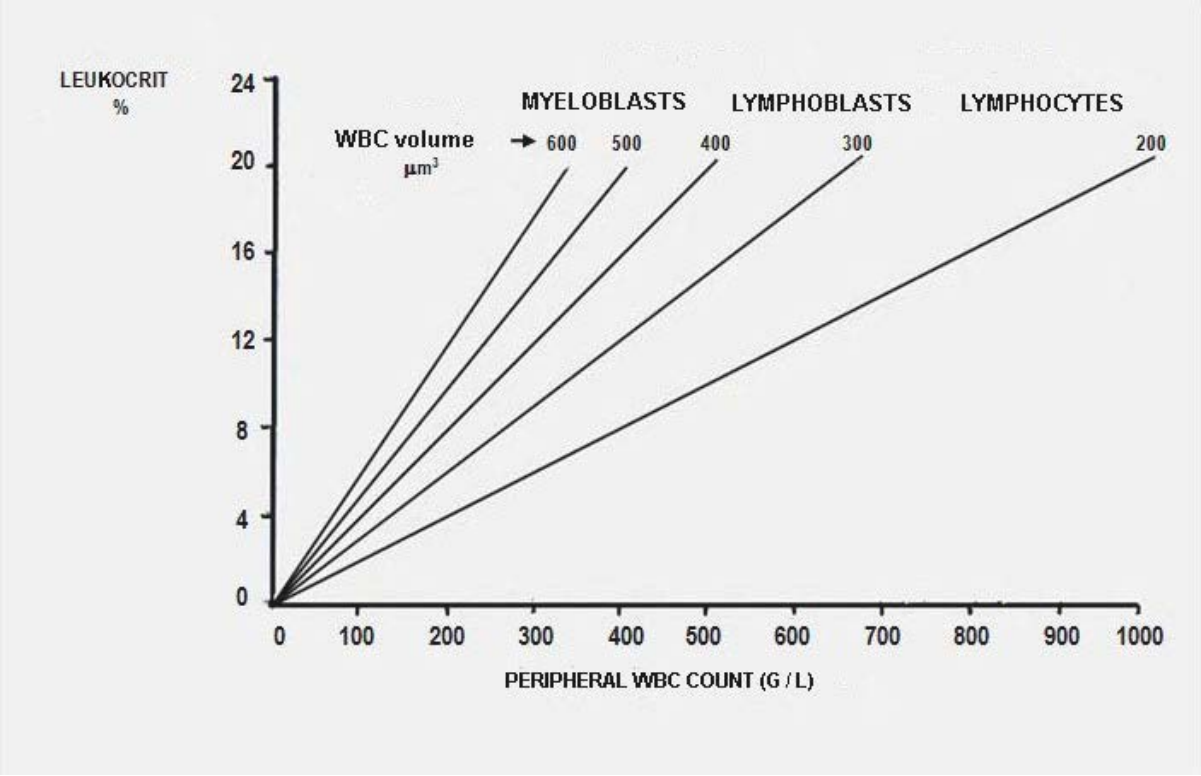
CLINICAL FEATURES OF ACUTE MYELOID LEUKEMIA (2)

DISSEMINATED INTRAVASCULAR COAGULATION : DIC

Mainly acute promyelocytic leukemia with t(15;17)(q22;q21); *PML-RARA*

LEUKOSTASIS

Mainly acute myelomonocytic, monoblastic or 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 or rare 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

CRITERIA

CYTOLOGY - CYTOCHEMISTRY - IMMUNOPHENOTYPING - CYTOGENETICS - MOLECULAR BIOLOGY

CLASSIFICATION

ACUTE MYELOID LEUKEMIA WITH RECURRENT CYTOGENETIC ABNORMALITIES

Cytogenetics	Rearrangement	Hematological features
t(8;21)(q22;q22)	***RUNX1-RUNX1T1***	AML generally with neutrophil lineage maturation
inv(16)(p13.1q22) ou t(16;16)(p13.1;q22)	***CBFB-MYH11***	Myelomonocytic AML with abnormal bone marrow eosinophils
t(15;17)(q22;q21)	***PML-RARA***	Acute promyelocytic leukemia and microgranular variant
t(9;11)(p22;q23)	***MLLT3-MLL***	AML usually associated with monocytic differentiation
t(6;9)(p22;q34)	***DEK-NUP214***	AML frequently with basophilia, multilineage dysplasia ± monocytosis
inv(3)(q21q26.2) or t(3;3)(q21;q26.2)	***RPN1-MECOM***	AML with often normal or ⇗ platelet count in peripheral blood; ⇗ of atypical megakaryocytes in the bone marrow; multilineage dysplasia
t(1;22)(p13;q13)	***RBM15-MKL1***	Peripheral blood and bone marrow similar to the acute megakaryoblastic leukemia NOS[1], cf.p. 156
Provisional entities : AML with NPM1 or CEBPA mutations (cf.p. 157)		

[1]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.p. 155-156
- 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

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 → neutrophil ≥ 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

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

ACUTE MYELOID LEUKEMIA, NOS (2)

With monoblastic or monocytic differentiation :	***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 +**
With erythroblastic differentiation :	***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 or without differentiation)** ***Pure erythroid leukemia :*** **≥ 80% of dysplastic erythroid precursors (basophilia, vacuoles, PAS +, glycophorin +), without myeloblastic component**
With megakaryoblastic differentiation :	**Blasts ≥ 20% of NMC; ≥ 5% 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

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, t(6;9), 3q26, 11q23 aberrations [except t(9;11) (p21;q23)]
Molecular genetic alterations	Mutations	*NPM1*[3] ,*CEBPA*[4]	*FLT3*-ITD[5], *MLL*-PTD[6], *WT1*[7], *c-KIT* (CD117) *NPM1 + FLT3*
	Overexpression	Apoptosis promoters (bax, ⇗ bax / BCL2 ratio)	*EVI1*[8] *BAALC*[9], Apoptosis inhibitors (BCL2) *ERG*[10], *MN1*[11]

[1] Karnofsky Index : patient performance index, cf. next page; [2] MDR : Multidrug Resistance; [3] *NPM1 :* Nucleophosmine, member 1; [4] *CEBPA* : CCAAT / Enhancer Binding Protein α ; [5] *FLT3*-ITD : Fms-Like tyrosine Kinase 3-Internal Tandem Duplication (Tyrosine kinase receptor); [6] *MLL*-PTD : Myeloid / Lymphoid or Mixed Lineage Leukemia-Partial Tandem Duplication; [7] *WT1* : Wilms' Tumor 1; [8] *EVI1* : Ecotropic Viral Integration site 1; [9] *BAALC* : Brain and Acute Leukemia, Cytoplasmic; [10] *ERG* : ETS (Erythroblast Transformation Specific)-Related Gene; [11] *MN1* : Meningioma 1

Modified from Schiffer C.A. : Prognosis of acute myeloid leukemia; Jamuary 2012, UpToDate.

KARNOFSKY PERFORMANCE STATUS

LEVEL OF PERFORMANCE	%	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

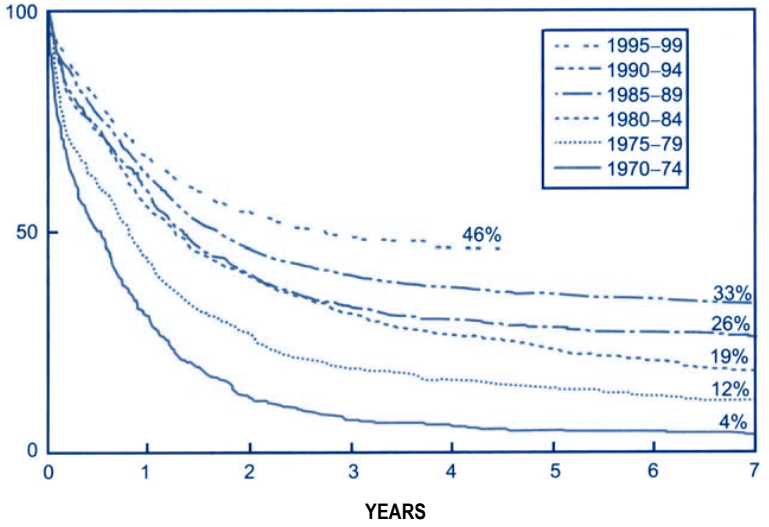
TREATMENT OF INFECTION
TRANSFUSION SUPPORT (RBC, platelets)

CHEMOTHERAPY

INDUCTION
CONSOLIDATION
INTENSIFICATION

HEMOPOIETIC STEM CELL / BONE MARROW TRANSPLANTATION

ALLOGENEIC ($\rightarrow$ 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

CYTARABINE + ANTHRACYCLIN (Daunorubicin, Idarubicin) : "7 + 3"

CYTARABINE + MITOXANTRONE

TAD (6-Thioguanine + Cytarabine + Daunorubicin); Etoposide

Complete remission rate (after 1st or 2nd induction cycle), survival rate after consolidation and intensification : highly variable in relation with presence of main adverse risk factors or not :

- **Age > 60 years**
- **Low perfomance index**
- **Adverse cytogenetic and / or molecular anomalies**
- **History of chemotherapy or radiation exposition**
- **History of myelodysplasia or other hematological disorder**

Improvement of survival after autologous or allogeneic hematopoietic stem cell transplantation (with reduced intensity conditioning for patients over 60)

- **Relapse free 5 year survival rate (allogeneic HLA-identical donor) : 18-59%**

ATRA (all-trans retinoic acid) + Cytarabine and Anthracyclin :

Acute promyelocytic leukemia t(15;17)(q22;q21); *PML-RARA*

TREATMENT OF RELAPSE[1]

Fludarabine, Decitabine, Clofarabine, inhibitors of farnesyltransferase (Tipifarnib), of MDR1[2], BCL2[3], FLT3[4], tyrosine kinase (by c-KIT mutation), antiangiogenic drugs (anti-VEGF : Bevacizumab), anti-CD33 (Gemtuzumab, Lintuzumab), Arsenic trioxide for acute promyelocytic leukemia

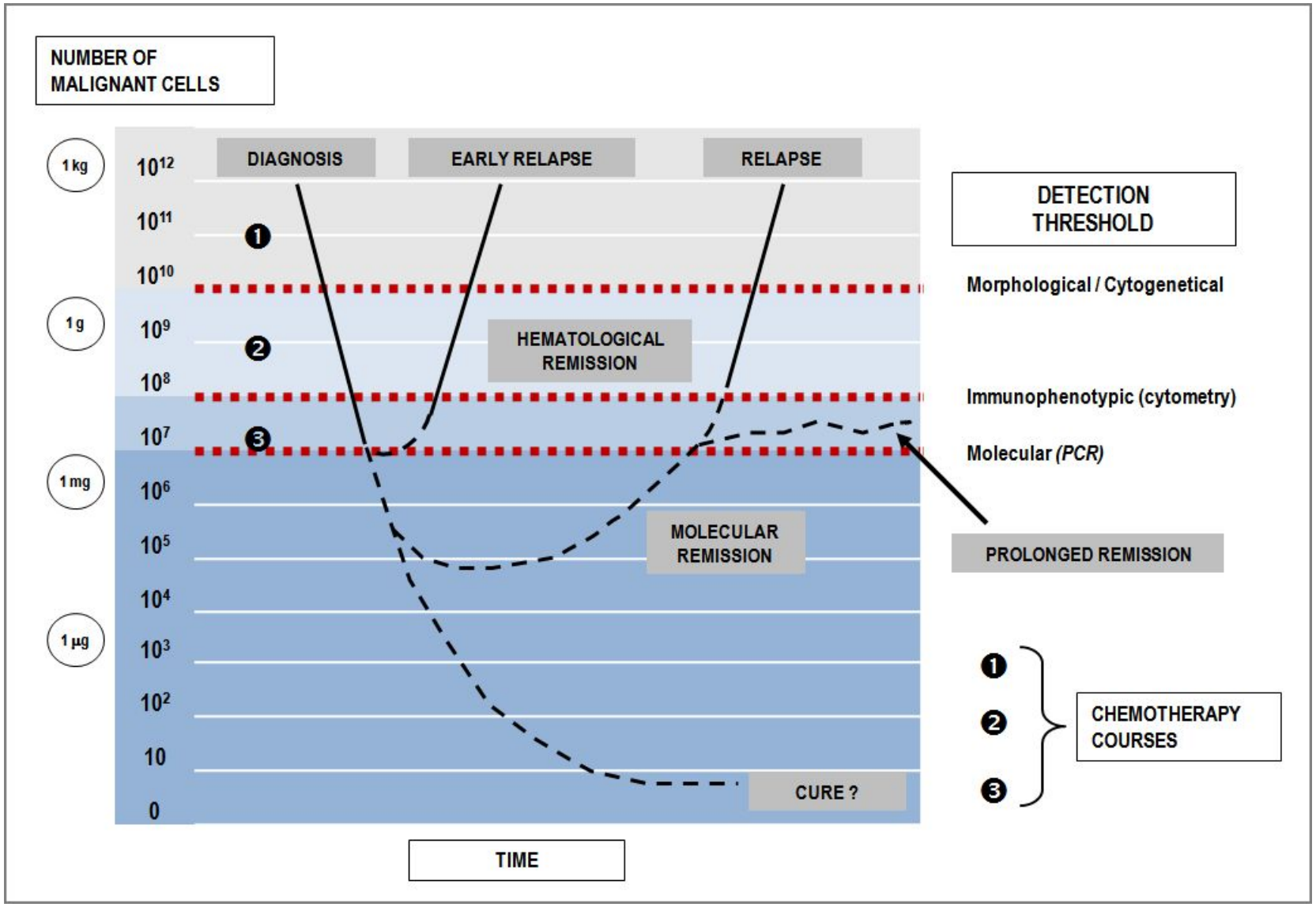
[1] Most mentioned new drugs (apart from arsenic trioxide) are still on clinical trials

[2] MDR : Multidrug Resistance

[3] BCL2 : B-Cell Leukemia / Lymphoma 2 (protooncogene, inhibitor of apoptosis)

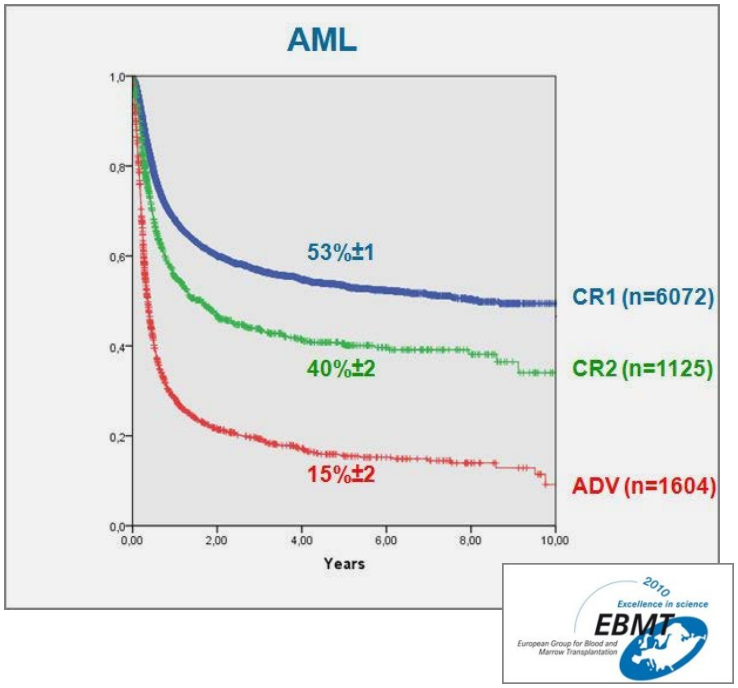
[4] FLT3 : Fms-Like tyrosine Kinase 3 (tyrosine Kinase receptor)

KINETICS OF LEUKEMIC CELLS UNDER TREATMENT

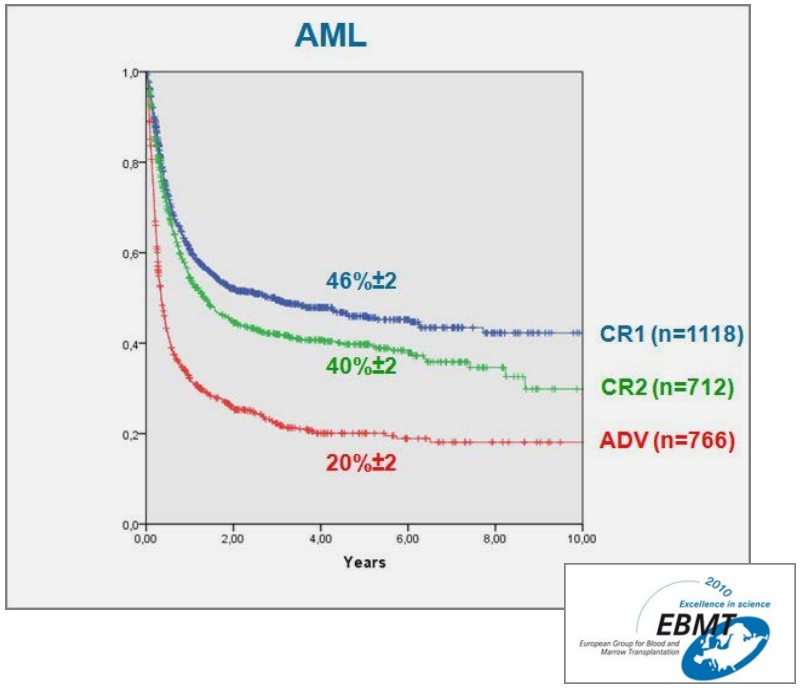


ACUTE MYELOID LEUKEMIA : ALLOGENEIC TRANSPLANTATION

ADULTS TRANPLANTED BETWEEN 1999 AND 2009
ALLOGENEIC TRANSPLANT
HLA COMPATIBLE SIBLING DONOR



ADULTS TRANSPLANTED BETWEEN 1999 AND 2009
ALLOGENEIC TRANSPLANT
UNRELATED HLA COMPATIBLE DONOR



Modified from EBMT Registry 2010 European Group for Blood and Marrow Transplantation.

LYMPHOID NEOPLASMS[1]
(WHO 2008)

PRECURSOR B-CELL OR T-CELL NEOPLASMS

B-cell lymphoblastic leukemia / lymphoma
T-cell lymphoblastic leukemia / lymphoma

MATURE B-CELL NEOPLASMS (cf.p. 174-194)

MATURE T-CELL AND NK-CELL NEOPLASMS (cf.p. 195-199)

HODGKIN LYMPHOMA (cf.p. 201-204)

IMMUNODEFICIENCY-ASSOCIATED LYMPHOPROLIFERATIVE DISORDERS

[1] Former lymphoproliferative syndromes, malignant lymphomas

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	EXTENSION
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
(Spinal tap : CSF[1] examination)

Evaluation of prognosis :

Histological type (low grade vs. high grade malignancy)
IPI[2] score (aggressive lymphoid neoplasms) : *(1 pt. / criterion)* or aaIPI[3]
- Age ≤ 60 years vs. > 60 years
- Clinical condition (ECOG[4] score) 0 - 1 vs. ≥ 2
- Ann Arbor I-II vs. III-IV
- Extranodal involvement 0-1 vs. > 1 site
- 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

IPI SCORE	TX WITHOUT RITUXIMAB OVERALL SURVIVAL AT 5 YEARS (%)	TX WITH RITUXIMAB OVERALL SURVIVAL AT 3 YEARS (%)
0 - 1	73	91
2	51	81
3	43	65
4 - 5	26	59

aaIPI SCORE	≤ 60 YEARS OLD OVERALL SURVIVAL AT 5 YEARS (%)	> 60 YEARS OLD OVERALL SURVIVAL AT 5 YEARS (%)
0	83	56
1	69	44
2	46	37
3	32	21

Modified from Freedman A.S .& Friedberg J.V. : Evaluation, staging and prognosis of non-Hodgkin lymphoma.; January 2012, UpToDate.

[1] CSF : Cerebrospinal fluid [2] IPI : International Prognostic Index [3] aaIPI : (age adjusted IPI), 3 prognostic factors : ECOG + Ann Arbor + LDH

[4] 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], ACVP[5], CHOP[1] + Rituximab (anti-CD20)
Intensification + autologous transplant

Overall 5 years survival about 30-40% (dependant on IPI score, cf. previous page)

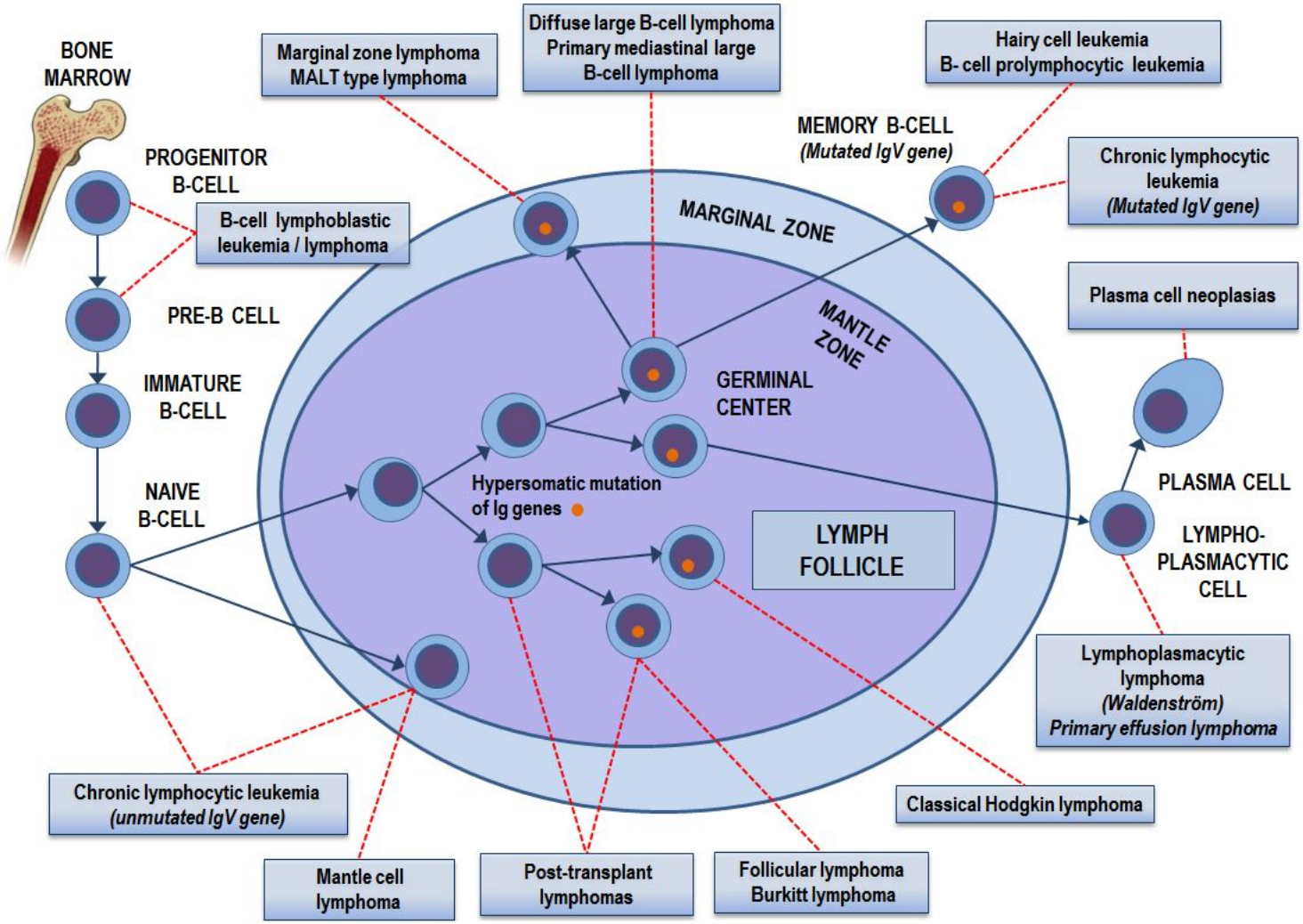
INDOLENT LYMPHOID NEOPLASM (e.g. follicular lymphoma grade 1-2)

Radiation therapy, α-Interferon, purine analogues (Fludarabine, Cladribine), monoclonal antibodies :
Rituximab (Mabthera®) alone or in combination, radioimmunoconjugates : Ibritumomab (Zevalin®), CVP[6], CHOP[1]

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] ACVP : Doxorubicin + Cyclophosphamide + Vincristine + Prednisone
[6] CVP : Cyclophosphamide + Vincristine + Prednisone

B-CELL DIFFERENTIATION
RELATIONSHIP TO MAJOR B-CELL NEOPLASMS



PRECURSOR B OR T-CELL LYMPHOID NEOPLASMS

LYMPHOBLASTIC LEUKEMIA / LYMPHOMA

B-cell lymphoblastic leukemia / lymphoma, NOS[1] (B-ALL / B-LL)

B-cell lymphoblastic leukemia / lymphoma with recurrent genetic anomalies

T-cell lymphoblastic leukemia / lymphoma

[1] NOS : Not Otherwise Specified

B-CELL LYMPHOBLASTIC LEUKEMIA / LYMPHOMA, NOS

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 very high

B LYMPHOBLASTIC LYMPHOMA (B-LBL)

Most frequent sites of involvement

- Skin
- Soft tissues
- Bone marrow
- Lymph nodes

B-CELL LYMPHOBLASTIC LEUKEMIA / LYMPHOMA WITH RECURRENT GENETIC ANOMALIES

CYTOGENETICS	FUSION TRANSCRIPT	PROGNOSIS
t(9;22)(q34;q11.2)	*BCR-ABL 1*	very poor
t(v;11q23)	*MLL rearranged*	poor
Hypodiploidy (< 46 chromosomes)		poor
t(1;19)(q23;p13.3)	*TCF3-PBX1*	intermediate
t(5;14)(q31;q32)	*IL3-IGH*	intermediate
t(12;21)(p13;q22)	*ETV6-RUNX1*	good[1]
Hyperdiploidy (51-65 chromosomes)		good[1]

[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 involvement at diagnosis

T-CELL 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 adverse prognostic cytogenetic anomalies

LYMPHOBLASTIC LEUKEMIA / LYMPHOMA

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 (type Burkitt ALL) cf.p. 185

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 - ANTHRACYCLINS - MITOXANTRONE - ASPARAGINASE

PRINCIPLES : Induction - Consolidation - Maintenance

RESULTS : Adults[1] (1991-2002) : CR* : 64-93%

DFS** : 20-42% (at 5 years)

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 patients with unfavorable risk factors, early autologous transplantation with peripheral blood progenitor cells

Nucleosidic analogues (Clofarabine, Nelarabine), FMdC (ribonucleotide reductase inhibitor), Trimetrexate (dihydrofolate reductase inhibitor, liposomal Vincristine, Flavopiridol (Cyclin-Dependent Kinase (CDK) inhibitor), 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 Philadelphia chromosome positive acute lymphoblastic leukemia in adults; January 2012, UpToDate.

[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.

MATURE B-CELL LYMPHOID NEOPLASMS

CHRONIC LYMPHOCYTIC LEUKEMIA (CLL)

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; January 2012, UpToDate.

CHRONIC LYMPHOCYTIC LEUKEMIA (3)

COURSE AND COMPLICATIONS

Infection secondary to :

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

Autoimmune manifestation[1]

- **Hemolytic anemia with positive direct Coombs test (advanced stage : 11%)**
- **Immune thrombocytopenia (early stage : 2-3%)**
- **Pure red cell aplasia / Erythroblastopenia (early stage : 6%)**

Prolymphocytoid transformation (~ 10%)

Transformation to diffuse large B-cell lymphoma (DLBCL) : Richter syndrome (1-10%)

⇗ risk of developing another neoplasm : bone, skin, thyroid, ENT region, lung

DIFFERENTIAL DIAGNOSIS

Viral or bacterial lymphocytosis (cf.p. 115)

Other lymphoid neoplasm

[1] *Diehl L.F., Ketchum L.H.: Autoimmune disease and chronic lymphocytic leukemia : autoimmune hemolytic anemia, pure red cell aplasia, and autoimmune thrombocytopenia. Semin Oncol 1998; 25 : 80-97.*

CHRONIC LYMPHOCYTIC LEUKEMIA (4)
PROGNOSTIC FACTORS

PARAMETER	FAVORABLE	UNFAVORABLE
Bone marrow lymphocytic infiltration	Focal	Diffuse
Peripheral lymphocytosis doubling time	> 12 months	< 12 months
Immunophenotyping	CD38 -, ZAP-70 -[1]	CD38 +, ZAP 70 +, ⇗ CD20, ⇗ CD52
Conventional cytogenetics or FISH	Normal karyotype Del 13q14	del 11q23 Del 17p / 53 anomalies
IgV genes (variable region of immunoglobulins)	Mutated	Unmutated
Others		Dysfunction or ⇗ of p53 expression ⇗ TNF-α, β_2-microglobulin, IL-6, 8, 10, LDH, VEGFR-2[2]

[1] ZAP-70 : Zeta chain-Associated Protein : tyrosine kinase restricted to T- and NK-lymphocytes under normal physiological conditions
[2] Vascular Endothelial Growth Factor Receptor-2

Modified from Rai K.R., Keating M.J. : Staging and prognosis of chronic lymphocytic leukemia; January 2012, UpToDate.

CHRONIC LYMPHOCYTIC LEUKEMIA (5)
TREATMENT

"Wait and watch" as long as possible

Alkylating agents ***(Chlorambucil)***

Purine analogues ***(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)***

Steroids

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 : 44%)

Splenectomy (possibly splenic irradiation) : ***in case of very large painful spleen with severe cytopenias***

[1] **cf.p. 167**

PROLYMPHOCYTIC B-CELL LEUKEMIA (PLL-B)

Large splenomegaly, few or absent lymphadenopathies

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

Large cells with prominent nucleolus :

Treatment : CHOP (cf.p. 167), purine analogues (fludarabine, cladribine), chemotherapy + Rituximab, splenectomy

Median survival : 30-50 months

Immunophenotype :	CD19 +, CD20 +, CD22 +, CD23 + (10-20%), cCD79a +, CD79b +, FMC-7 +, CD5 + (20-30%)
Cytogenetics :	del 17p, mutations p53 (~ 50%), del 13q14 (~ 25%)

HAIRY CELL LEUKEMIA (HCL)

Splenomegaly without lymphadenopathies

Pancytopenia

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

Presence of tricholeukocytes, TRAP + (*Tartrate Resistant Alkaline Phosphatase*)

Bone marrow fibrosis

Complications : Recurrent infections
Vasculitis or other immune disease
Neurological disorders
Bleeding occurrence
Bone lesions

Immunophenotype :	CD19 +, CD11c +, CD22 +, CD25 +, CD103 +, CD123 +
Immunohistochemistry :	Annexin A1 +, Cyclin D1 ±

Treatment : Purine analogues (+ Rituximab), α-interferon, splenectomy, anti-CD22 immunotoxins, anti-CD25

Overall survival at 10 years : > 90%

SPLENIC B-CELL MARGINAL ZONE LYMPHOMA (SMZL)

Splenomegaly
Variable presence in peripheral blood of villous lymphocytes
Occasionally autoimmune thrombocytopenia or anemia
Small monoclonal serum paraprotein (1/3 of cases)
Clinical course indolent
Treatment : splenectomy

Immunophenotype : CD20 +, cCD79a +, CD5 -, CD25 + / -, CD11c + / -, CD103 -, CD123 - (~ 3% of cases +)

B-CELL LEUKEMIA / LYMPHOMA, UNCLASSIFIABLE

Splenic diffuse red pulp small B-cell lymphoma

Frequently massive splenomegaly
Usually low lymphocytosis, presence of villous lymphocytes
Sometimes cutaneous infiltration (pruritic papules)
Indolent lymphoma, not curable; beneficial effect of splenectomy

Immunophenotype : CD20 +, CD25 -, CD5 -, CD103 -, CD123 -, CD11c -, CD10 -, CD23 -, IgG +, IgD -
Immunohistochemistry : Annexin A1 -

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

Cytochemistry : TRAP - or weakly +
Immunophenotype : identical to classical HCL except : CD25 -, CD123 - / +

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

LYMPHOPLASMACYTIC LYMPHOMA
WALDENSTRÖM MACROGLOBULINEMIA

Lymphoplasmacytic bone marrow infiltration

Splenomegaly, hepatomegaly and / or adenopathy in 15-30% of patients

Peripheral blood may be involved : mixture of small and large lymphocytes, sometimes with eccentric nucleus and pronounced cytoplasmic basophilia

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

Possible cryoglobulinemia (~ 10%) (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

Differential diagnosis :	**IgM MGUS[2] (IgM < 30 g / L, no anemia, hepatosplenomegaly, adenopathies nor general symptoms; bone marrow lymphoplasmacytic cells < 10%)**
Treatment :	**Plasmapheresis if hyperviscosity syndrome** **Rituximab alone or combined with purine analogues (Fludarabine, Cladribine)** **CHOP[3], corticosteroids, splenectomy** **Relapse : Bortezomib, Everolimus (immunosuppressive drug), Imatinib, Alemtuzumab, BCL2 anti-sense oligonucleotides, Perifosine (Akt inhibitor), allotransplant**
Median survival :	**5-10 years**

[1] Myelin Associated Glycoprotein
[2] MGUS : Monoclonal Gammapathy od Unknown Significance
[3] cf.p. 167

FOLLICULAR LYMPHOMA

~ 20% of non Hodgkin lymphomas, median age : 60 years, sex ratio 1 : 1.7

Origin : Centrocytes and centroblasts from the germinal center of the lymph follicle

Histology : Follicular architecture with centrocytes (cells of small to medium size with cleft nuclei) and centroblasts
Aggressiveness dependent on the proportion of centroblasts : 1) grade I : 0-5 centroblasts / field;
2) grade II : 6-15 centroblasts / field; 3) grade III : > 15 centroblasts / field (magnification : 40x)

Localisations : Peripheral lymphadenopathies, hilar, mediastinal, spleen (40%), liver (50%), bone marrow (60-70%)
Tumor bulks of the digestive tract, urinary tract, with symptoms or not, epidural

B symptoms in 20% of cases : fever, sweats, weight loss

Immunophenotype : sIg + (IgM : 50-60%, IgG : 40%), HLA-DR +, CD19 +, CD20 +, CD79a +, CD21 +, CD10 + (60%), CD5 -, CD11c -, CD23 - / +, CD43 -

Cytogenetics : t(14;18)(q32;q21) : ~ 85% of cases, with IgH / BCL2 rearrangement (overexpression of BCL2[1]), 3q27 anomalies, less frequent variants
t(2;18)(p12;q21), t(8;22)(q21;q11) and / or rearrangement BCL6 : 5-15% (more frequent in grade III aggressive follicular lymphomas)

Prognostic :

***Risk factors** (1 point / factor)* :

Age > 60 years
⇗ LDH
Hb < 120 g / L
Ann Arbor stages III-IV
lymphatic sites > 4

FLIPI[2] (Follicular Lymphoma International Prognostic Index)

Score	Risk groups	Survival rate at 5 years (%)	Survival rate at 10 years (%)
0-1	Low	91	71
2	Intermediate	78	51
3-5	High	52	36

Treatment :

Localized, asymptomatic type : "wait and watch"
Localized and symptomatic type : radiotherapy, possiblity surgical excision
Aggressive type : Rituximab, radio-immunoconjugate anti CD20 (Ibritumomab, Ositumomab),
CVP or CHOP (cf.p. 167) + Rituximab, Fludarabine + Rituximab
Allogeneic transplant (young patient with HLA identical donor)

[1] Oncogene inhibitor of apoptosis

[2] *Modified from Solal-Céligny P., Roy P., Colombat P. et al. : Follicular Lymphoma International Prognostic Index. Blood 2004; 104 : 1258-1265.*

MANTLE CELL LYMPHOMA

~ 7% of non Hodgkin lymphomas, median age : 68 years, sex ratio : 3:1

Origin : Naïve B Lymphocytes of the mantle zone of lymphatic follicle

Histology : 1) Small cleaved cells, centrocytic type; 2) blastoid aggressive variant; 3) pleiomorphic aggressive variant

Localizations : Lymphadenopathies, splenomegaly (40-60%), bone marrow (> 60%), peripheral blood, digestive track, Waldeyer ring

B symptoms in > 1/3 of cases : fever, sweats, weight loss

Immunophenotype : sIgM ± IgD, λ light chains, CD19 +, CD20 +, CD5 + (rarely -), CD43 +, FMC-7 +, CD10 -, BCL6 -, CD23 - (or low +)

Immunohistochemistry : Cycline D1 (BCL1) + (> 90%)

Cytogenetics : Rearrangement of Ig, t(11;14)(q11;q32) : 50-65% by conventional genetics, ~ 100% by FISH or PCR

Prognosis : Depends on FLIPI[1] (Follicular Lymphoma International Prognostic Index) cf. previous page and on Ki67 expression, (proliferation index)

Risk factors *(1 point / factor)* **:**
- Age > 60 years
- ⇗ LDH
- Hb < 120 g / L
- Ann Arbor, stages III-IV
- # lymphatic sites > 4

Score	Risk group	Survival rate at 5 years (%)
0-1	Low	65
2	Intermediate	42
≥ 3	High	8

Treatment : Indolent type (absence of tumor bulk or general symptoms : "wait and watch"

Low aggressive type (scores 1-2) : CHOP or CVP (cf.p. 167) ± Rituximab

Aggressive type (scores ≥ 3) : Hyper-CVAD (Cyclophosphamide + Vincristine + Doxorubicin + Dexamethasone) ± Rituximab, autologous graft

Refractory or relapsed disease : Bortezomib, Bendamustine + Rituximab, FCM (Fludarabine + Cyclophosphamide + Mitoxantrone) ± Rituximab, Cladribine, Temsirolimus (m-TOR inhibition), Thalidomide, Lenalidomide, Non myeloablative allogeneic transplant

[1] **Appears more reliable than IPI (cf.p. 165) or MIPI (Mantle Cell Lymphoma Prognostic Index) : age + performance index + LDH + leukocyte count**

Møller M.B. and coll. : Mantle Cell lymphoma : prognostic capacity of the Follicular Lymphoma International Prognostic Index. Br J Haematol 2006; 133 : 43-49.

BURKITT LYMPHOMA

Types : 1) Endemic (Africa); 2) Sporadic; 3) Linked to AIDS
Association to EBV (Epstein-Barr Virus), mostly in endemic type

Localization : Frequent involvement of central nervous system in all 3 types
Involvement of jaw and other facial bones in the endemic type
Abdominal involvement (ileocecal region), ovaries, kidneys, breasts in the sporadic type
Lymphadenopathies and bone marrow involvement in AIDS linked type

Rapidly progressive, frequently bulky : important abdominal tumor masses

Treatment : CODOX-M[1] / IVAC[2] + intrathecal Methotrexate
EPOCH[3] + Rituximab (patients > 60 years)

Immunophenotype :	**sIgM, CD19 +, CD20 +, CD22 +, CD10 +, BCL6 +, CD38 +, CD77 +, CD43 +, BCL2 ± (20%), TdT -, Ki67 +**
Cytogenetics :	**t(8;14)(q24;q32), variants t(2;8)(p12;q24), t(8;22)(q24;q11)**

Overexpression of c-Myc oncogene, mostly through translocation to an immunoglobulin heavy chain gene

Variant type : Acute lymphoblastic leukemia Burkitt type
Blood and bone marrow involvement
Blast cells with hyperbasophilic cytoplasm with vacuoles
Frequent involvement of CNS at diagnosis
Treatment : cf.p. 174 (treatment of lymphoblastic leukemia / lymphoma)
Extreme chemosensitivity *(risk of acute tumor lysis syndrome)*

[1] CODOX-M : Cyclophosphamide + Vincristine + Doxorubicin + Methotrexate high dose
[2] IVAC : Ifosfamide + Cytarabine + Etoposide
[3] EPOCH : Etoposide + Vincristine + Doxorubicin + Cyclophosphamide + Prednisone

DIFFUSE LARGE B-CELL LYMPHOMA (DLCBL)

~ 25% of non-Hodgkin lymphomas, more common in males than in females, median age at diagnosis : 68 years

Features : Cervical lymph node bulk ou abdominal mass with rapid growth
B symptoms (fever, sweats, weight loss) in 30% of cases
Stage I-II (~ 40%), III-IV (~ 60%) at initial presentation
Extranodal and extramedullary involvement (> 40%) :
Digestive track (stomach and ileocecal region)
Bone, testis, breast, spleen, Waldeyer ring, salivary gland, thyroid, liver, kidney, adrenal, skin, bone marrow (11-27%)

Morphology : large cells, prominent nucleoli and basophilic cytoplasm
Main variants: Centroblastic
Immunoblastic
Anaplastic

Molecular subgroups: Germinal Centre B-cell-like : GCB
Activated B-cell-like : ABC

Immunophenotype :	sIg (50-75%) : sIgM > sIgG > sIgA, CD19 +, CD20 +, CD22 +, CD79a +, CD45 +, CD10 + (30-60%), CD5 - (10% +)
Immunohistochemistry :	expression of BCL2 + (25-80%), BCL6 + (60-90%), rearrangement of BCL6, Ki67 + (proliferation index) : > 40%
Cytogenetics :	t(14;18)(q32;q21) with BCL2 gene translocation (20-30%), 3q27 anomalies (BCL6 gene), c-Myc rearrangement (> 10%)

DLBCL subgroups : 1) T-cell / histiocyte rich DLBCL; 2) Primary CNS DLBCL; 3) Primary cutaneous leg type DLBCL;
4) Chronic inflammation associated DLBCL

Prognosis : Depends on aaIPI (age adjusted International Prognostic Index). cf.p. 166

Treatment : Initial : CHOP (cf.p. 167) or CEOP[1] + Rituximab, R-CEPP[2], chemotherapy + radiotherapy ("Bulky")
Intrathecal chemotherapy, surgery in case of spinal cord compression
Refractoriness or relapse : R-ICE[3] followed by autologous stem cell transplant

[1] CEOP : Cyclophosphamide + Epirubicin + Vincristine + Prednisone; [2] R-CEPP : Rituximab + Cyclophosphamide + Etoposide + Procarbazine + Prednisone
[3] R-ICE : Rituximab + Ifosfamide + Carboplatin + Etoposide

PLASMA CELL NEOPLASMS

Clonal expansion of mature B cells, after isotypic switch of heavy chains, secreting a homogeneous immunoglobulin (= paraprotein)
Occasional biclonality

Presence of paraprotein is also called monoclonal gammopathy

1) IgG, IgA and light chains gammopathies :
Plasma cell neoplasms

2) IgM and heavy chains gammopathies :
a) Lymphoplasmacytic lymphoma (Waldenström macroglobulinemia, cf.p. 182

b) Heavy chain deposition diseases

WHO CLASSIFICATION 2008

Monoclonal gammopathy of undetermined significance / MGUS

Plasma cell myeloma
- Asymptomatic ("smoldering") plasma cell myeloma
- Symptomatic plasma cell myeloma
- Non secretory plasma cell myeloma
- *Plasma cell leukemia*

Plasmacytoma
- Solitary plasmacytoma of bone
- Extraosseous (extramedullary) plasmacytoma

Immunoglobulin deposition diseases
- Primary amyloidosis
- Systemic light and heavy chain deposition diseases

Osteosclerotic myeloma (POEMS) :
- *Polyneuropathy*
- *Organomegaly : spleen, liver, lymph nodes*
- *Endocrinopathy : diabetes, gynecomastia, testicular atrophy*
- *M-component : monoclonal gammopathy*
- *Skin : hyperpigmentation, hypertrichosis*

	HISTOLOGY	CLINICAL SITES
γ heavy chain disease	Lymphoplasmacytic lymphoma	Lymph nodes, Waldeyer ring, bone marrow, spleen, liver, blood
μ heavy chain disease	Chronic lymphoid leukemia	Spleen, liver, bone marrow, blood
α heavy chain disease (IPSID)[1]	Extranodal marginal zone lymphoma of mucosa associated lymphoid tissue (MALT)[2]	Small bowel, mesenteric lymph nodes

[1] IPSID : Immunoproliferative small intestinal disease
[2] MALT : Mucosa-Associated Lymphoid Tissue

DIAGNOSTIC WORK-UP

Paraprotein pattern

Protein electrophoresis, immunofixation, quantitative immunoglobulins dosage (serum)
Free light chains (FLC), κ / λ ratio (serum)
Protein electrophoresis, immunofixation (urine)[1]

Peripheral blood examination

(inclusive platelets, reticulocytes and microscopic blood smear examination)

Blood chemistry:

Creatinin, Calcium, Albumin, LDH, β_2-microglobulin, CRP, alkaline phosphatase, ALAT, ASAT

Bone marrow examination

Cytology and histology, immunophenotyping, cytogenetics and FISH[2]

Radiology work-up

Conventional Xray examination : spine, skull, pelvis and long bone
CT / IRM (e.g. whole body) / PET-CT
(Bone scintigram is poorly reliable)

[1] Not necessary in case of serum free light chains dosage with κ / λ ratio, except for amyloidosis work-up
[2] FISH : Fluorescent In Situ Hybridization

TYPES OF PARAPROTEINS[1] / FREQUENCY

TYPE	%	TYPE	%
IgG	50	IgD, IgM, biclonal	<10
IgA	20	Absence of paraprotein	~3
Light chains	20	IgE	<1

[1] PARAPROTEIN = MONOCLONAL IMMUNOGLOBULIN

PLASMA CELL NEOPLASMS
FREE SERUM LIGHT CHAINS (FLC) AND κ / λ FLC RATIO

Immunonephelometric measurement of free kappa (κ) or lambda (λ) monoclonal light chains in serum (FLC) is of diagnostic, prognostic and monitoring relevance

The result can also be expressed as the ratio of κ to λ free light chains amounts

Reference range :

FLC κ : 3.3 – 19.4 mg / L
FLC λ : 5.7 – 26.3 mg / L
κ / λ ratio : 0.26 – 1.65

Examples:

- FLC κ : **9.6 mg / L** FLC λ **: 16.5 mg / L**
  κ / λ ratio : **9.6 / 16.5 = 0.58** *(normal)*

- FLC κ : **2.5 mg / L** FLC λ : **32.8 mg / L**
  κ / λ ratio : **2.5 / 32.8 = 0.076** *(< 0.26)*[1]

- FLC κ : **28.0 mg / L** FLC λ **: 6.25 mg / L**
  κ / λ ratio : **28.0 / 6.24 = 4.48** *(> 1.65)*[2]

[1] Low abnormal by excess of λ FLC

[2] High abnormal by excess of κ FLC

INDICATIONS TO FLC AND κ / λ FLC RATIO MEASUREMENT

Replaces quantitative measurement of urine light chains by immunofixation in the work-up algorithm of monoclonal gammopathy documented by serum electrophoresis and immunofixation (*except for amyloidosis work-up*)

Diagnostic parameter of non secretory (or low secretory) plasma cell myeloma

Complementary diagnostic parameter of plasma cell myeloma with complete paraprotein

Risk parameter for MGUS evolution to plasma cell myeloma

Risk parameter for smoldering plasma cell myeloma to symptomatic myeloma

Risk parameter for progression of solitary plasmacytoma

Prognostic parameter (independant risk factor) for plasma cell myeloma

Monitoring parameter during and after treatment of plasma cell myeloma :

Early treatment response indicator

Indicator of response quality

(normalization of values allows the definition of a "stringent" complete remission)

Early relapse indicator

Modified from : Dispenzieri A. & al. International Myeloma Working Group guidelines for serum free light chain analysis in multiple myeloma and related disorders. Leukemia 2009; 23 : 215-224.

MGUS AND PLASMA CELL MYELOMA
DIFFERENTIAL DIAGNOSIS / COURSE

DIFFERENTIAL DIAGNOSIS OF MGUS, SMOLDERING AND SYMPTOMATIC PLASMA CELL MYELOMA

	MGUS	SMOLDERING MYELOMA	SYMPTOMATIC MYELOMA
Plasma cells (Bone marrow)	< 10%	≥ 10%	>10%
Monoclonal immunoglobulin (Ig)	< 30 g / L ⇘ other Ig : 30-40% of cases FLC[1] no / slight ⇗	> 30 g / L[2] ⇘ other Ig : > 90% of cases FLC[1] ⇗. κ / λ ratio abnormal	> 30 g / L[2] ⇘ other Ig usual FLC[1] ⇗ ⇗. κ / λ ratio abnormal
CRAB[3]	0	0	CRAB[3] + / ++

[1] FLC : Free Light Chain (serum). κ / λ ratio : ratio of FLC κ amount to FLC λ amount
[2] A paraprotein level > 30 g / L is not mandatory. Lower levels do not exclude plasma cell myeloma if other criteria present
[3] CRAB : Myeloma related organ involvement : Hypercalcemia (C), Renal failure (R), Anemia (A), Bone lesions (B)

The measurement of FLC and κ / λ ratio ist a key parameter for the follow-up of MGUS or indolent plasma cell myeloma. It is a reliable, independent risk factor

Initial measurement allows to define a patient **group with excellent prognosis** for whom follow-up may be done at large intervals (e.g. yearly)

RISK OF MGUS OR SMOLDERING MYELOMA PROGRESSION RELATION TO κ / λ RATIO

	PROGNOSTIC CRITERIA	RISK OF PROGRESSION	% PATIENTS
MGUS 3 - 5 % of patients > 70 years	normal κ / λ ratio[1] paraprotein < 15 g / L IgG type	< 5% at 30 years	± 40%
	κ / λ ratio 0.25 – 4.0	± 20% at 30 years	± 60%[2]
	κ / λ ratio < 0.25 / > 4.0	± 45% at 30 years	± 30%
SMOLDERING MYELOMA	κ / λ ratio 0.125 – 8.0	± 50% at 15 years	-
	κ / λ ratio < 0.125 ou > 8.0	± 80% at 15 years	-

[1] Normal κ / λ ratio : 0.26 –-1.65
[2] Including the 40% of excellent prognosis

PLASMA CELL MYELOMA
PROGNOSTIC FACTORS

Paraprotein serum level : IgG or IgA
Type of paraprotein : IgA unfavorable
Level of serum free light chains and κ / λ ratio
β_2- microglobulin level (serum)
Hypercalcemia (C)
Renal failure (R)
Anemia ≤ 100 g / L (A)
Bone lesion(s) (B)

C R A B

Bone marrow infiltration > 50%
Performance index ≥ 3

Cytogenetics (or FISH) of bone marrow plasmocytes[1]

Bad prognosis : *del17p, t(14;16), t(14;20)*

Intermediate prognosis : *hypodiploidy, t(4;14), del13q (by karyotyping)*

Standard prognosis : *hyperdiploidy, t(11;14), t(6;14)*

Genomics : *GEP*[2] *"high risk signature"*

(Risk stratification with these criteria is in constant evolution)

[1] *Based on S. Vincent Rajkumar : Multiple Myeloma : 2012 update on diagnosis, risk-stratification and management. Am. J. Hematol 2012; 87 : 79-88.*

[2] Gene Expression Profile

DURIE & SALMON STAGES

STAGE	DESCRIPTION
I	**Low tumor mass** *All following criteria* Hemoglobin > 100 g / L IgG serum < 50 g / L or IgA serum < 30 g / L Normal calcemia Urine paraprotein < 4 g / day No generalized bone lesions
II	**Values intermediate between I and III**
III	**High tumor mass** *One or more following criteria* Hemoglobin < 85 g / L IgG serum > 70 g / L or IgA serum > 50 g / L Calcemia > 3 mMol / L Urine paraprotein > 12 g / day
A	Creatinin (serum) < 170 µMol / L
B	Creatinin (serum) > 170 µMol / L

PLASMA CELL MYELOMA
PROGNOSTIC FACTORS (2)

ISS (International Staging System) : 8'449 patients[1]

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

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

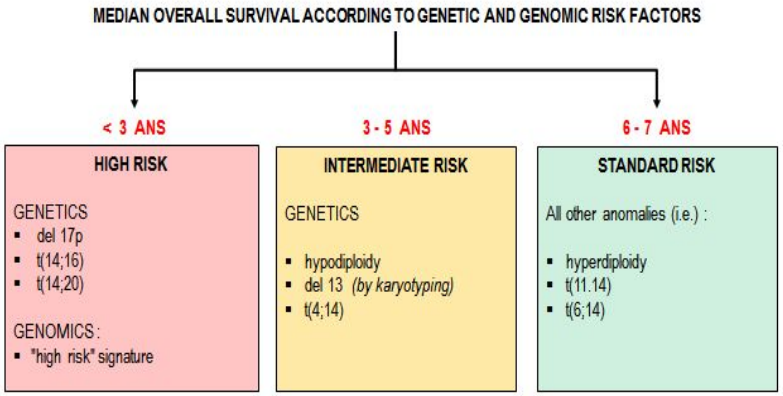
[2] β_2-m : β_2-microglobulin

Prognostic impact of κ / λ ratio[3] on ISS

RISK GROUP	1 YEAR SURVIVAL %	5 YEARS SURVIVAL %	MEDIAN SURVIVAL (MONTHS)
ISS Stage I			
κ / λ ratio 0.03 - 32	87.6	41.5	51
κ / λ ratio < 0.03 / > 32	88.9	29.8	41
ISS Stage II			
κ / λ ratio 0.03 - 32	83.2	35.2	40
κ / λ ratio < 0,03 / > 32	77.5	20.5	30
ISS Stage III			
κ / λ ratio 0.03 - 32	67.6	24.4	17
κ / λ ratio < 0.03 / > 32	62.5	15.3	23

[3] κ / λ ratio of serum Free Light Chains (FLC)

Modified from Snozek C.L.H., Katzmann J.A., Kyle R.A. & al. Leukemia 2008; 22 : 1933–1937.



Based on Rajkumar S.V. : Multiple Myeloma : 2012 update on diagnosis, risk-stratification and management. Am. J. Hematol. 2012; 87 : 79-88.

COMPLICATIONS

Hyperviscosity syndrome (mostly IgA, IgG3)

Neurologic : **compression (spinal or radicular)**

Renal : **light chain, calcic or uric nephropathy, amyloidosis, plasma cell infiltration**

Infectious

Hematological : **bone marrow failure, thrombopathy**

PLASMA CELL MYELOMA
TREATMENT

INDICATION : Symptomatic plasma cell myeloma *(with CRAB type symptoms)*
Presence at diagnosis of unfavorable risk factor(s) is not by itself an indication to treatment

Bortezomib, Lenalidomide, Thalidomide, possibly in combination or with high dose Dexamethasone

Bortezomib + Cyclophosphamide + Dexamethasone ***(high or reduced dosage)***

Radiotherapy ***(solitary plasmocytoma)***

Supportive care ***(transfusions of RBC, platelets, antibiotics, analgesics, bisphosphonates)***

Plasmapheresis ***(hyperviscosity syndrome)***

Intensification with autologous HST[1] ≤ 70 years[2]

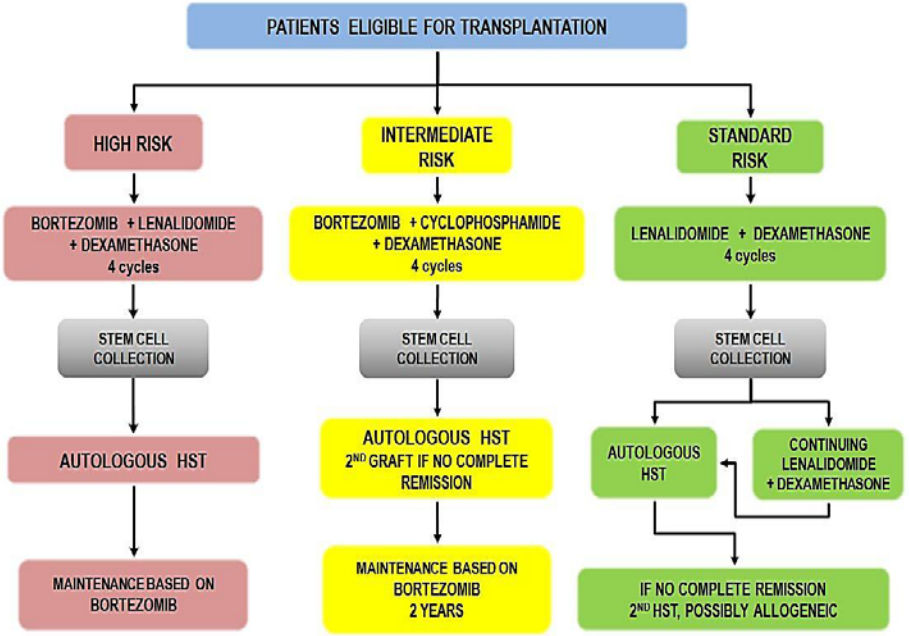
Allogeneic transplant ***(stem cell or bone marrow)*** **< 55 years, possible cure, important treatment related mortality, GVH +++. Allograft with reduced intensity conditioning**

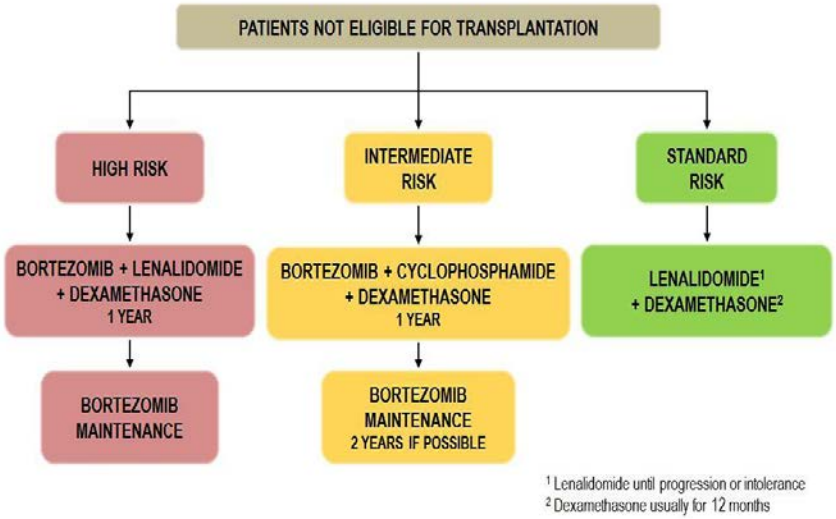
As reserve : Melphalan + Prednisone, VAD[3], VBAP[4], VMCP[5] , VDT-PACE[6]

[1] Hematopoietic Stem cell Transplantation (peripheral blood stem cells or bone marrow)
[2] In relation with adequate clinical status and performance index : ≤ 78 years of age
[3] VAD : Vincristine + Doxorubicine + Dexamethasone "high dose"
[4] VBAP : Vincristne + BCNU + Doxorubicine + Prednisone
[5] VMCP : Vincristine + Melphalan + Cyclophosphamide + Prednisone
[6] VDT-PACE : Bortezomib + Dexamethasone + Thalidomide + Cisplatine + Doxorubucine + Cyclophosphamide + Etoposide

PLASMA CELL MYELOMA
TREATMENT (2)

EXAMPLES OF RISK RELATED TREATMENT ALGORITHMS





High and intermediate risk
t(14;16), t(14;20), del17p, del13, t(4;14), hypodiploidy

Eligibility for transplant :

- Autologous : age ≤ 70 years[1]. Good performance index. Acceptable risk of treatment related complications

- Allogeneic : age ≤ 55 years. Good performance index. High risk of autologous transplant failure or relapse after autologous transplant
In case of doubt consider transplant with reduced intensity conditioning

[1] In very favorable situations ≤ 78 ans

MATURE B-CELL LYMPHOID NEOPLASMS

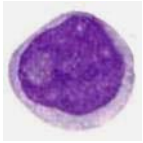
Contribution of immunological markers, cytogenetics and molecular biology

	sIg	CD19	CD5	CD23	CYTOGENETICS	OTHERS
CLL	+ / -	+	+	+		
B-PLL	+	+	- / +	- / +		
HCL	+	+	-	-		TRAP + CD11c + CD25 + CD103 +
SMZL	+	+	- / +	-		
FL	+	+	-	-	t(14;18)(q32;q21)	CD10+ BCL2
MCL	+	+	+	-	t(11;14)(q13;q32)	Cyclin D1

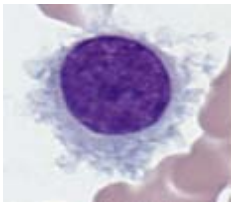
	CD123[1]	CD25	CD11c	CD103
HCL	22 / 23 95%	24 / 25 96%	25 / 25 100%	25 / 25 100%
HCL VARIANT	1 / 11 9%	0 / 11 0%	11 / 11 100%	4 / 11 36%
SMZL	1 / 29 3%	18 / 28 64%	10 / 26 38%	0 / 25 0%

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

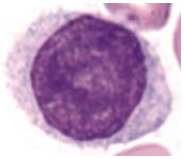
The contribution of morphology remains paramount for the differential diagnosis of B-cell prolymphocytic leukemia, hairy cell leukemia and its variant form as for splenic B-cell marginal zone lymphoma



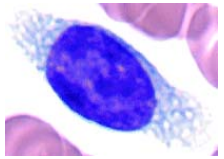
Prolymphocytic leukemia
(Cell with big nucleolus)



Hairy cell leukemia
("Hairy" pattern of cytoplasm)



Hairy cell leukemia variant
("Hairy" pattern of cytoplasm + big nucleolus)



Splenic marginal zone B-cell lymphoma
(Villous lymphocytes : hairy pattern at the poles of cytoplasm

[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.*

MATURE T-CELL AND NK-CELL LYMPHOID NEOPLASMS

T-CELL PROLYMPHOCYTIC LEUKEMIA (T-PLL)

Hepatosplenomegaly, generalized lymphadenopathy, occasionally effusion of serous membranes (pleura)

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

Skin involvement (20% of cases)

Aggressive disease, median survival < 1 year

Treatment : anti-CD52 (alemtuzumab)

Immunophenotype :	CD2 +, CD3 + (sometimes weakly), CD7 +, CD52 + CD4 + / CD8 - (60%); coexpression CD4 / CD8 (25%); CD4 - / CD8 + (15%) CD1a - even with 25% of cases CD4 + / CD 8 +
Cytogenetics :	inv(14)(q11;q32), t(14;14)(q11;q32), t(X;14)(q28;q11), i(8)(q10) t(8;8)(p23;q11), +8, del(6q), del(11q) Rearrangement of TCR genes

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

Indolent clinical course, median survival ~ 13 years

Immunophenotype :	CD3 +, CD2 +, CD8 +, CD4 -/+, CD57 + and CD 16 + (> 80% of cases)
Cytogenetics :	rearrangement of TCR genes

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

Clinical course generally indolent : rare cases of spontaneous complete remission or of tranformation in aggressive NK-cell leukemia

Immunophenotype :	CD3 -, CD4 -, CD8 -, CD16 +, CD56 + (usually weak), CD57 -
Cytogenetics :	absence of TCR genes rearrangement

AGGRESSIVE NK-CELL LEUKEMIA

Rare, prevalent in Asia, median age : 42 years

Strong association with EBV

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

Fulminant clinical course (coagulopathy, hemophagocytosis syndrome)

Median survival : < 2 months

Immunophenotype :	CD2 +, CD3 -, CD56 +, CD 57 -
Cytogenetics :	del (6)(q21q25), del 11q , TCR genes in germline configuration

ADULT T-CELL LEUKEMIA / LYMPHOMA (ATLL)

Japan (1977), Caribbean region, Central Africa

Clinical variants : Acute (most common)
Lymphomatous
Chronic
Smoldering

Lymphadenopathy, hepatosplenomegaly

Skin involvement (rash, papules, nodules)

Leukocytes : 5 – 100 G / L

Lymphocytes with lobated nucleus

Association with HTLV-1 virus

Hypercalcemia

Immunophenotype :	CD2 +, CD3 +, CD5 +, usually CD4 +, CD 7 -, CD8 - CD 25 +, CD30 +
Cytogenetics :	rearrangement of TCR genes

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

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)

Secondary infiltration of tissues and organs

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

Aggressive disease

Overall survival rate : 10-20% at 5 years

Immunophenotype :	CD2 +, CD3 +, CD5 +, CD4 + (usually) CD8 -, CD26 -, CD7- (or weakly +)
Cytogenetics :	TCR genes rearrangement

Stages of Mycosis fungoides and Sézary syndrome

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

Modified from : Baumann Conzett K. et coll. : Lymphomes cutanés. Classification et recommandations thérapeutiques. Forum Med Suisse 2009, 42 : 744-749.

MATURE T-CELL AND NK-CELL LYMPHOID NEOPLASMS

Contribution of immunological markers, cytogenetics and molecular biology

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

HODGKIN LYMPHOMA

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 type
  - Lymphocyte-rich type
  - Mixed cellularity type
  - Lymphocyte-depleted type

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 of 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
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, ICE

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

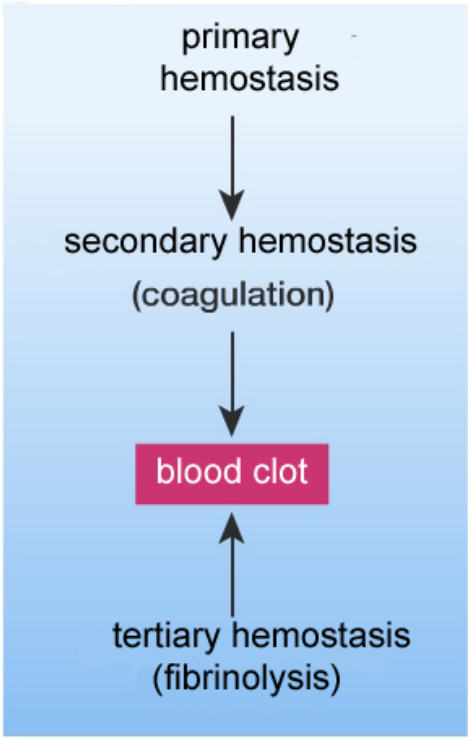
Response after 2 courses of ABVD by FDG-PET imaging is a relevant prognostic indicator in advanced stage disease[1]

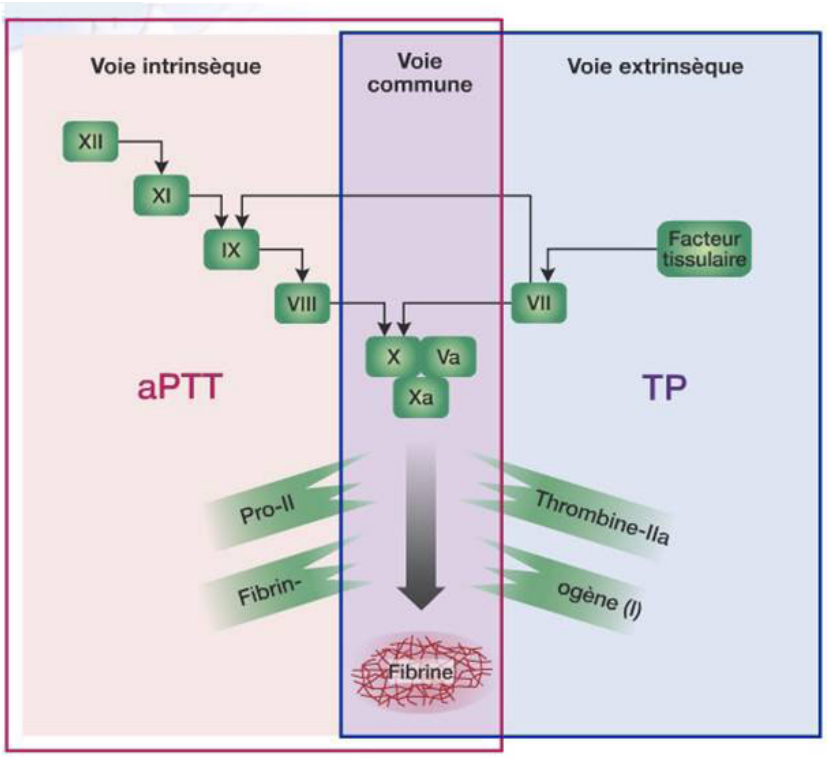
M(C)OPP :	**Mustard gas analog (or Cyclophosphamide) + Vincristine + Procarbazine + Prednisone**
ABVD :	**Adriamycin + Bleomycin + Vinblastine + Dacarbazine (DTIC)**
MIME :	**Mitoguazone + Ifosfamide + Methotrexate + Etoposide**
CEP :	**Lomustine + Etoposide + Prednimustin**
DHAP :	**Dexamethasone +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

[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



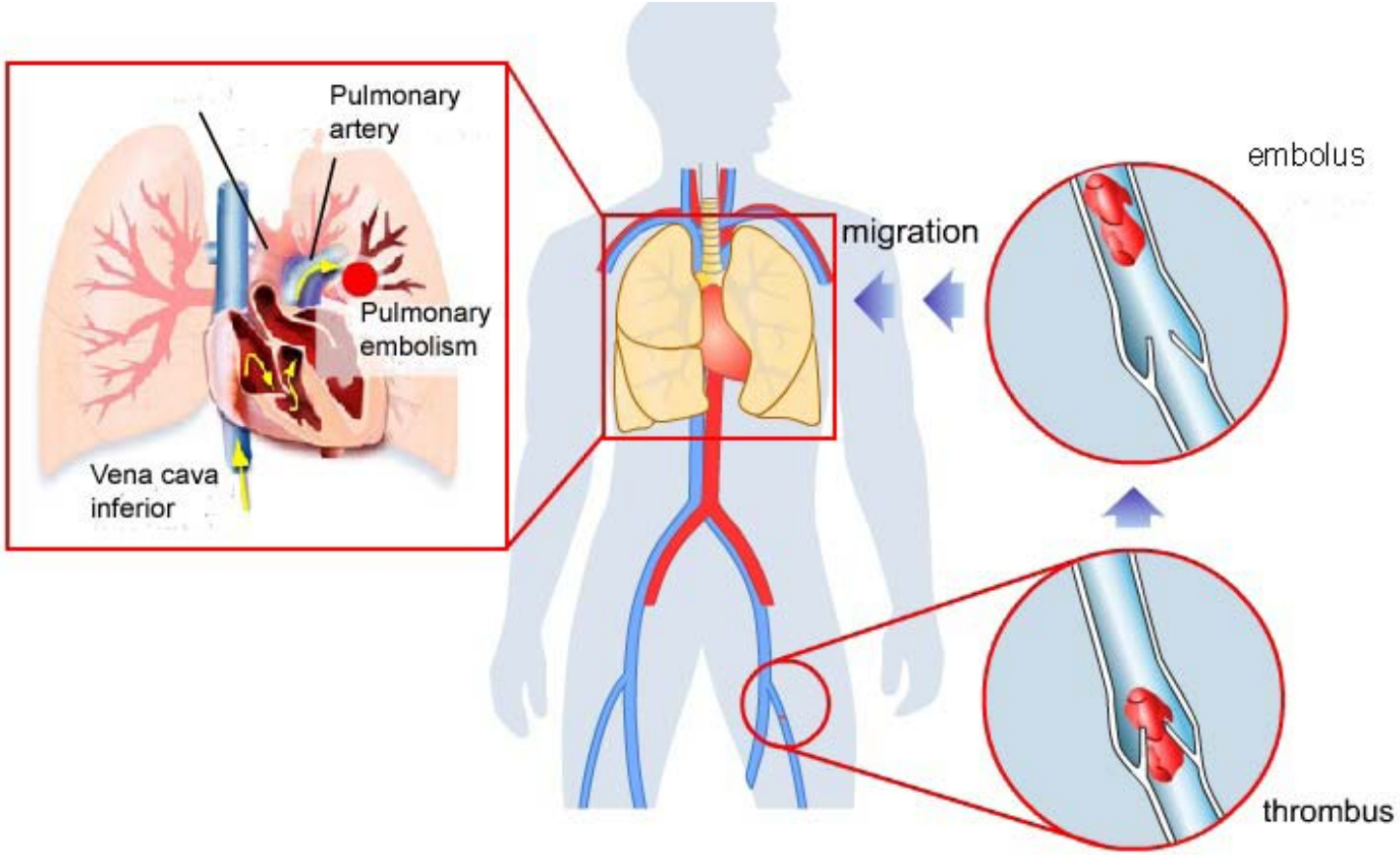


HEMOSTASIS
EXPLORATION METHODS

PRIMARY HEMOSTASIS	**Capillary resistance**
	Platelet count (RI : 150 – 350 G / L)
	PFA-100™ [1]
	Platelet functions (ADP, arachidonic acid, adrenalin-heparin, collagen, TRAP-6, U46619, ristocetin)
	Measure of platelet secretion
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 level
	Investigation of factor XIII deficiency (fibrin stabilizing factor)
	Investigation of activation ***(Fibrin monomers and D-dimers)***
TERTIARY HEMOSTASIS	**Euglobulins lysis time**
	Fibrinogen level
	D-Dimers level
	Plasminogen level
	α2-antiplasmin level
	PAI-1 level (Plasminogen Activator Inhibitor-1)

[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**

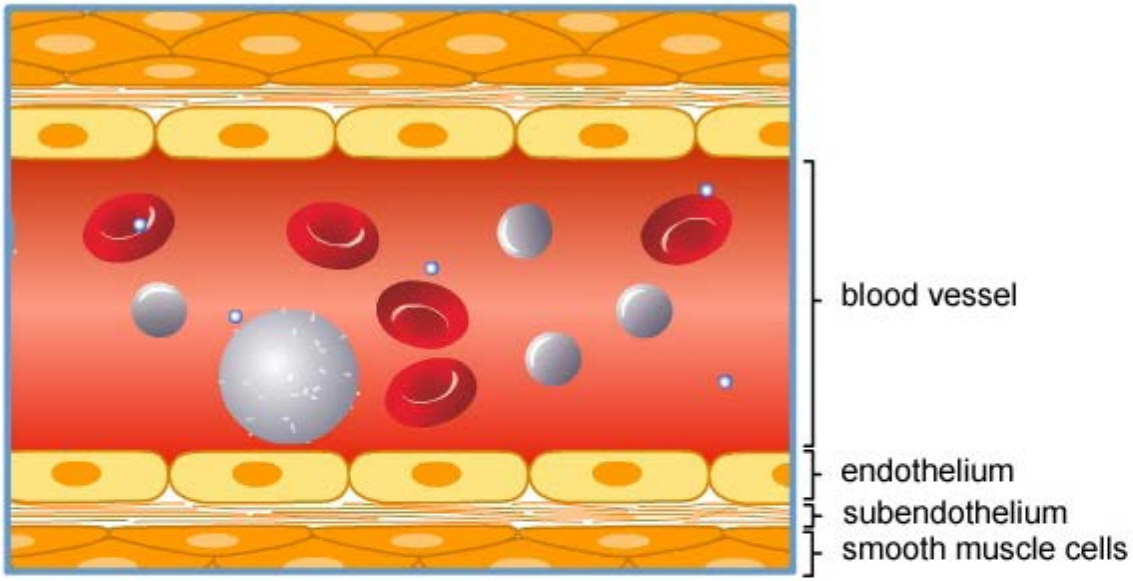
THROMBUS AND EMBOLUS

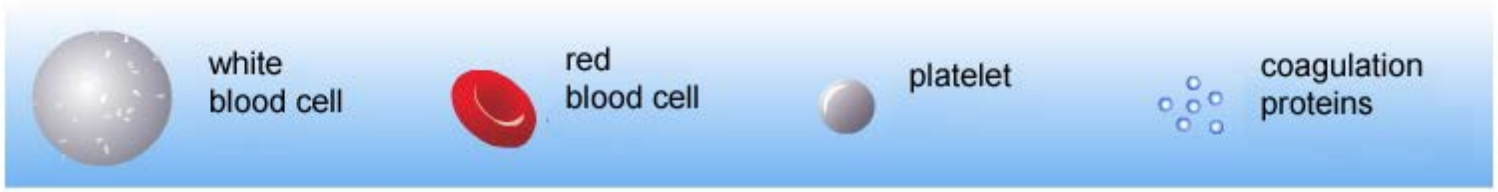


Thrombus : inappropriate clot formation in a blood vessel (artery or vein)
Embolus : migrating thrombus

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 and stabilization

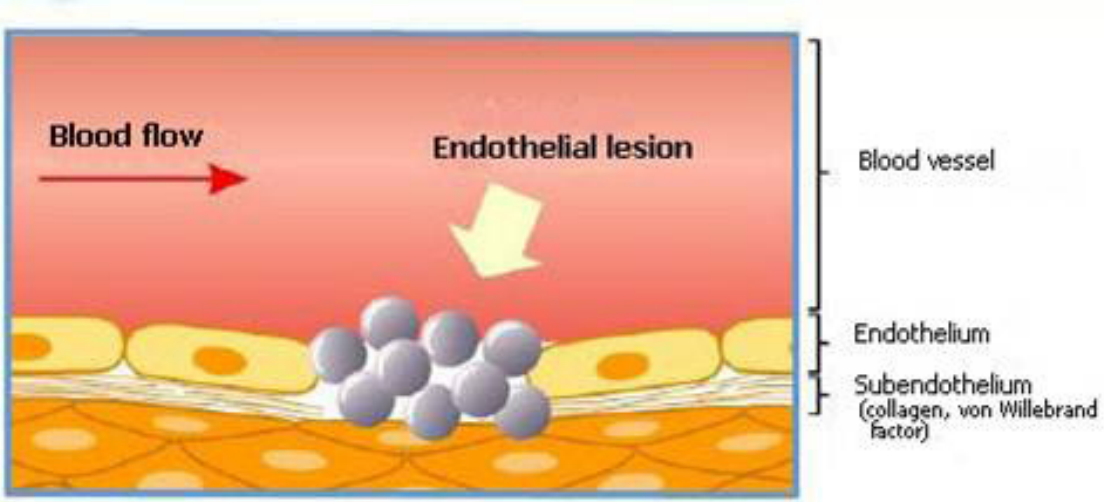
SECONDARY HEMOSTASIS (coagulation)

Coagulation cascade
Clot formation

TERTIARY HEMOSTASIS (fibrinolysis)

Clot lysis

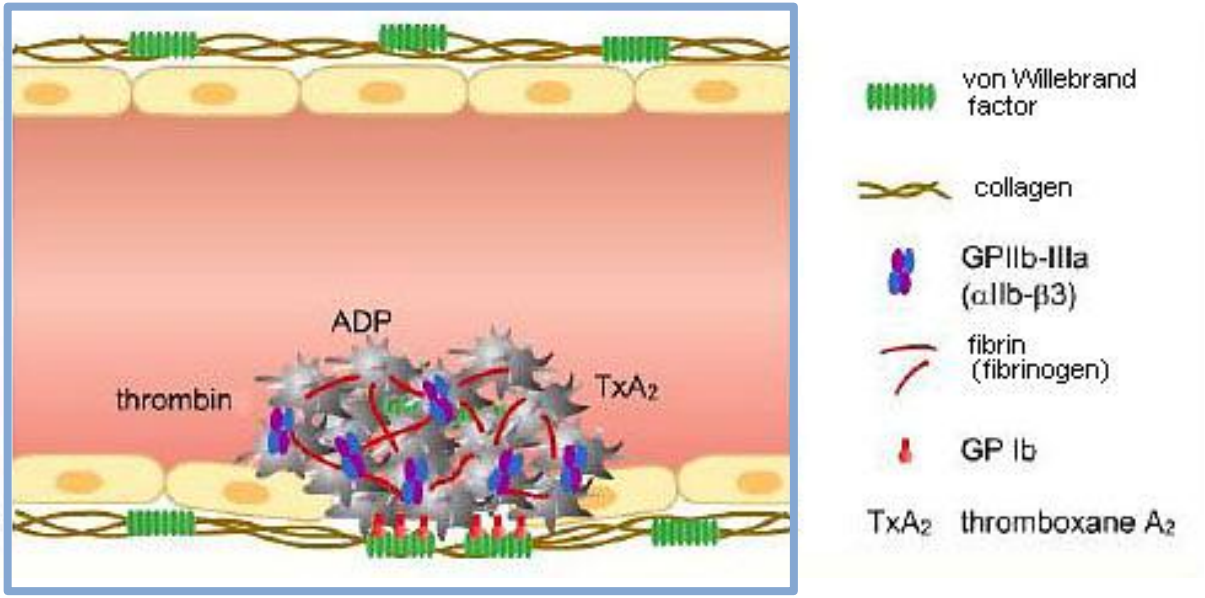
STEPS OF PRIMARY HEMOSTASIS



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 (ADAMTS 13), leading to prevention of spontaneous platelet aggregates formation (cf. TTP, p. 89-90)

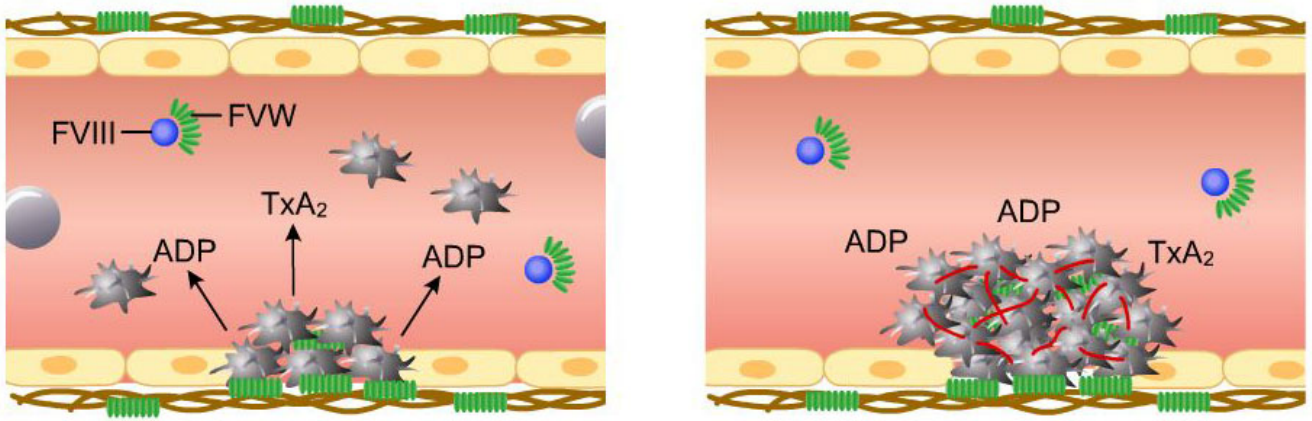
Involved, *in vitro,* in the process of platelet adhesion to subendothelial fibers

Mandatory for *in vitro* ristocetin induced platelet aggregation

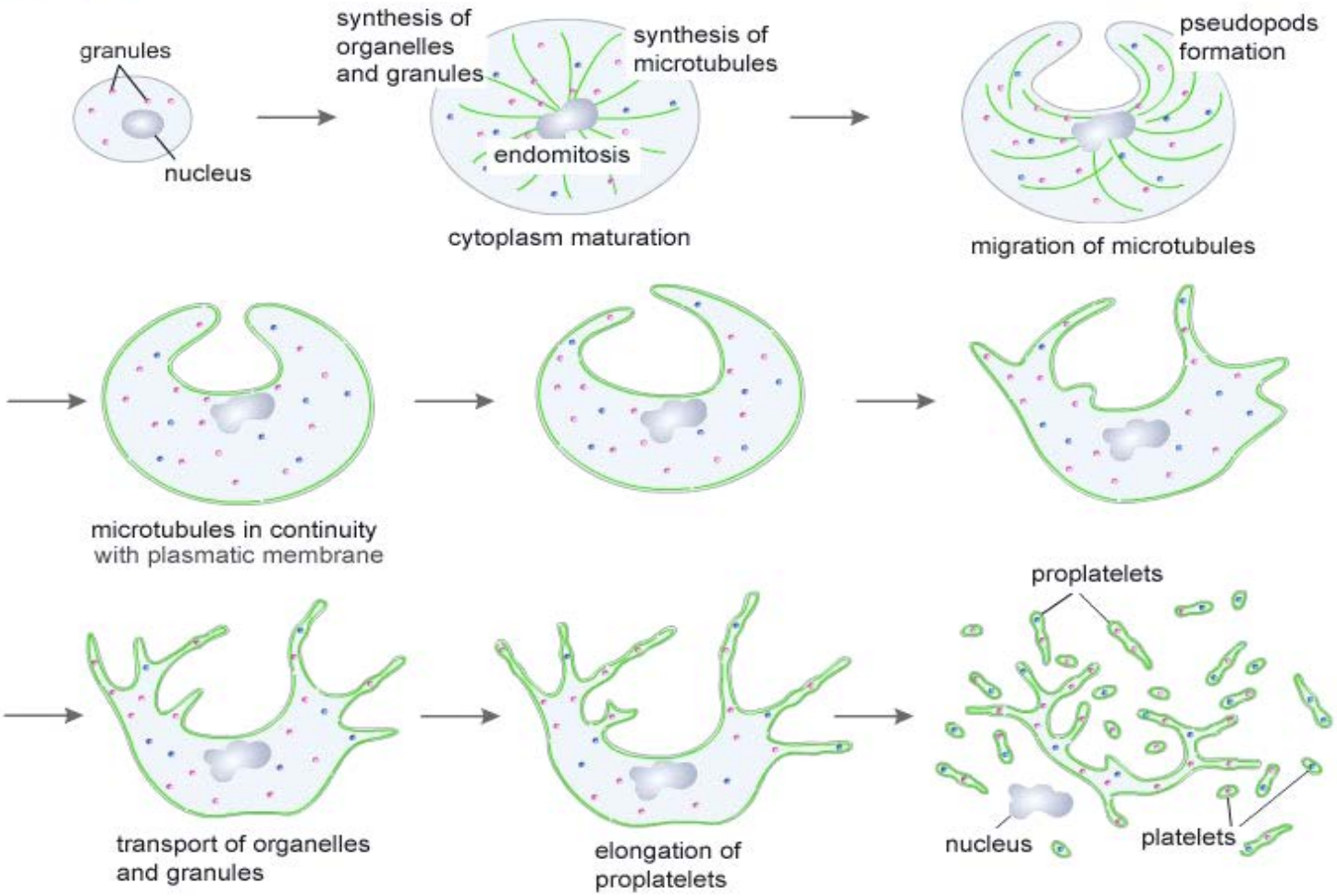
Transport of factor VIII to vascular lesion

Bound to factor VIII, it prolongs its life span

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

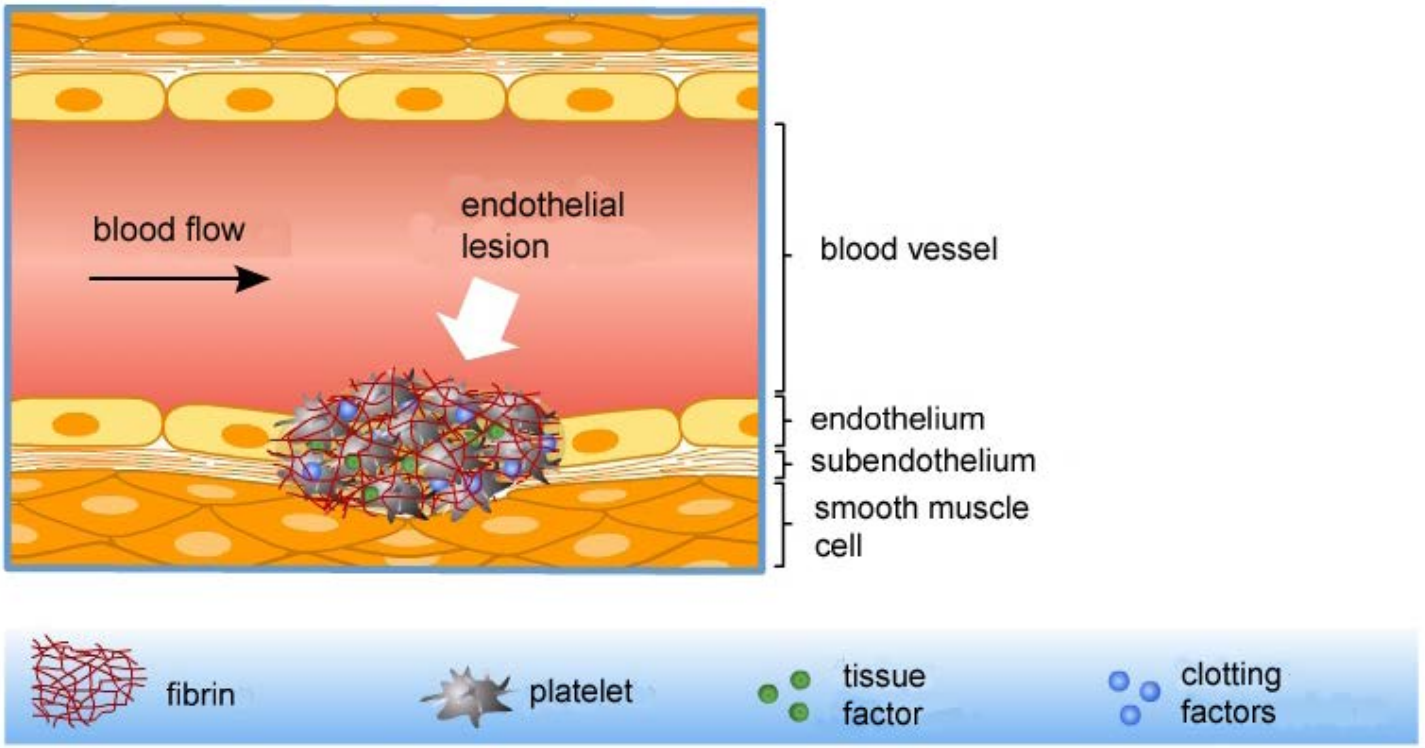
Coagulation (blood clotting) needs interaction of :

Plasmatic proteins (coagulation factors and inhibitors)

A tissular protein (tissue factor)

Platelets

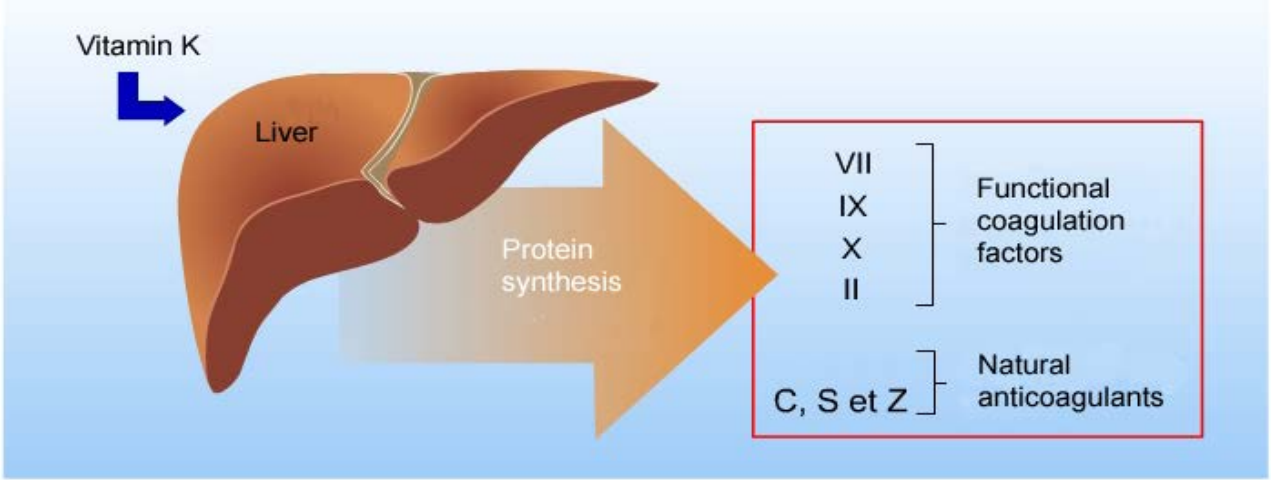
Calcium



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	**α subunit :** *monocytes, megakaryocytes, platelets* **β subunit** : *liver*	–
Factor vW	von Willebrand factor	15	Endothelium Megakaryocytes	–

VITAMIN K DEPENDENT FACTORS



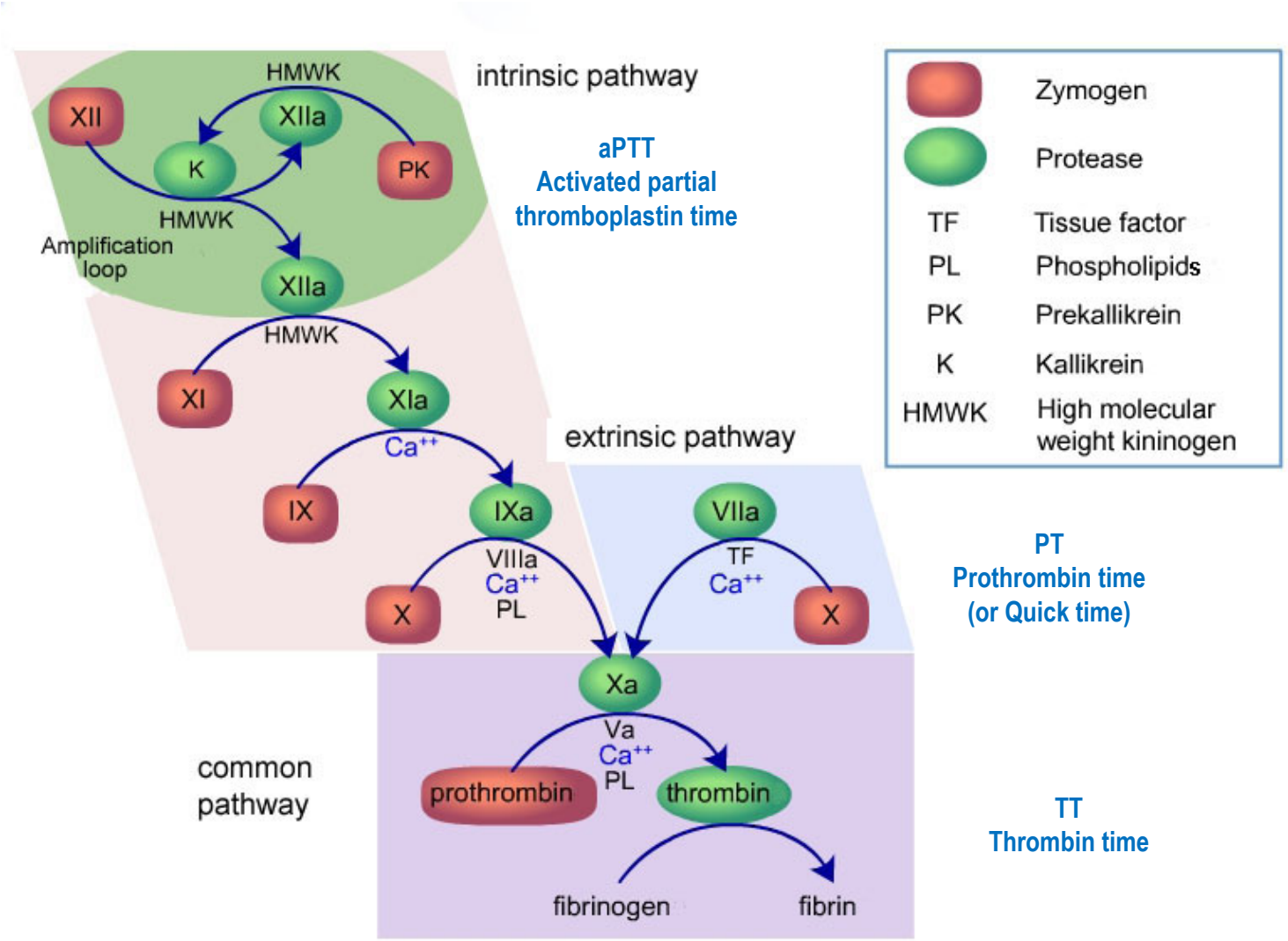
These coagulation factors are synthetized by hepatocytes

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
CLASSICAL SCHEME



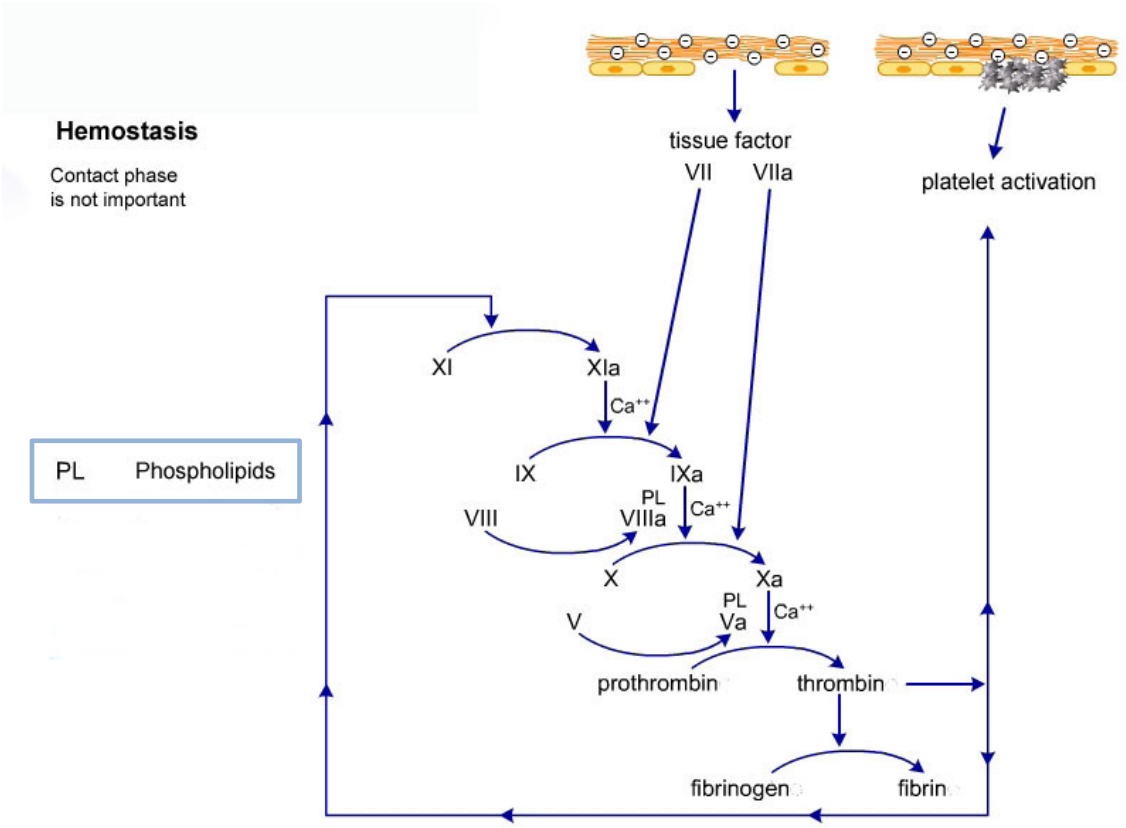
aPTT
Activated partial thromboplastin time

PT
Prothrombin time
(or Quick time)

TT
Thrombin time

Fibrinogen
Functional or quantitative dosage

COAGULATION CASCADE (2)
CONCEPTUAL CHANGES



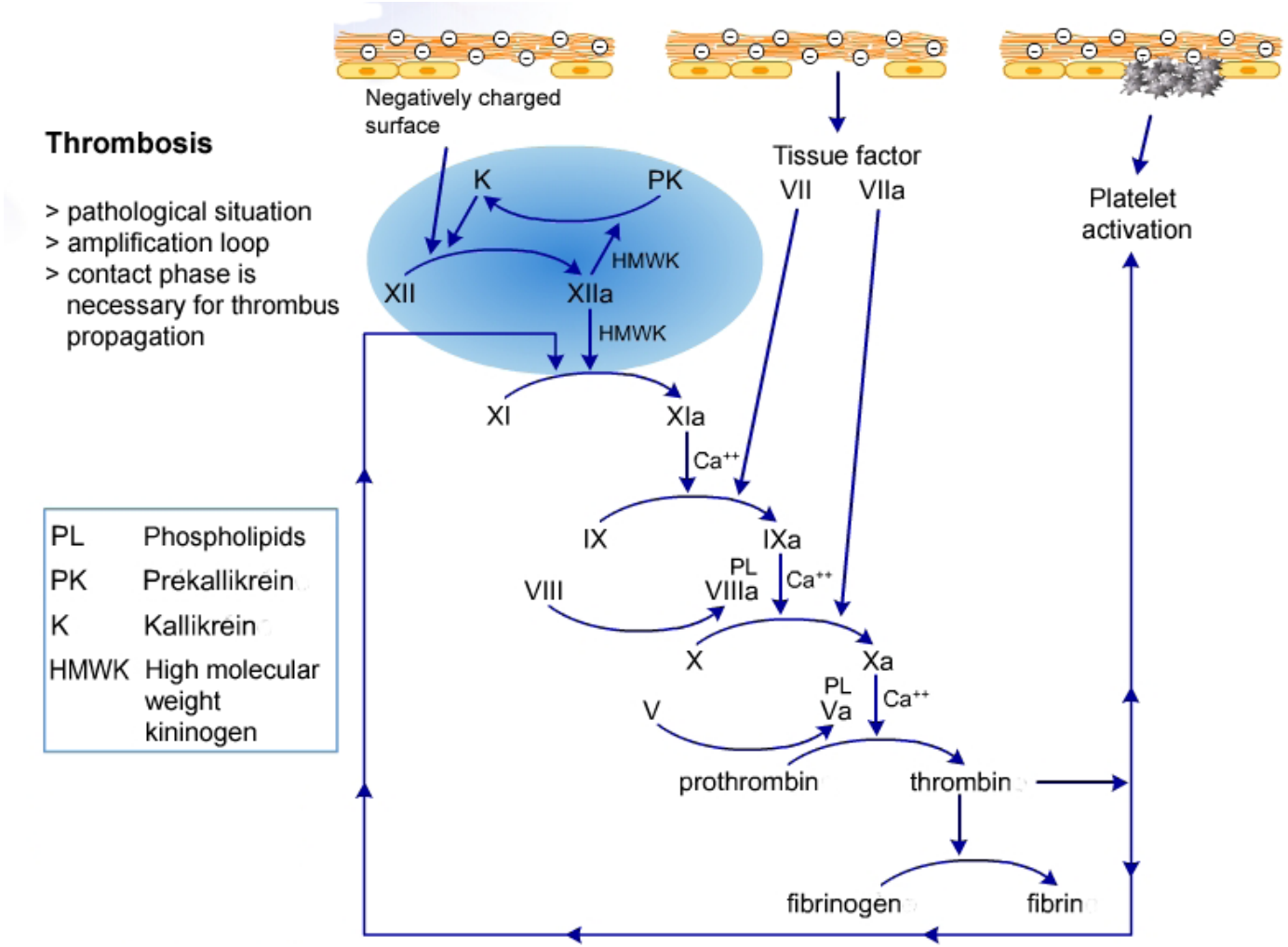
Factor XI may be activated by thrombin as well as by factor XIIa

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

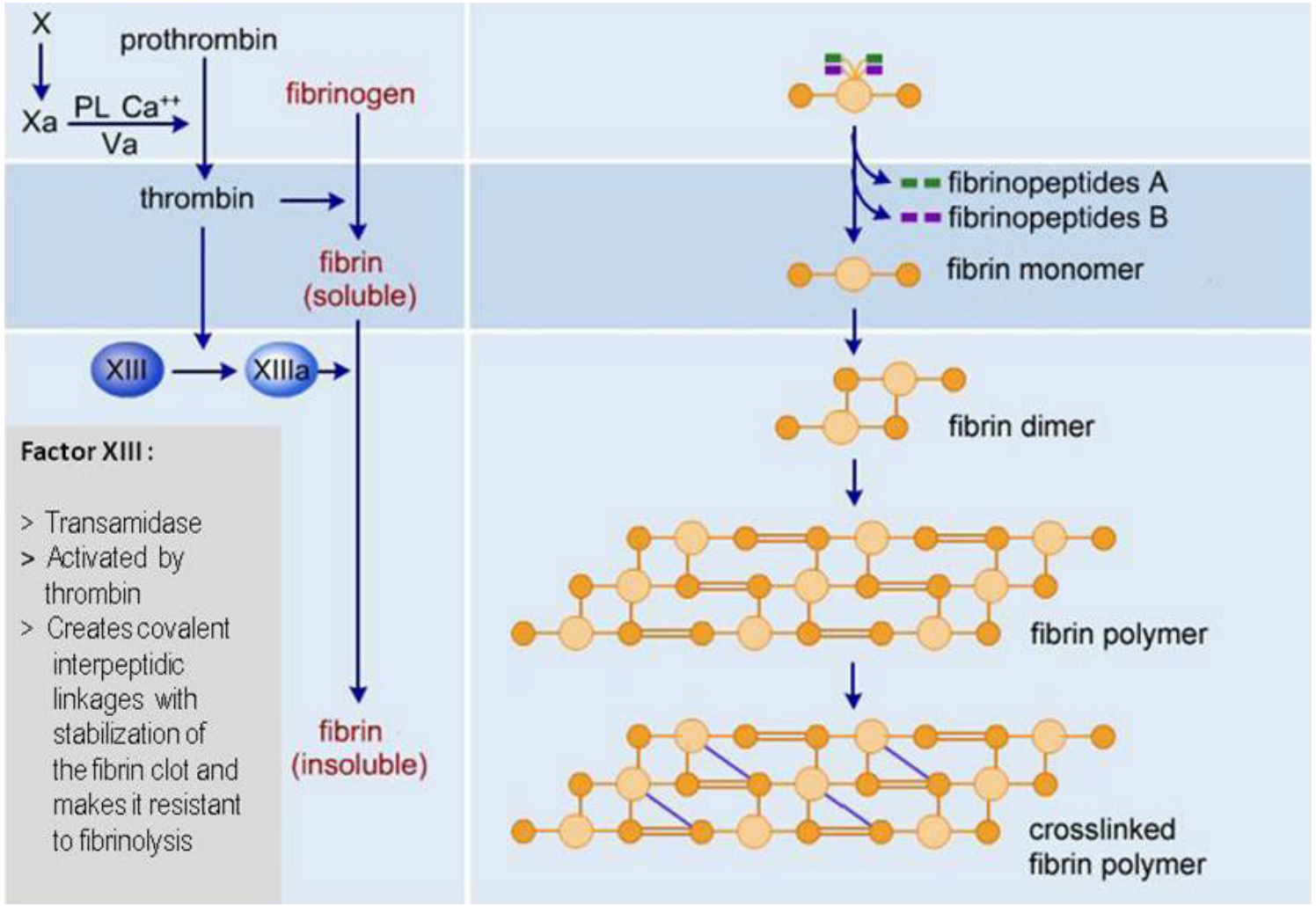
In experimental models factor XI and factor XII deficiencies have antithrombotic effect

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

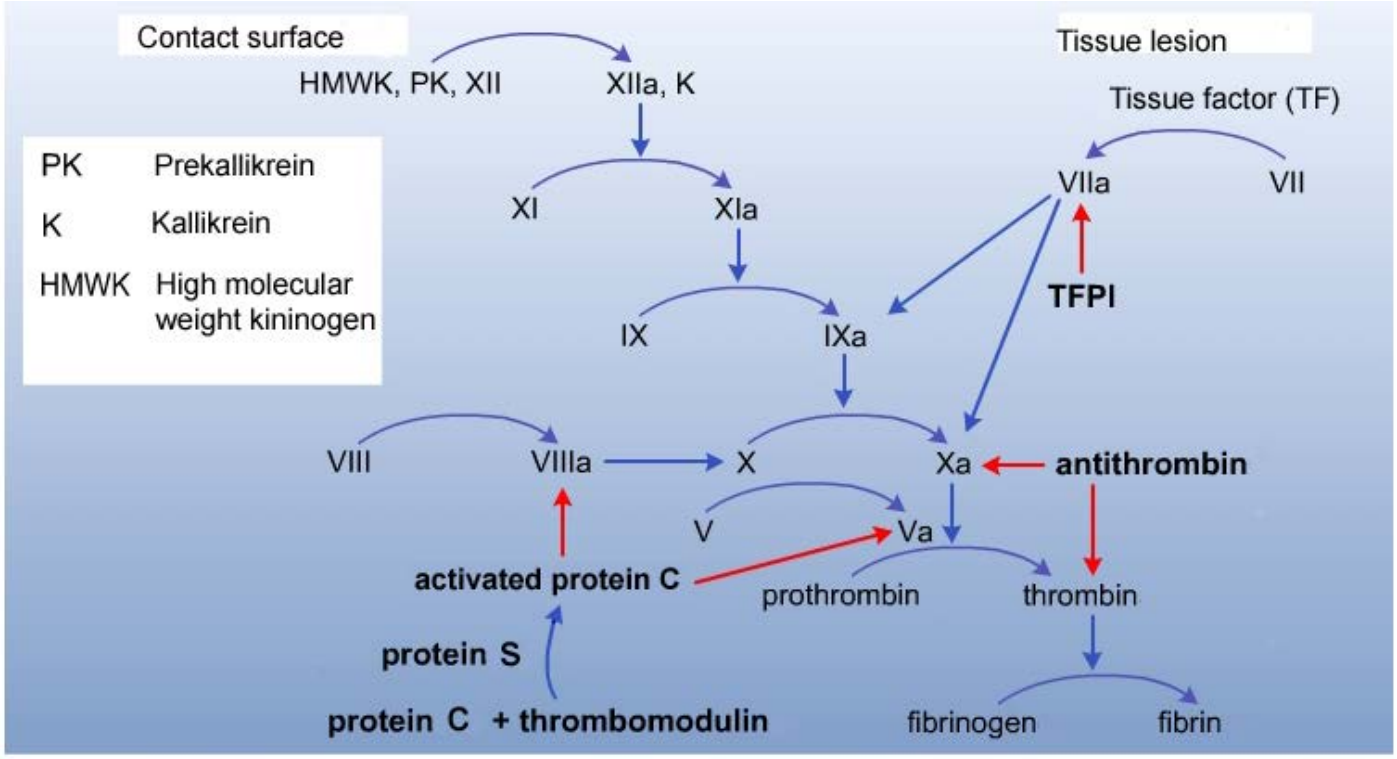
COAGULATION CASCADE (3)
CONCEPTUAL CHANGES (2)



FACTOR XIII AND FIBRIN STABILIZATION



NATURAL ANTICOAGULANTS



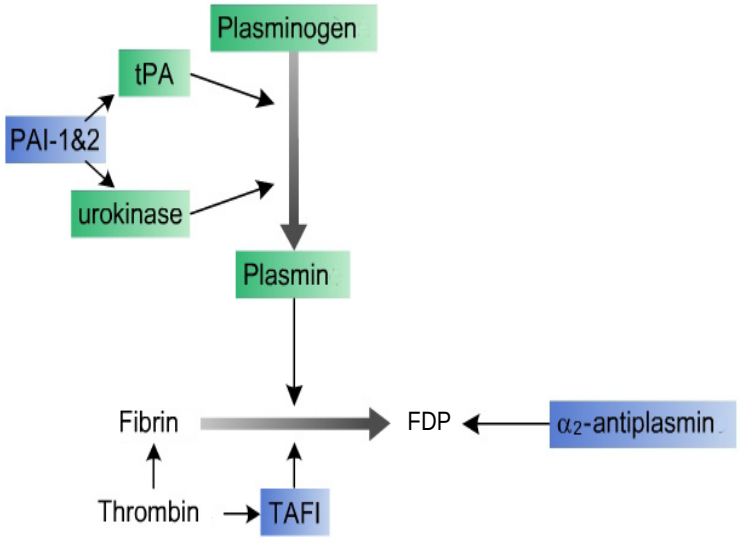
TFPI *(Tissue Factor Pathway Inhibitor)* is an effective inhibitor of factor VII - Tissue factor complex

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

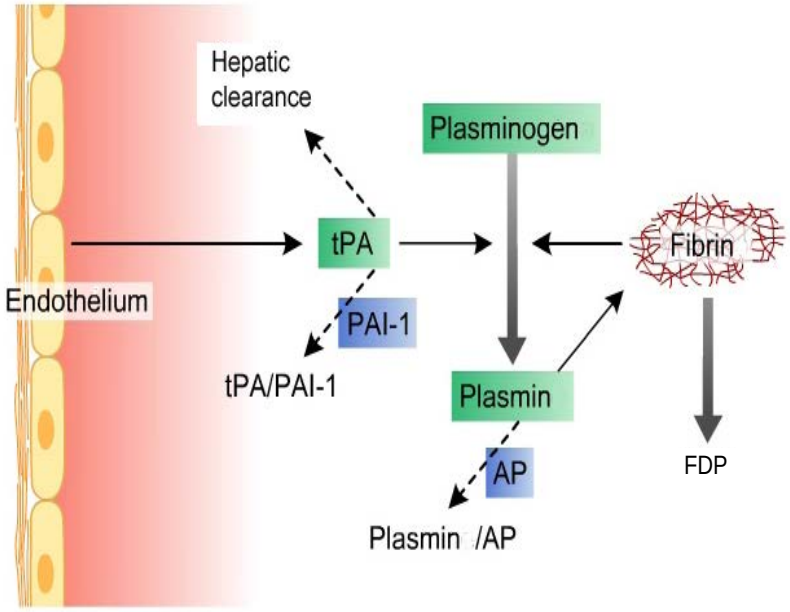
The protein C - protein S system inhibits factors Va and VIIIa

Protein S acts also as TFPI cofactor

TERTIARY HEMOSTASIS
FIBRINOLYSIS



Intravascular fibrinolysis



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

Profibrinolytic proteins
Antifibrinolytic proteins

HEMORRHAGIC SYNDROME
PRIMARY HEMOSTASIS

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-Schönlein)
- 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 neoplasms (cf.p. 121-137)

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, 2011

[2] cf.p. 225
[3] Col / EPI : Collagen / Epinephrin
[4] Col / ADP : Collagen / Adenosin-5'-diphosphate

ACQUIRED THROMBOPATHY

DRUGS

Aspirin	irreversible inhibition of the cyclo-oxygenase
Clopidogrel *(Plavix®)*	irreversible binding of metabolite to ADP receptors type $P2Y_{12}$ on platelets
Prasugrel *(Efient®)*	
Ticagrelor (Brilique®)	reversible antagonist of ADP receptors type $P2Y_{12}$ on platelets
Abciximab *(ReoPro®)*	Fab fragment of humanized chimeric antibody against glycoprotein IIb-IIIa (GP) receptors
Eptifibatide *(Integrilin®)*	Reversible inhibition GPIIb-IIIa receptors
Tirofiban *(Agrastat®)*	

RENAL FAILURE

PARAPROTEINEMIA

MYELOPROLIFERATIVE NEOPLASM OR MYELODYSPLASTIC SYNDROME

HEREDITARY THROMBOPATHY

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

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.p. 228-230

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

SOLITARY PERIPHERAL THROMBOCYTOPENIA
NON IMMUNOLOGICAL

BY ANOMALY OF PLATELET DISTRIBUTION

Hypersplenism

BY PLATELET DESTRUCTION

Alcohol
Disseminated Intravascular Coagulation (DIC)
Extracorporeal circulation
Thrombotic Thrombocytopenic Purpura (TTP)[1]
Hemolytic Uremic Syndrome (HUS)[2]
HELLP[3] syndrome (10% of preeclampsias)
Renal transplant rejection
Allogeneic stem cell or bone marrow transplantation

[1] *TTP : Thrombotic Thrombocytopenic Purpura*
[2] *HUS : Hemolytic Uremic Syndrome*
[3] *HELLP : Hemolysis, Elevated Liver function tests, Low Platelets (in pregnancy)*

SOLITARY PERIPHERAL THROMBOCYTOPENIA (2)
IMMUNE

PRIMARY

Primary immune thrombocytopenia (PIT), cf. next page

SECONDARY

Due to autoantibody or immune complexes

Drugs : Quinine

Heparin : Heparin-induced thrombocytopenia (HIT[1])

Type I : Early onset thrombocytopenia (< 24 h) and transient

Type II : 0.5-5% of patients treated by UFH[2]

Thrombocytopenia onset on treatment day 4 to 20

Thrombotic complications

Presence of anti-PF4[3]-Heparin (IgG) antibodies

Infection *(Helicobacter Pylori, hepatitis C, HIV, CMV, varicella, herpes zoster, malaria)*

Autoimmune disease *(SLE[4], Evans syndrome[5])*

Common variable type immune deficiency

Lymphoid neoplasm, cancer

Bone marrow / hematopoietic stem cell transplantation

Due to alloantibody

Neonatal thrombocytopenia

Posttransfusion purpura

[1] HIT : Heparin Induced Thrombocytopenia
[2] UFH : Unfractionated Heparin
[3] PF4 : Platelet Factor 4
[4] Systemic lupus erythematosus
[5] Autoimmune hemolytic anemia and thrombocytopenia

PRIMARY IMMUNE THROMBOCYTOPENIA (Primary ITP[1])

Acquired solitary thrombocytopenia (platelets < 100 G / L) of immunological origin

Antibodies directed against platelets and megakaryocytes, probable ↘ of thrombopoietin (TPO)

Diagnosis by exclusion of all other causes of thrombocytopenia

Clinical presentation :

Children : Often preceded by viral infection
Course usually benign with frequent spontaneous remission

Adults : Persisting thrombocytopenia, often relapsing or chronic
Depending on duration : Newly diagnosed : ≤ 3 months
Persistent : 3-12 months
Chronic : > 12 months

Bone marrow examination :

Age > 60 : Exclusion of myelodysplastic syndrome
Age < 60 : If signs of neoplasm or systemic disorder
Treatment refractoriness, relapse < 6 months
Prior to splenectomy or other second line therapy

Treatment :

Minor bleeding	Prednisone 1-2 mg / kg qd orally, Dexamethasone 40 mg orally for 4 d
Major bleeding	Prednisone orally or Methyprednisolone 125-1'000 mg IV, d 1-5 Immunoglobulins IV : 0.4 g / kg d 1-5 or 1 g / kg, d 1-2 If necessary platelet transfusion(s)
Refractory ITP	Splenectomy Rituximab, TPO receptor agonists (Romiplostim, Eltrombopag) Azathioprine, Micophenolate mofetil, Danazol, Cyclosporin A, Cyclophosphamide, Alemtuzumab (humanized anti-CD52), combined chemotherapy, Etanercept (TNF-α inhibitor), allogeneic HST

[1] ITP : Immune 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, HCV, EBV, CMV)

SLE[1] serology

Thyroid function tests

Helicobacter pylori screening (to be considered in refractory or relapsing ITP[2])

Anti-HLA antibodies

Antiplatelet antibodies *(this test is frequently difficult to realise, as it needs a platelet count rarely high enough at diagnosis)*

[1] **Systemic lupus erythematosus**
[2] **ITP : Primary Immune Thrombocytopenia**

HEMORRHAGIC SYNDROME
SECONDARY HEMOSTASIS (COAGULATION)

CONSTITUTIONAL ANOMALIES

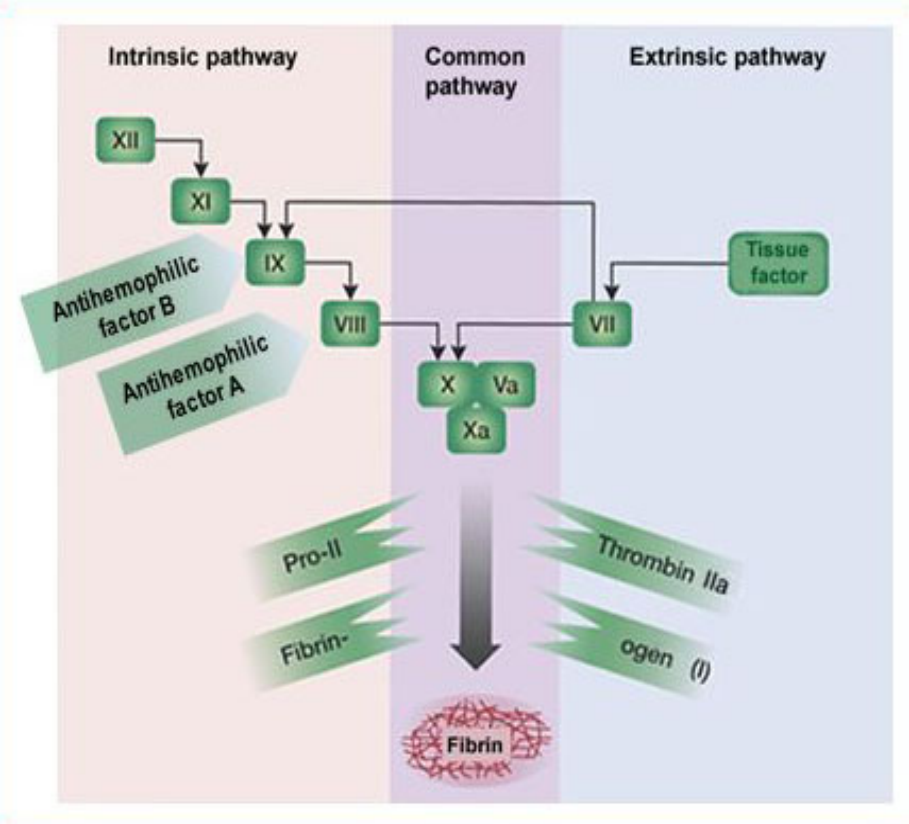
- Hemophilias (factors VIII, IX), von Willebrand disease, cf.p. 233-235
- Fibrinogen, factors II, V, VII, X, XI, XIII deficiencies

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;q21)
  - Obstetrical complications
    - Amniotic liquid embolism
    - Placental retention
    - Eclampsia
    - Septic abortion
  - Invasive surgery
  - Extended burns
  - Transfusion complications
  - Vascular malformations (Kasabach-Merritt syndrom)

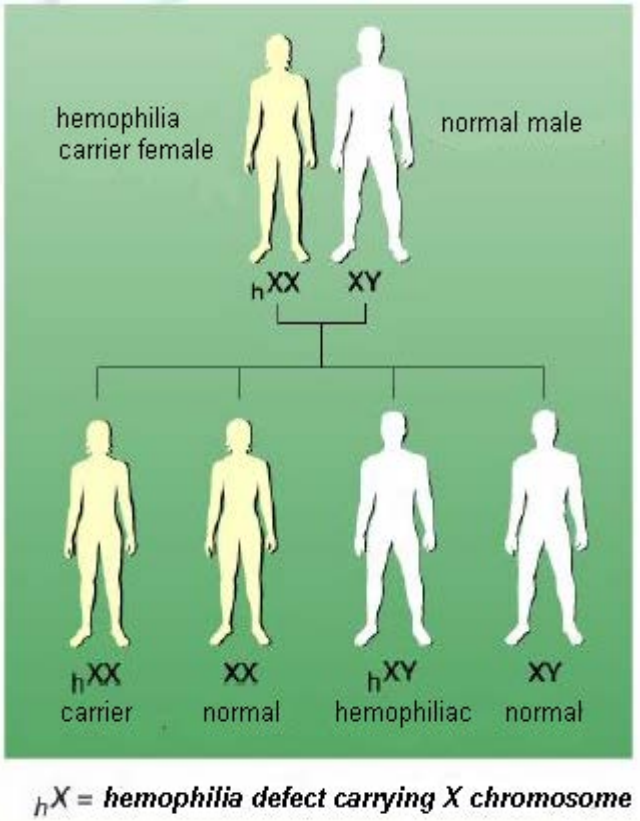
- Coagulation inhibitors (circulating anticoagulants)
  - Alloaantibodies against factor VIII (5-10% of hemophilia patients)
  - Autoantibodies against factor VIII (acquired hemophilia A) : pregnancy, postpartum, rheumatoid arthritis, lupus erythematosus, cancer, drugs

HEMOPHILIA



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, codeine, opiates; aspirin and NSAID[3] absolutely contraindicated except Celecoxib, Celebrex®*

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 (NovoSeven ®), FEIBA NF® ("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, thereof only approximately 1/10'000 with symptoms)

Mucosal and cutaneous bleeding (epistaxis, menorrhagia)

Biological signs : PFA-100^{TM} prolonged, PT *(Prothrombin time*) normal, prolonged aPTT, ↘ Factor VIII, ↘ Factor von Willebrand *(antigen and activity)*

THROMBOEMBOLIC DISEASE

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

MAIN RISK FACTORS

Arterial thrombosis :
- Arterial hypertension
- Hyperlipidemia, diabetes mellitus
- Tobacco smoking

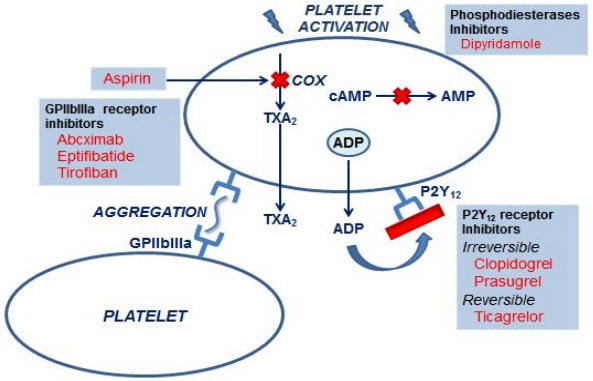
Venous thrombosis :
- Stasis (bed rest, dehydration, ⇗ plasma viscosity, varicose veins
- Surgery (in particular hip and abdomen)
- Pregnancy and post-partum
- Estrogens, contraceptive pills
- Cancer
- Behçet disease
- Constitutional coagulations anomalies *(cf. table)*

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	3 – 10	15	3 – 7
Factor V Leiden homozygoous	0.06 – 0.25	1.5	50 – 80
Heterozygous prothrombin gene mutation G20210A	1 – 3	5 – 6	2 – 4

Venous or arterial thrombosis :
- Myeloproliferative neoplasm
- Heparin induced thrombocytopenia (HIT)
- Hyperhomocysteinemia
- Lupus anticoagulant, antiphospholipid syndrome :
  - Paradoxical aPTT prolongation in the context of venous or arterial thrombosis, recurrent fetal loss or other pregnancy related complications
  - Primary or secondary : SLE ("Lupus anticoagulant"), infections, neoplasms, drugs
  - Treatment : cf.p. 240 Treatment algorithm

THROMBOEMBOLIC DISEASE
TREATMENT AND PREVENTION

ANTIPLATELET DRUGS



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

Clopidogrel (Plavix®) **and** ***Prasugrel (Efient®)*** **cause irreversible inhibition of $P2Y_{12}$ ADP receptor**

Ticagrelor (Brilique®) **is a reversible antagonist of $P2Y_{12}$ ADP receptor**

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

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

Etifibatide (Integrilin®) **and** ***Tirofiban (Agrastat®)*** **reversibly inhibit 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**
Low molecular weight : Nadroparin *(Fraxiparin® (Fraxiforte®),* Dalteparin *(Fragmin®),* Enoxaparin *(Clexane®),* Certoparin *(Sandoparin®)*	**Fixation and activation of AT III[1], 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[1], anti-Xa activity, no effect on platelets**
Hirudin analogues : Lepirudin *(Refludan®),* Bivalirudin *(Angiox®)*	**Direct inhibition of thrombin**
Argatroban *(Argatra®)* **Dabigatran *(Pradaxa®)***	
Pentasaccharide : Fondaparinux *(Arixtra®)* **Rivaroxaban *(Xarelto®)*** **Apixaban *(Eliquis®)***	**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. and 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)*

UNFRACTIONATED HEPARIN[1,2] :
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

LOW MOLECULAR WEIGHT HEPARIN :
e.g. : Enoxaparin = 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
Caution by creatinin clearance < 30 mL / min

FONDAPARINUX (Arixtra®) :
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 control of platelet count needed

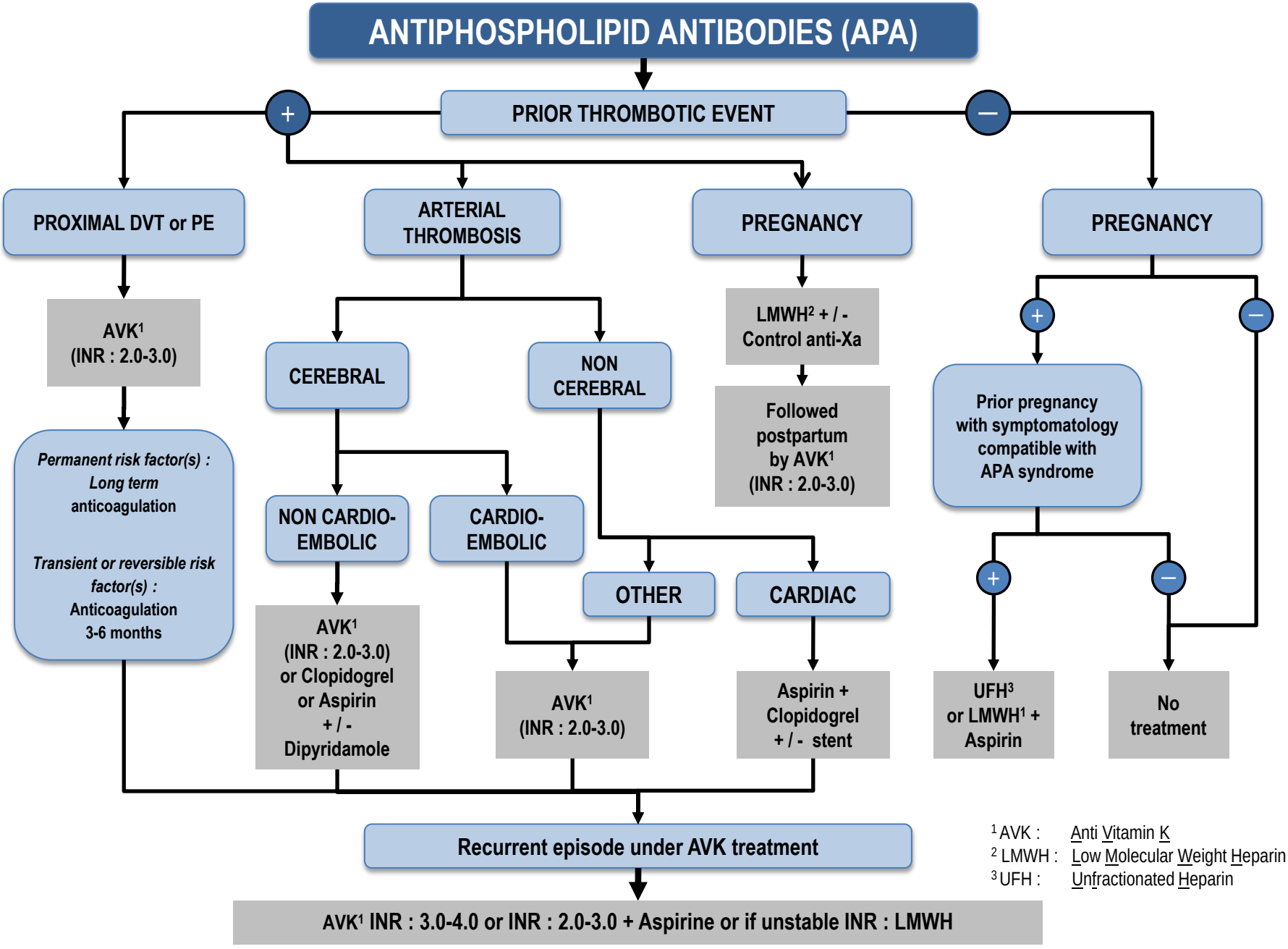
EARLY SWITCH TO ANTIVITAMIN K DRUGS *(Acenocoumarol : Sintrom®)*

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 and 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 week
Proximal deep vein thrombosis / Secondary pulmonary embolism	3 months
Deep vein thrombosis / Idiopathic pulmonary embolism	6-12 months (or more if persisting risk factor without increased bleeding risk)
Recurrent 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)

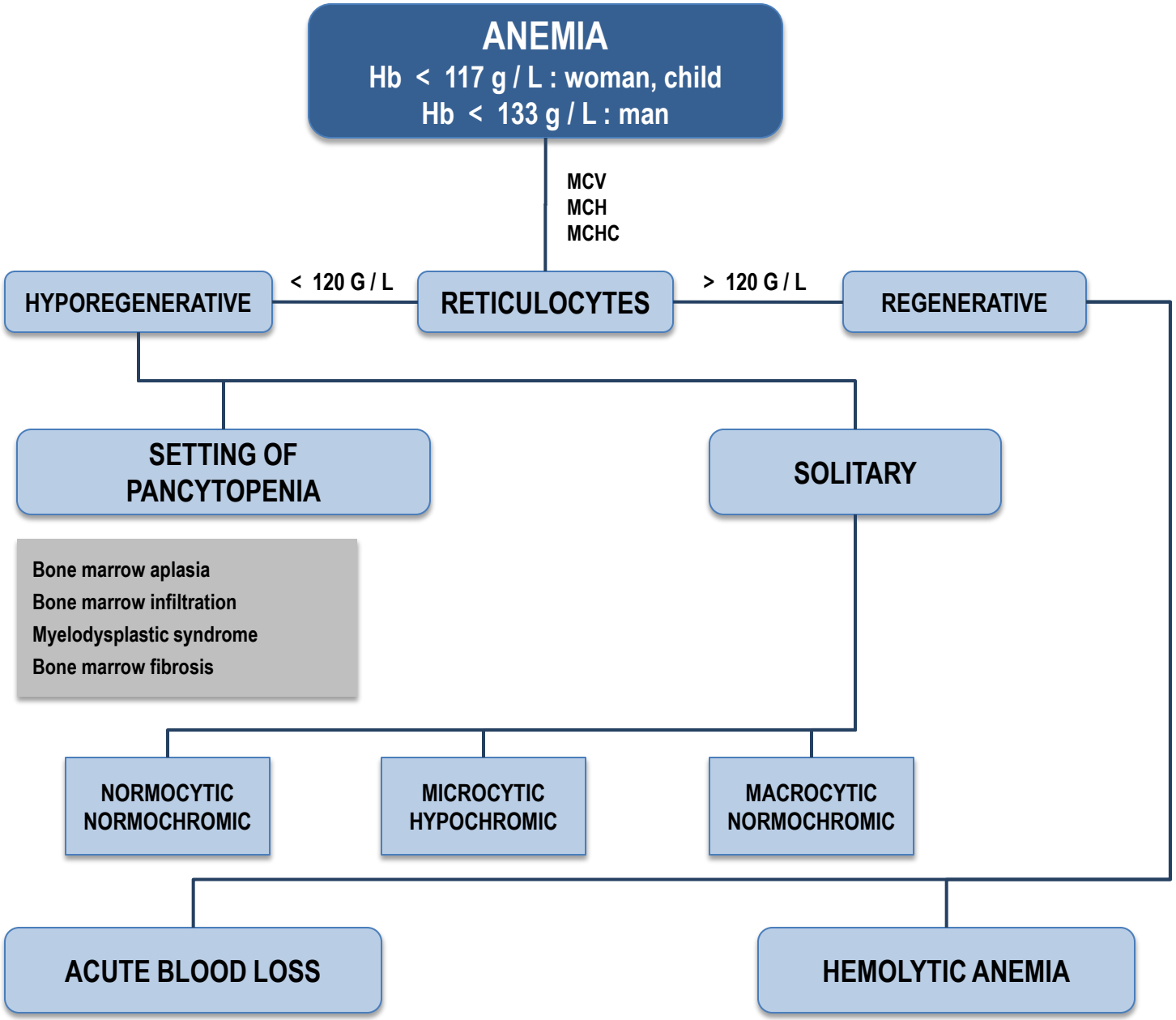


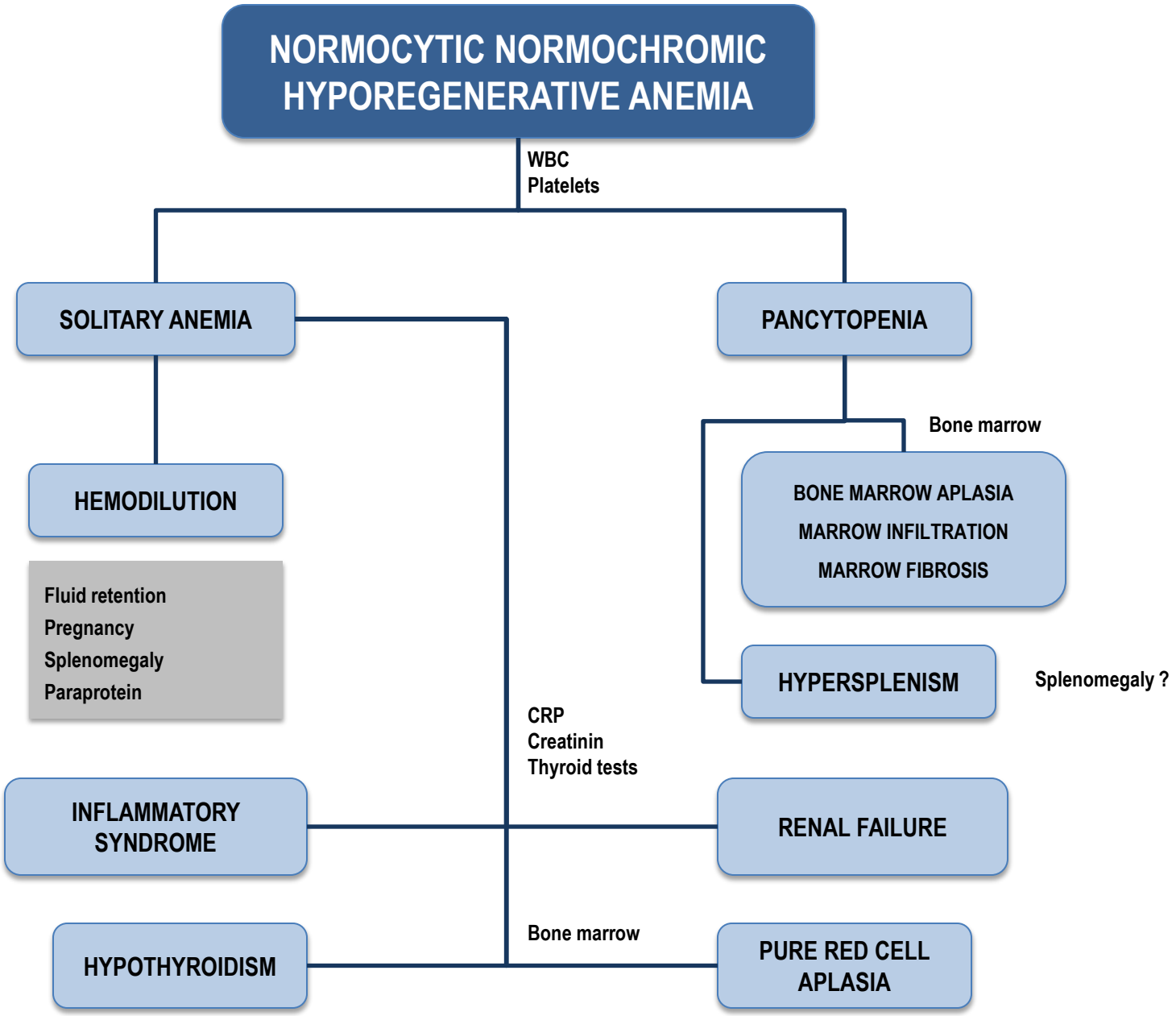
[1] AVK : Anti Vitamin K
[2] LMWH : Low Molecular Weight Heparin
[3] UFH : Unfractionated Heparin

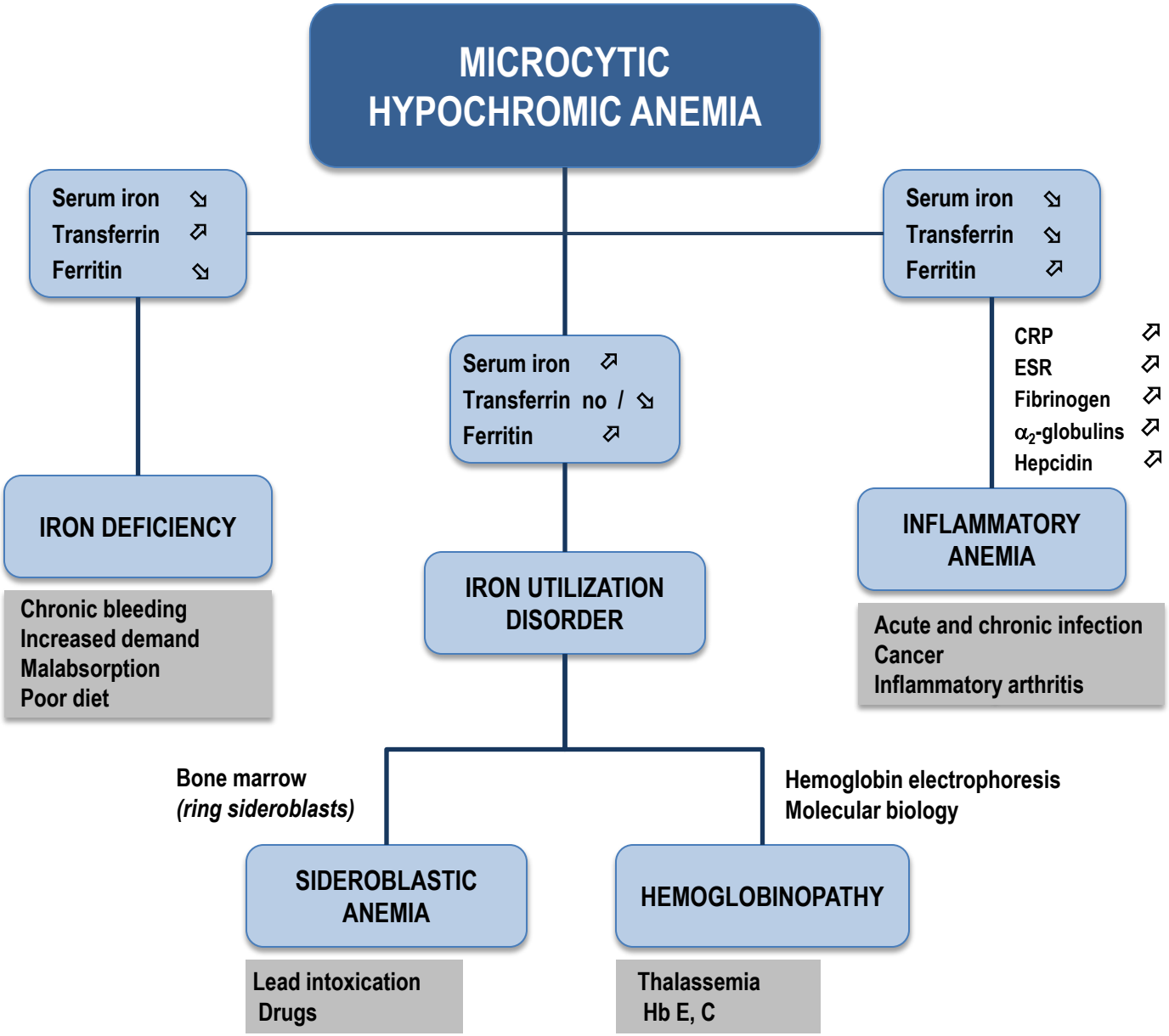
Modified from Giannakopoulos B., Krilis S.A.: How I treat the antiphospholipid syndrome. Blood 2009; 114 : 2020-2030.

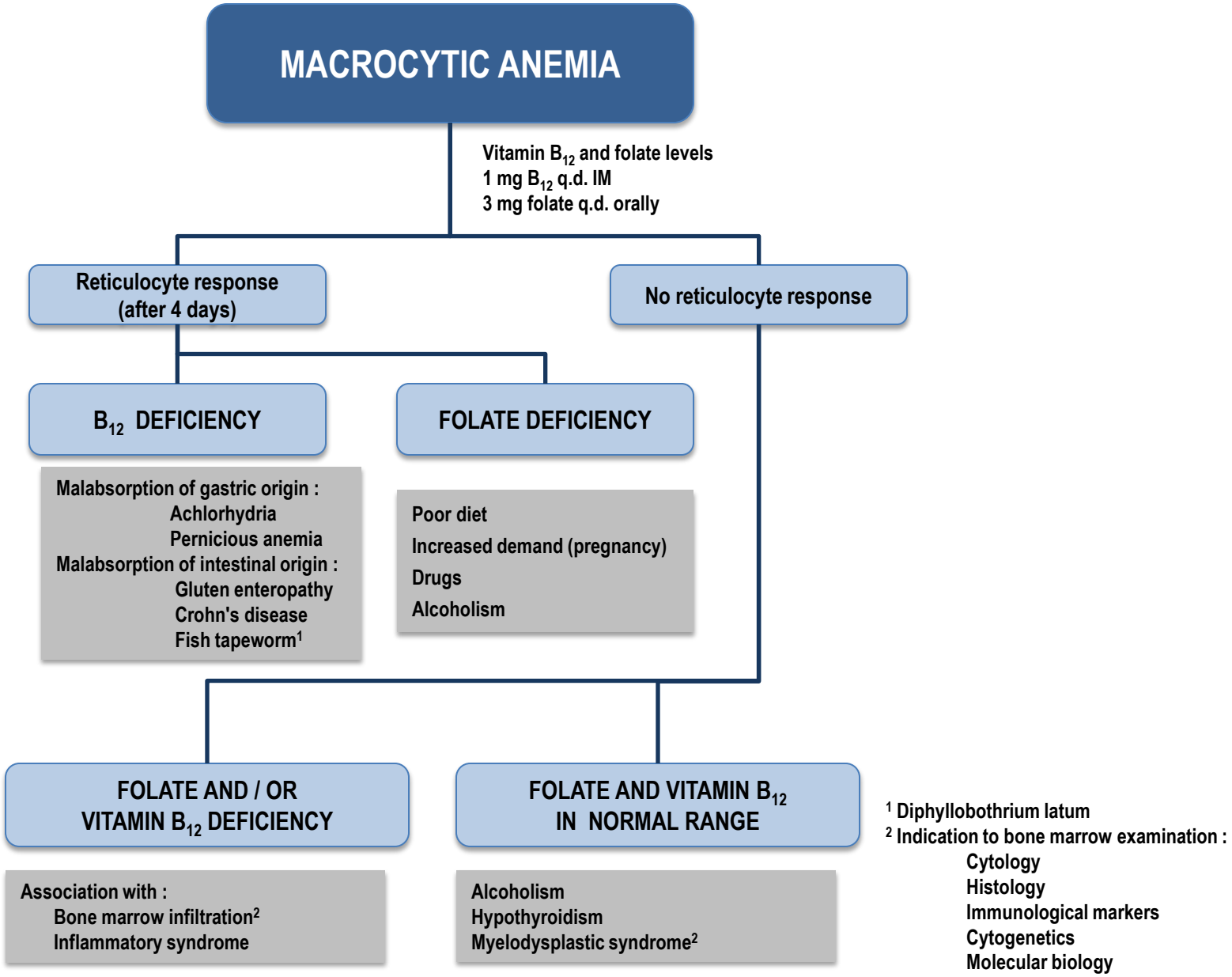
Part 4

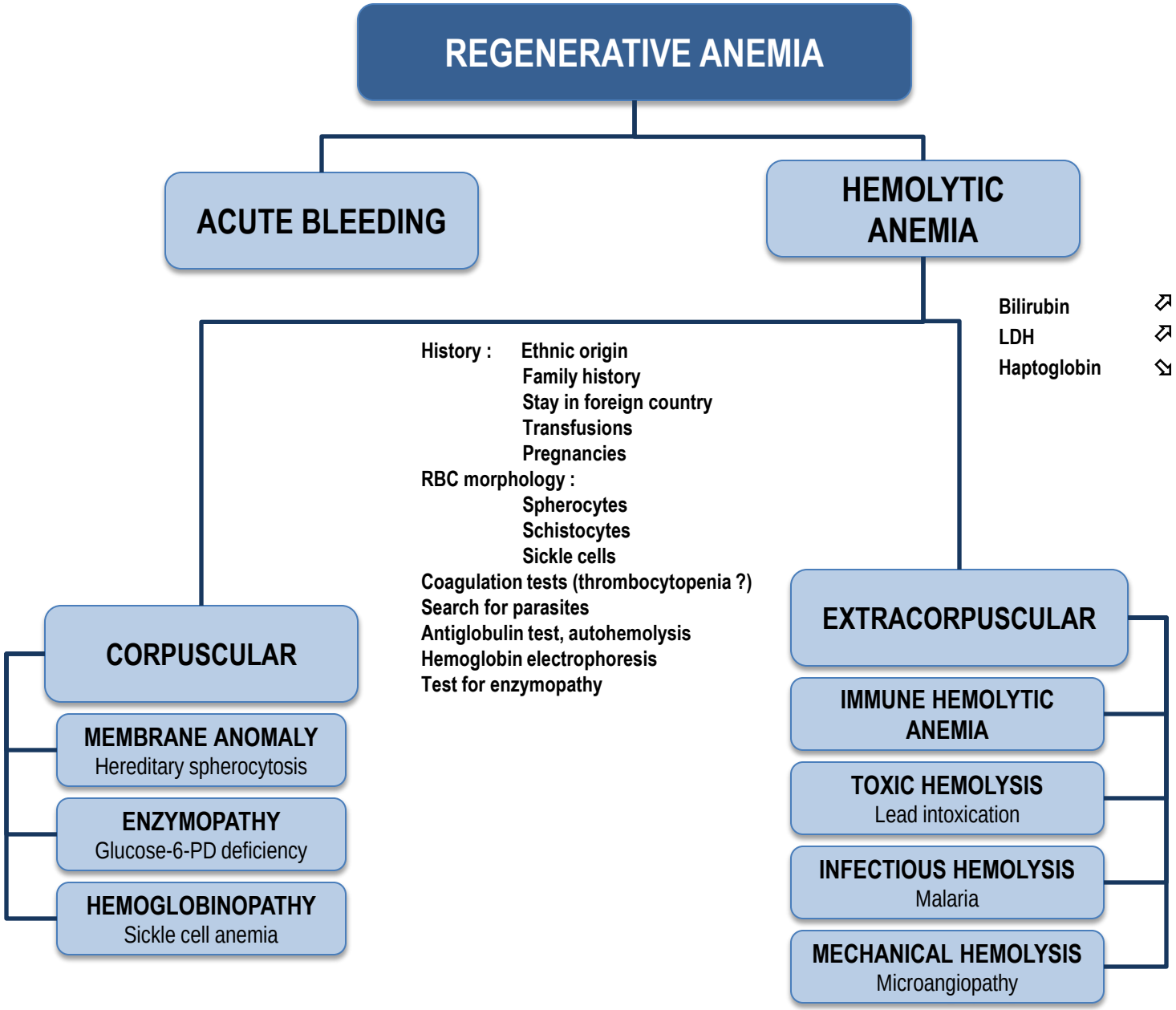
DIAGNOSTIC ALGORITHMS

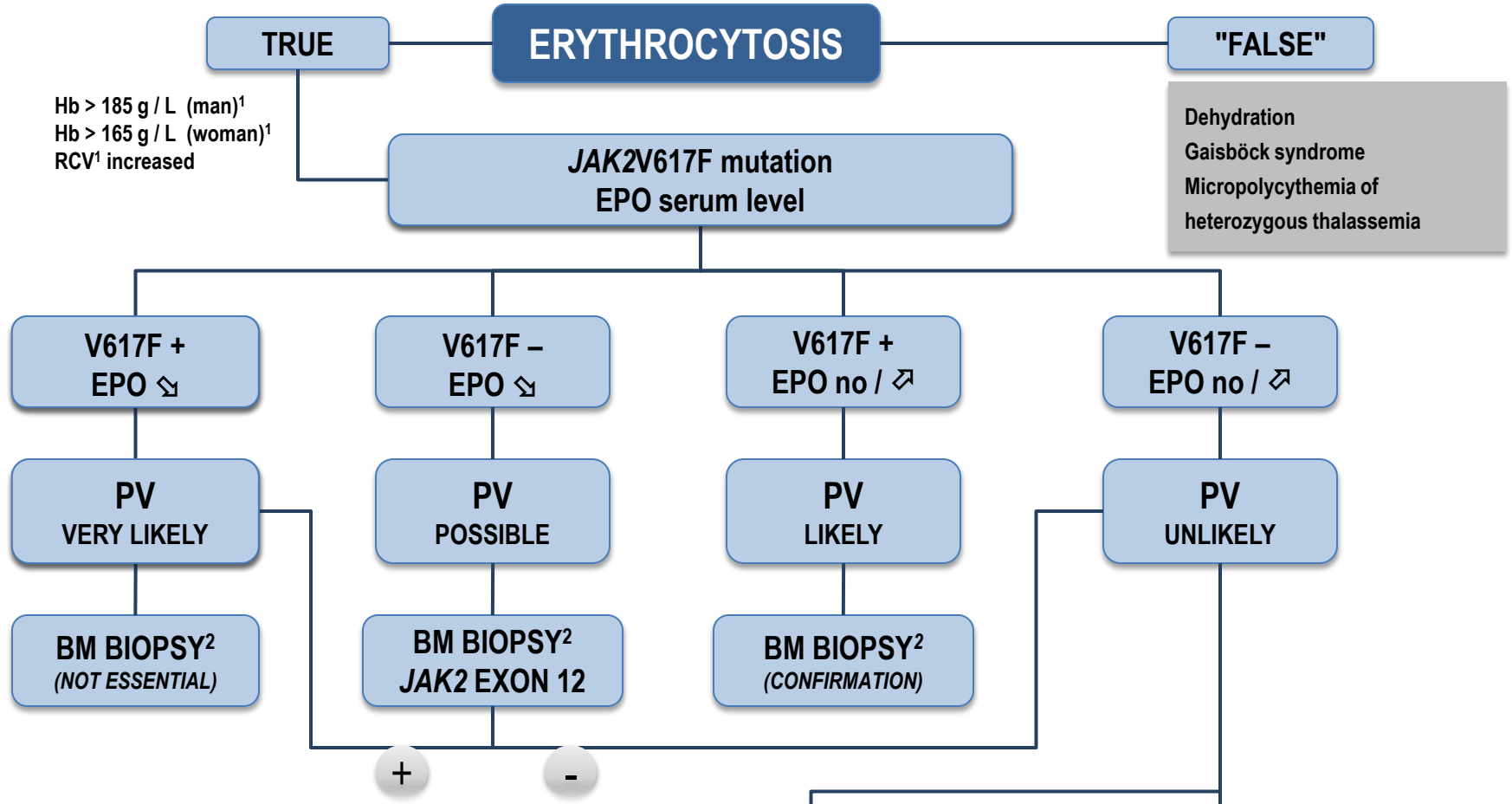

ANEMIA
Hb < 117 g / L : woman, child
Hb < 133 g / L : man
MCV
MCH
MCHC
HYPOREGENERATIVE
< 120 G / L
RETICULOCYTES
> 120 G / L
REGENERATIVE
SETTING OF PANCYTOPENIA
SOLITARY
Bone marrow aplasia
Bone marrow infiltration
Myelodysplastic syndrome
Bone marrow fibrosis
NORMOCYTIC NORMOCHROMIC
MICROCYTIC HYPOCHROMIC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

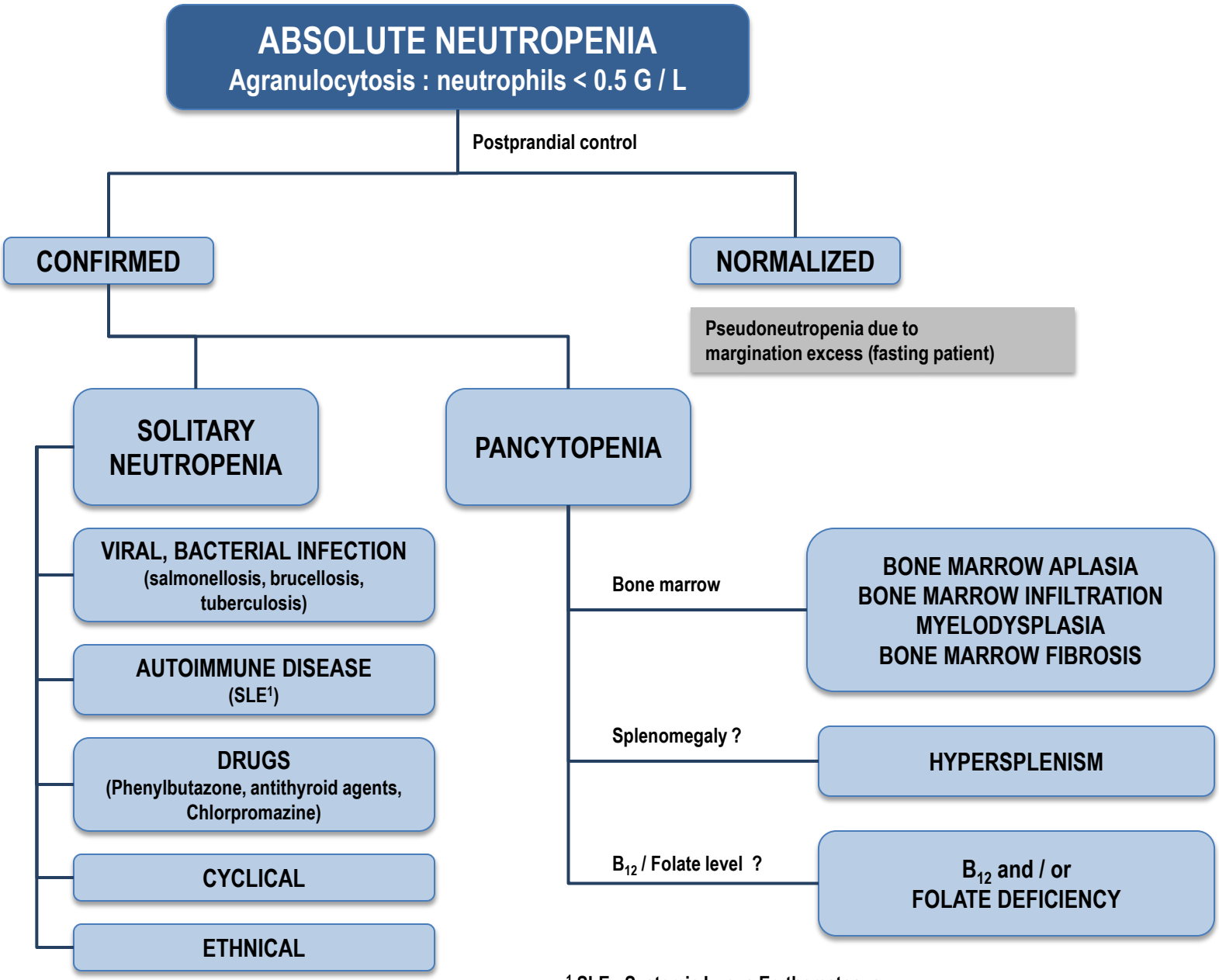

MACROCYTIC ANEMIA
Vitamin B12 and folate levels
1 mg B12 q.d. IM
3 mg folate q.d. orally
Reticulocyte response (after 4 days)
No reticulocyte response
B12 DEFICIENCY
FOLATE DEFICIENCY
Malabsorption of gastric origin :
Achlorhydria
Pernicious anemia
Malabsorption of intestinal origin :
Gluten enteropathy
Crohn's disease
Fish tapeworm1
Poor diet
Increased demand (pregnancy)
Drugs
Alcoholism
FOLATE AND / OR VITAMIN B12 DEFICIENCY
FOLATE AND VITAMIN B12 IN NORMAL RANGE
Association with :
Bone marrow infiltration2
Inflammatory syndrome
Alcoholism
Hypothyroidism
Myelodysplastic syndrome2
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

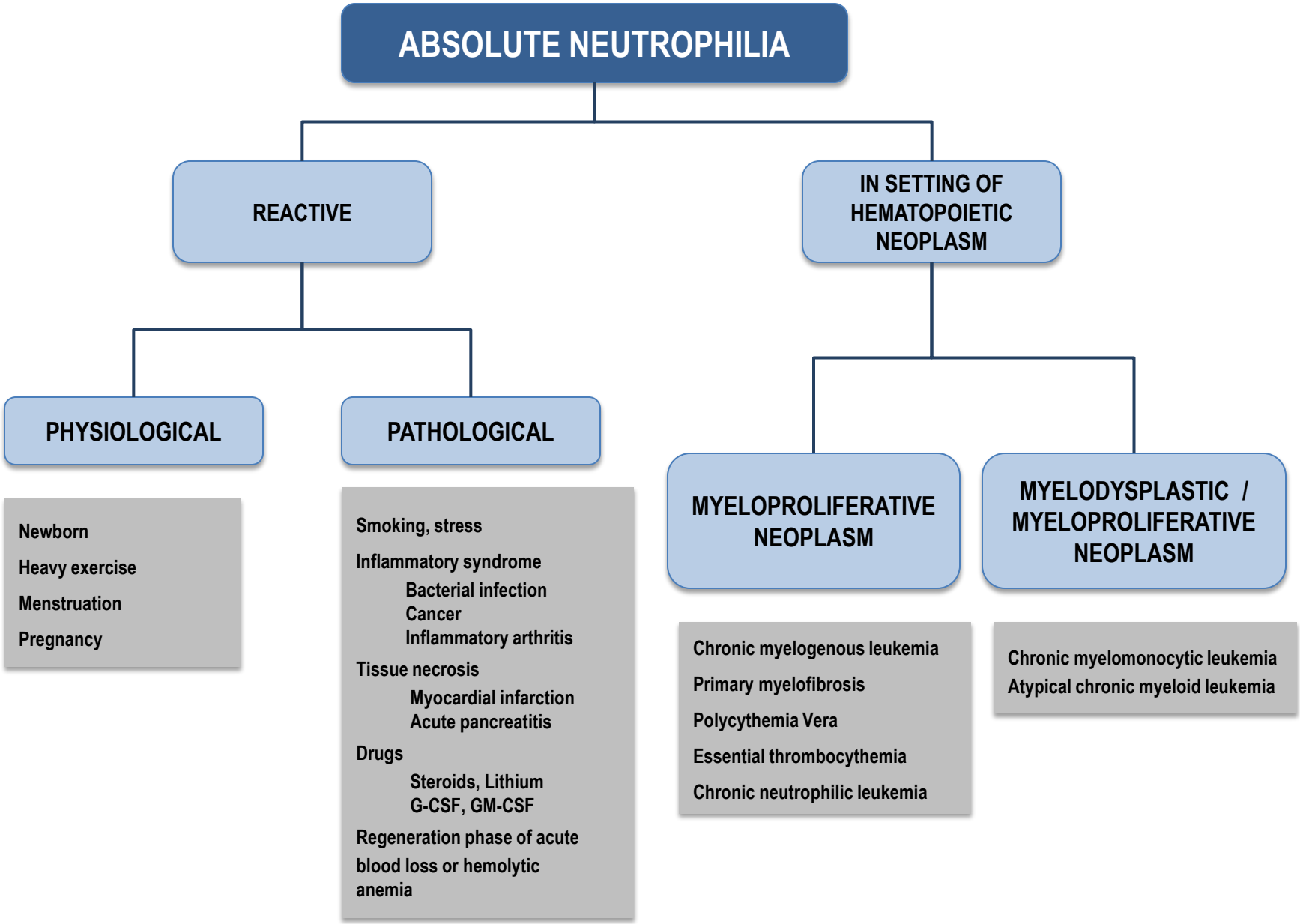


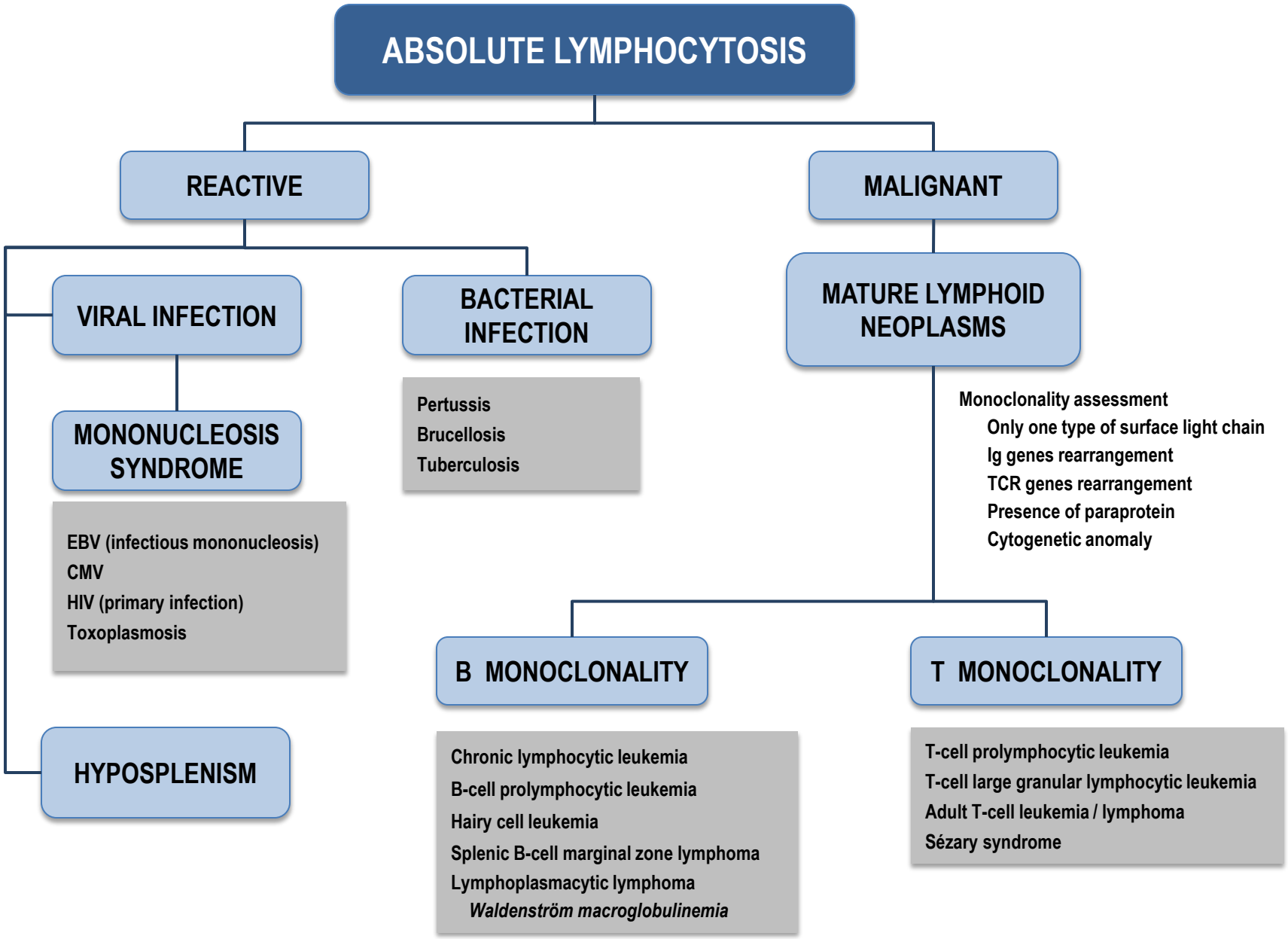
[1] or : Hb or Hct > 99th percentile of reference range for age, gender or altitude of residence
or : Hb > 170 g / L (men) or > 150 g / L (women) in case of sustained increase of ≥ 20 g / L from baseline that is not caused by correction of iron deficiency
or : RCV : Red Cell Volume > 25% over normal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

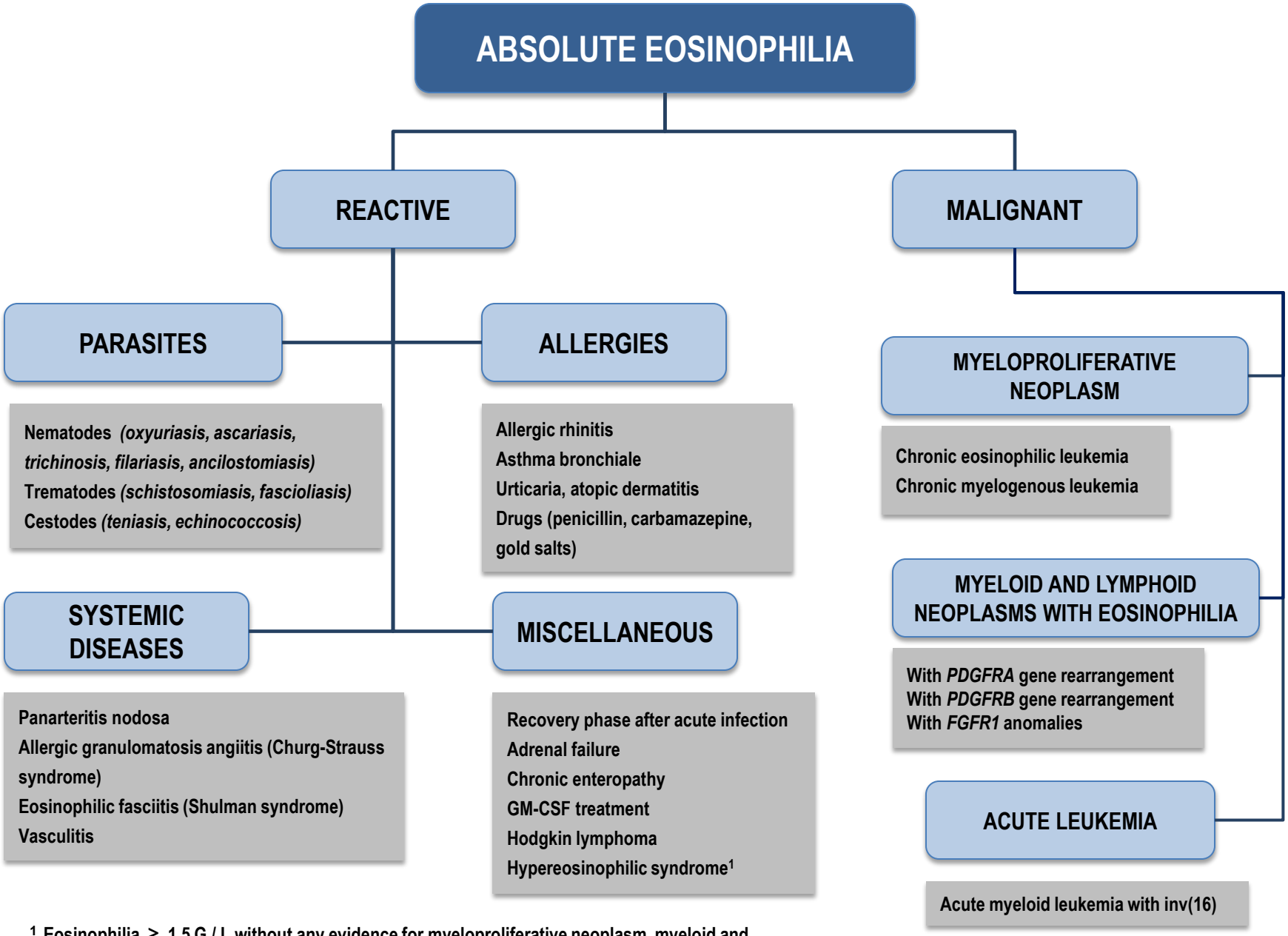
[2] Hypercellularity, increased number of megakaryocytes with morphological anomalies, reticulin fibrosis



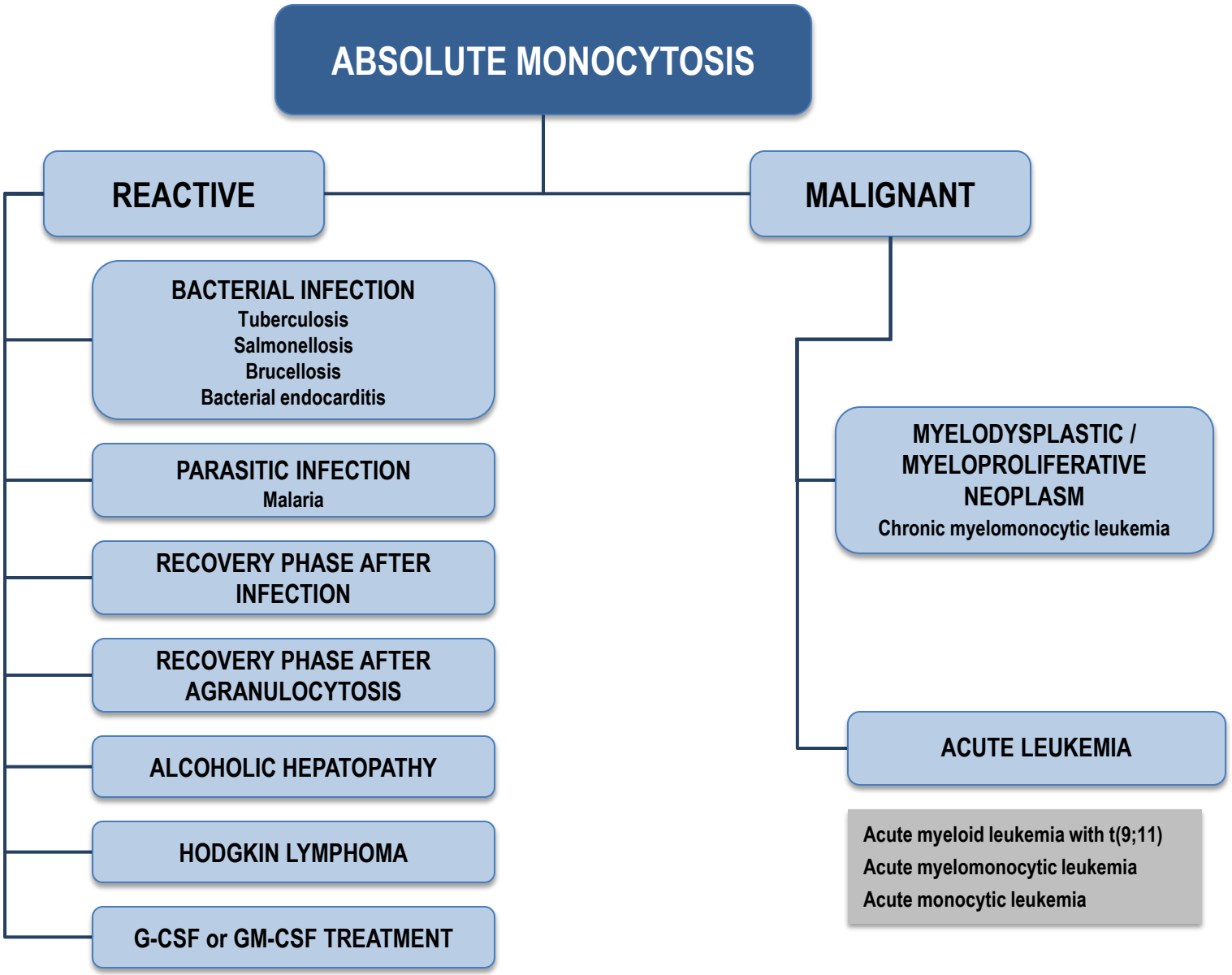
[1] SLE : Systemic Lupus Erythematosus

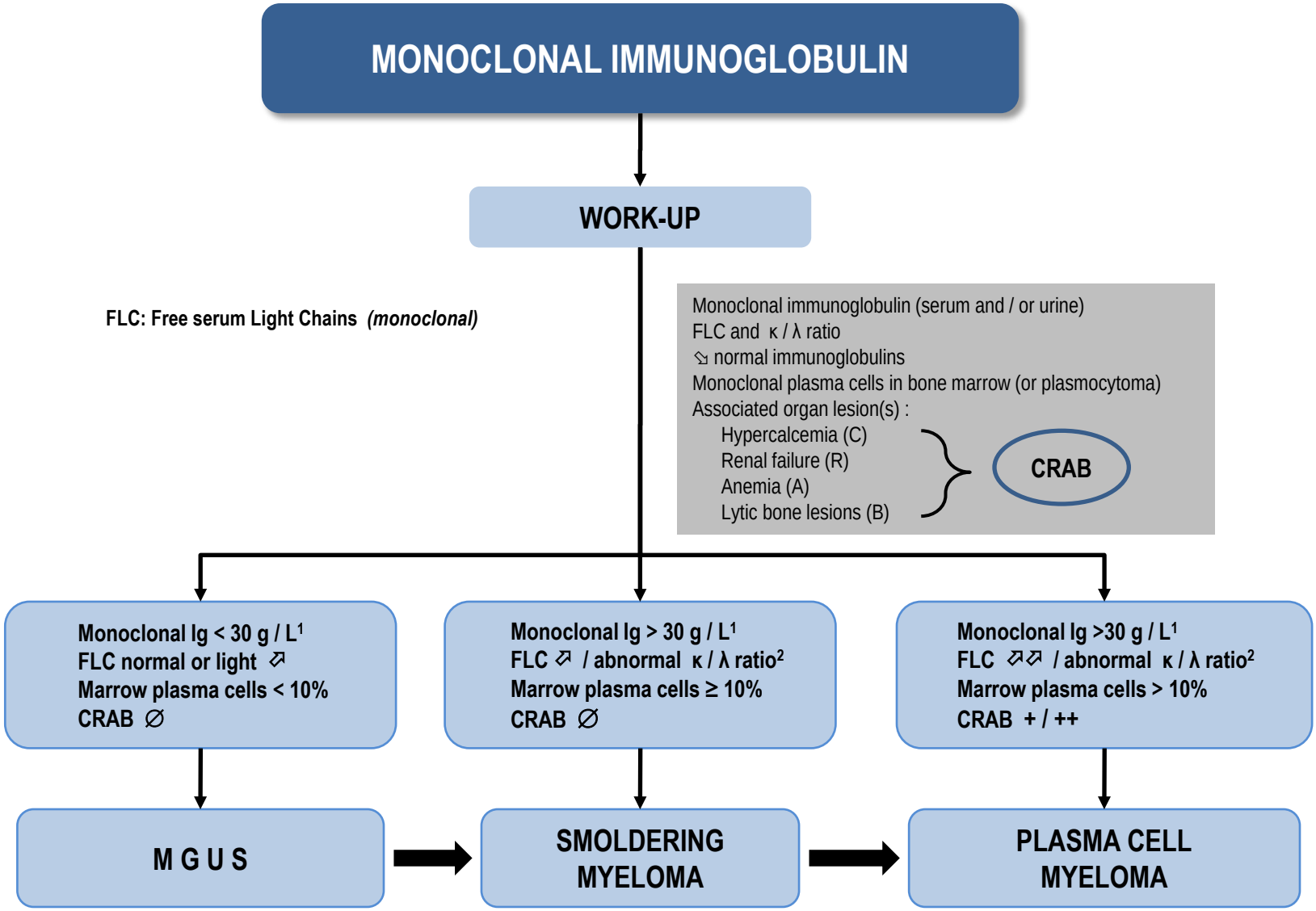

ABSOLUTE NEUTROPHILIA
REACTIVE
IN SETTING OF HEMATOPOIETIC NEOPLASM
PHYSIOLOGICAL
PATHOLOGICAL
MYELOPROLIFERATIVE NEOPLASM
MYELODYSPLASTIC / MYELOPROLIFERATIVE NEOPLASM
Newborn
Heavy exercise
Menstruation
Pregnancy
Smoking, stress
Inflammatory syndrome
Bacterial infection
Cancer
Inflammatory arthritis
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



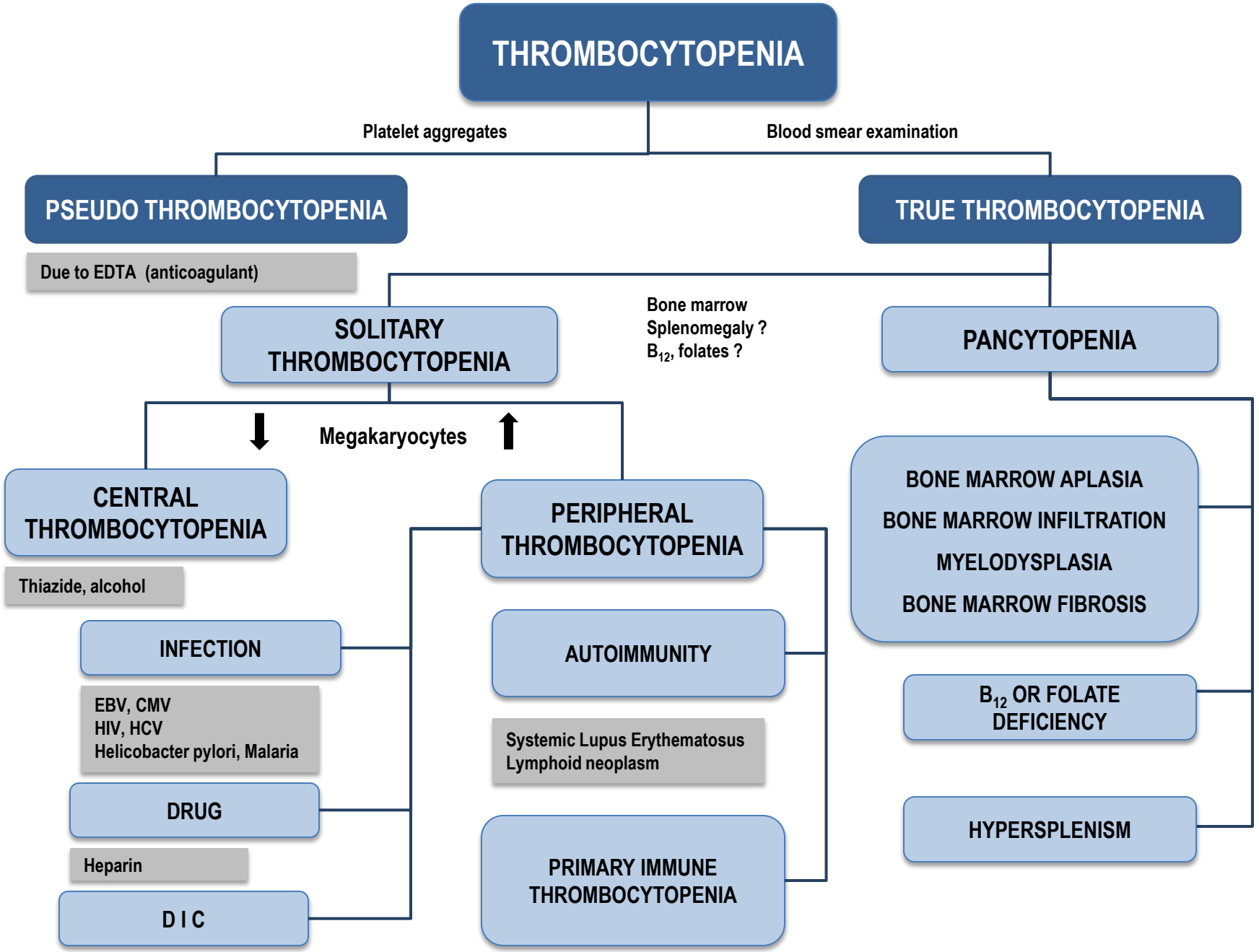
[1] Eosinophilia ≥ 1.5 G / L without any evidence for myeloproliferative neoplasm, myeloid and lymphoid neoplasm with eosinophilia and *PDGFRA*, *PDGFRB* or *FGFR1* anomaly, or AML

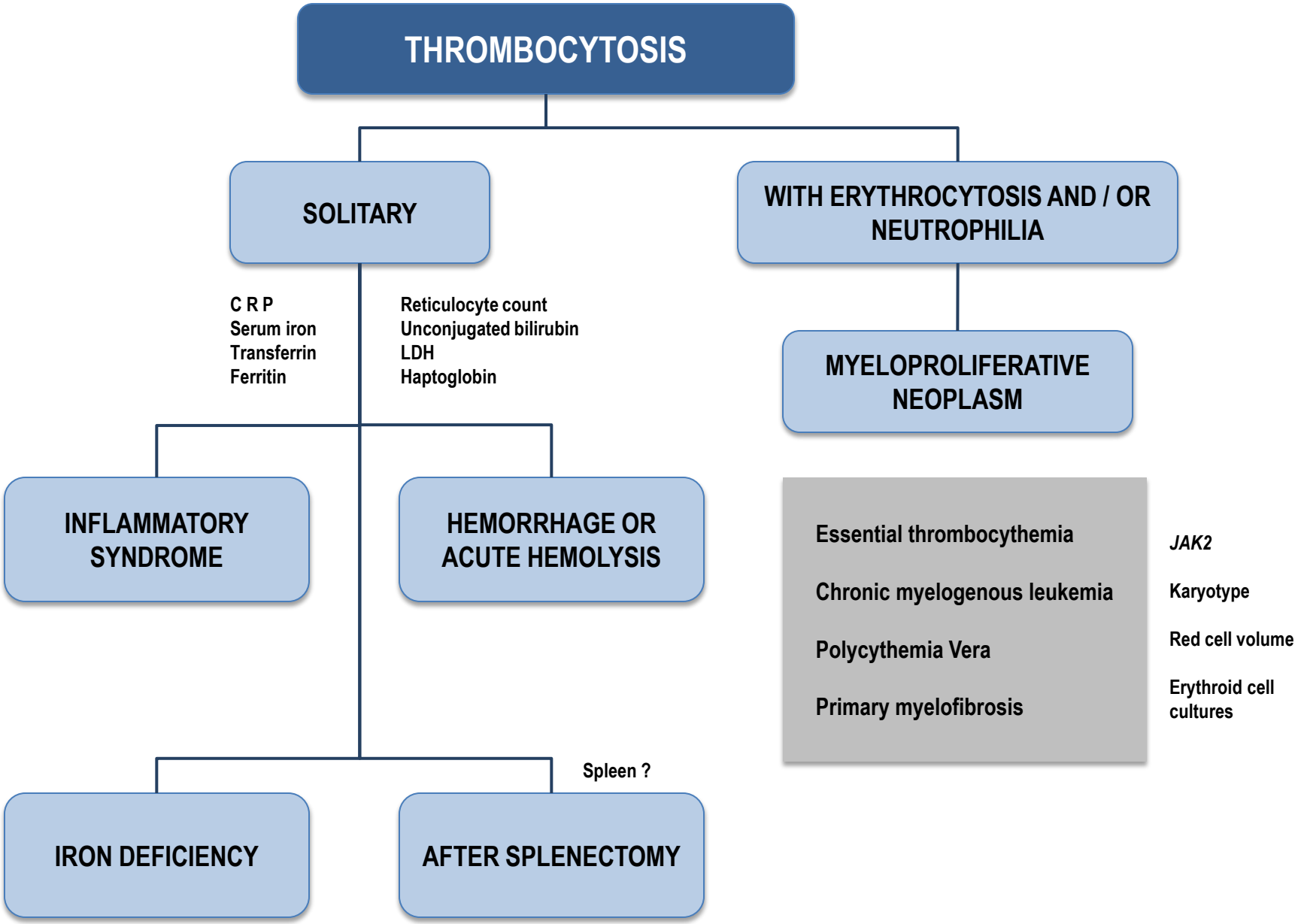

ABSOLUTE MONOCYTOSIS
REACTIVE
MALIGNANT
BACTERIAL INFECTION
Tuberculosis
Salmonellosis
Brucellosis
Bacterial endocarditis
PARASITIC INFECTION
Malaria
RECOVERY PHASE AFTER INFECTION
RECOVERY PHASE AFTER AGRANULOCYTOSIS
ALCOHOLIC HEPATOPATHY
HODGKIN LYMPHOMA
G-CSF or GM-CSF TREATMENT
MYELODYSPLASTIC /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

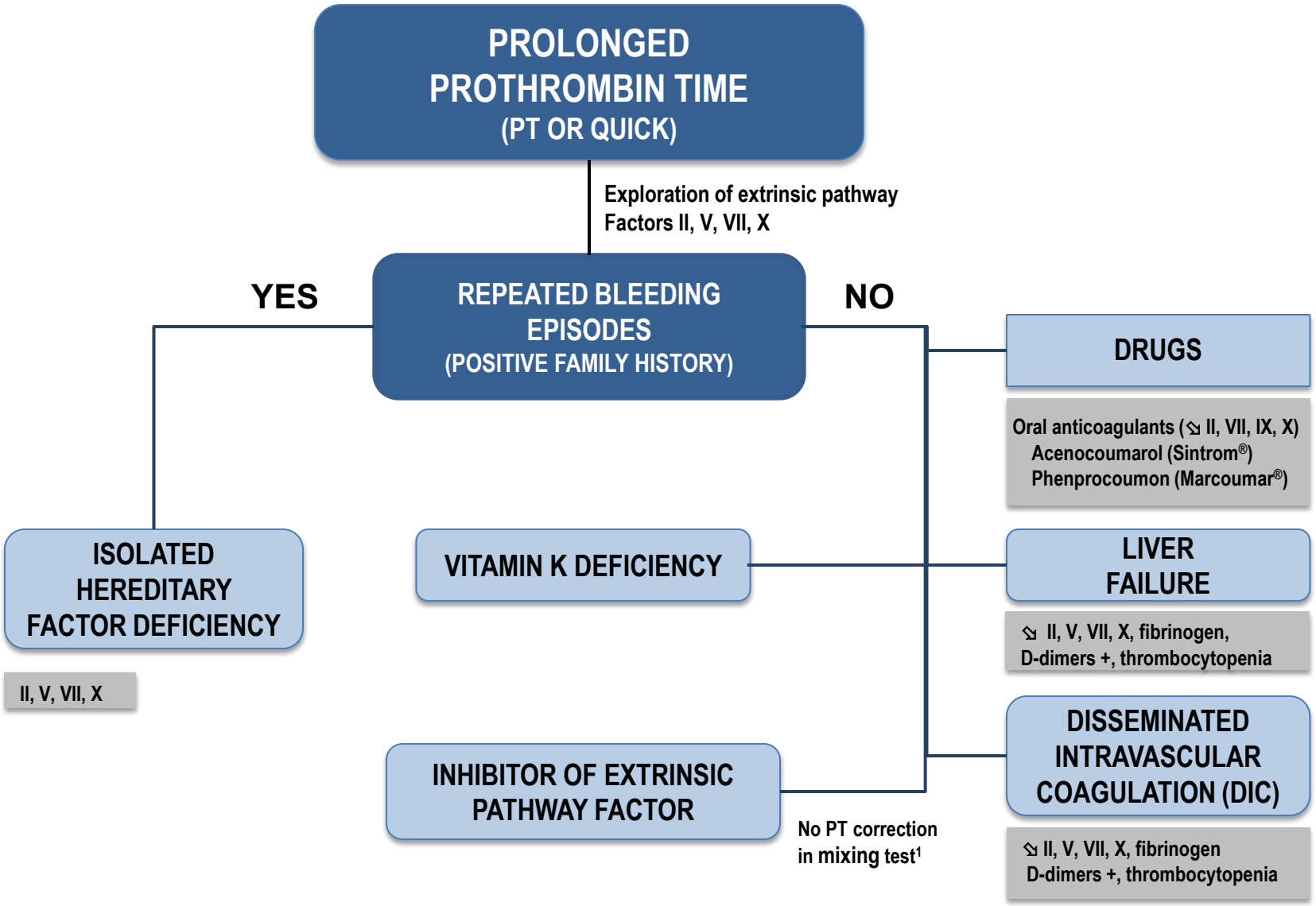


[1] Ig level may be lower for diagnosis if other criteria are present

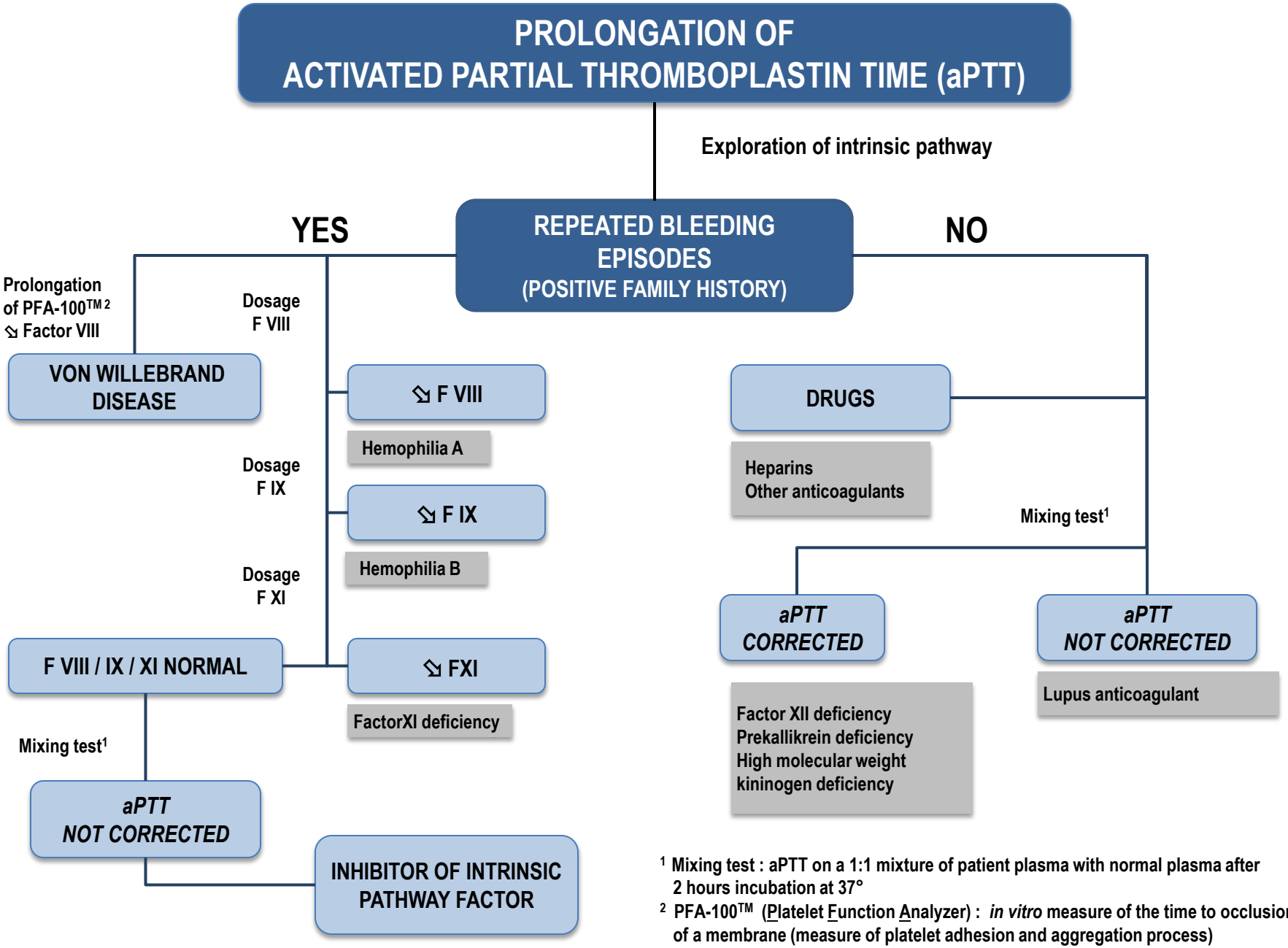
[2] ↗ ratio if kappa (κ) light chains increased
↘ ratio if lambda (λ) light chains increased


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, CMV
HIV, HCV
Helicobacter pylori, Malaria
AUTOIMMUNITY
Systemic Lupus Erythematosus
Lymphoid neoplasm
B12 OR FOLATE DEFICIENCY
DRUG
Heparin
D I C
PRIMARY IMMUNE THROMBOCYTOPENIA
HYPERSPLENISM


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



[1] Mixing test : PT / Quick on a 1:1 mixture of patient plasma with normal plasma after 2 hours incubation at 37°



[1] Mixing test : aPTT on a 1:1 mixture of patient plasma with normal plasma after 2 hours incubation at 37°

[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

Authors and Collaborators :

Pierre-Michel Schmidt, MD, *Hematology Service, Centre Hospitalier Universitaire Vaudois (CHUV), Lausanne (Switzerland)*

Pierre Cornu, MD, *Chairman, Board for Postgraduate and Continuous Medical Education, Swiss Society of Hematology*

Anne Angelillo-Scherrer, **MD and PhD,** *Assistant Professor, Service and Central Laboratory of Hematology, CHUV*

Valérie Parlier, PhD, *Unité de Cytogénétique du Cancer, Service de Génétique Médicale du CHUV*

Stéphane Quarroz, *Technician in Biomedical Analyses, Head of Unit, Central Hematology Laboratory (LCH), CHUV*

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

Remarks or suggestions for improvement of this document are welcome and may be addressed to the authors :

Pierre-Michel Schmidt : pmschmidt@vtx.ch

Pierre Cornu : pierre.cornu@hin.ch

Anne Angelillo-Scherrer : anne.angelillo-scherrer@chuv.ch

April 2012