Lecture_1

Introduction to hematology

Fifth-year students

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Anatomy and physiology

- Blood flows throughout the body in the vascular system, and
- Blood makes up **~8%** of total body weight
- **~55%** plasma **~45%** formed elements
- **4 - 5 L** for average
- Plasma: 90% are water and 10% (salt and minerals)

- **Formed Elements**
  - Red Blood Cells (RBCs, erythrocytes)
  - White Blood cells (WBCs, leukocytes)
  - Platelets (thrombocytes)

➢RBCs make up about **45%** of blood volume WBCs and platelets make up about **1%**

Haematopoiesis

- Is the process of formation of normal circulating blood cells and can rapidly increase in response to demands such as bleeding or infection.

- During development, haematopoiesis occurs in the yolk sac, liver and spleen, and subsequently in red bone marrow in the medullary cavity of all bones.
- Later at the end of childhood, red marrow is replaced by fat (yellow marrow)
- in adults, normal haematopoiesis is restricted to the vertebrae, pelvis, sternum, ribs, clavicles, skull, upper humeri and proximal femora
- However, red marrow can expand in response to increased demands for blood cells.

- BM contains a range of immature haematopoietic precursor cells and a storage pool of mature cells for release at times of increased demand.
- In normal marrow
- RBC precursors cluster around a central macrophage, which provides iron and also phagocytoses nuclei from red cells prior to their release into the circulation.
- Megakaryocytes are large cells that produce and release platelets into vascular sinuses.
- WBC precursors are clustered next to the bone trabeculae; maturing cells migrate into the marrow spaces towards the vascular sinuses
- Plasma cells are antibody-secreting mature B cells, represent < 5% of the marrow population

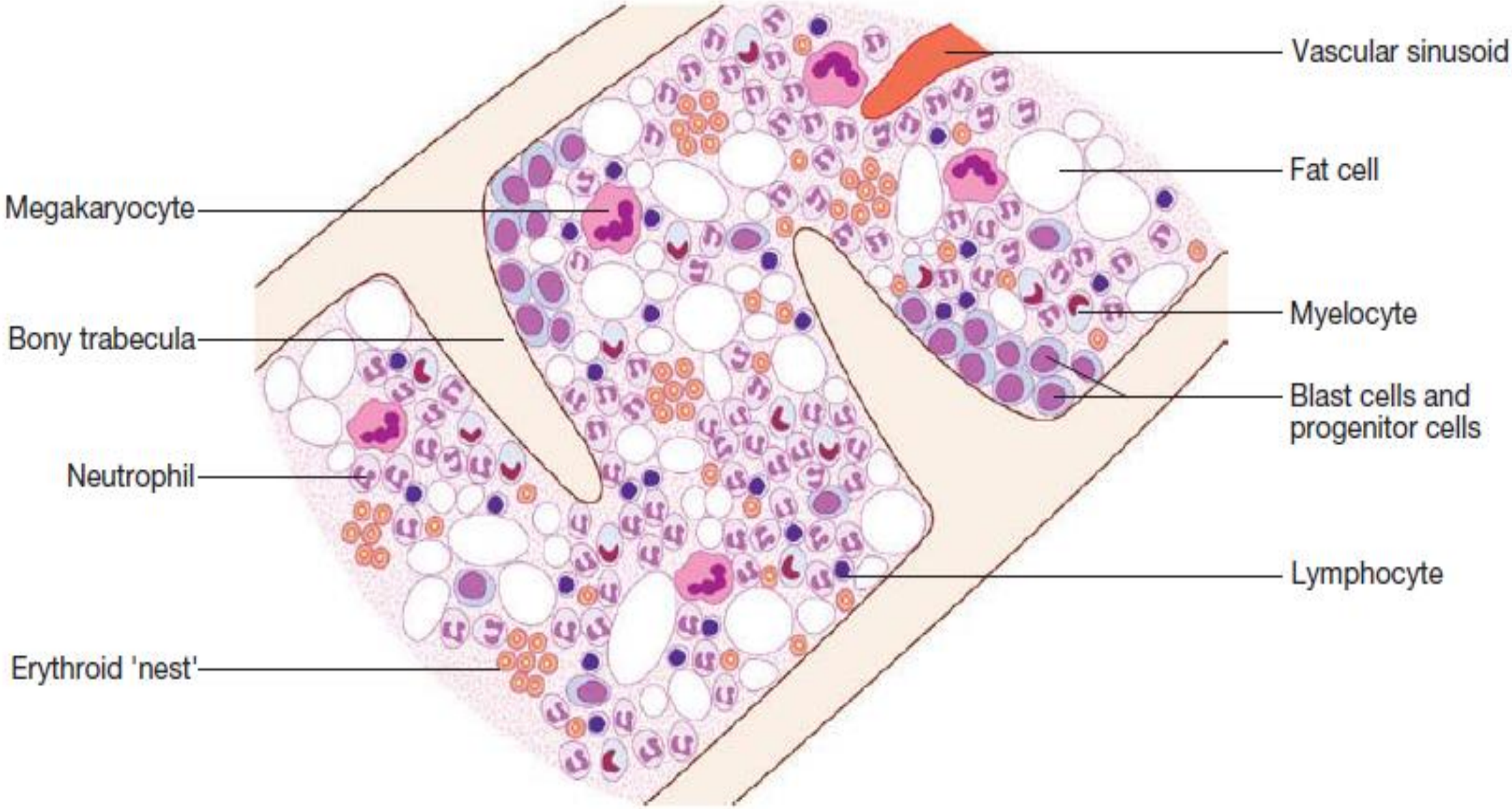
Stem cells

- Pluripotent haematopoietic stem cells comprise **0.01%** of the total BM, they can self-renew or differentiate to produce a hierarchy of lineage-committed progenitor cells.
- The resulting primitive progenitor cells cannot be identified morphologically, so they are named according to the types of cell (or colony) they form during cell culture experiments.
- **CFU–GM** (colony-forming unit – granulocyte, monocyte) is a progenitor cell that produces granulocytic and monocytic lines
- **CFU–E** produce erythroid cells
- **CFU–Meg** produce megakaryocytes and ultimately platelets.

- Growth factors, produced in bone marrow and elsewhere, control the survival, proliferation, differentiation and function of stem cells and their progeny, such as:
  - **interleukin-3 (IL-3)**
  - **stem cell factor (SCF)**
  - **Granulocyte macrophage–colony-stimulating factor (GM–CSF)**
  - **Erythropoietin**
  - **granulocyte–colony-stimulating factor (G–CSF)**
  - **thrombopoietin (Tpo), are lineage-specific.**
- act on a wide number of cell types at various stages of differentiation.

In addition, BM contains stem cells that can differentiate into non-haematological cells.

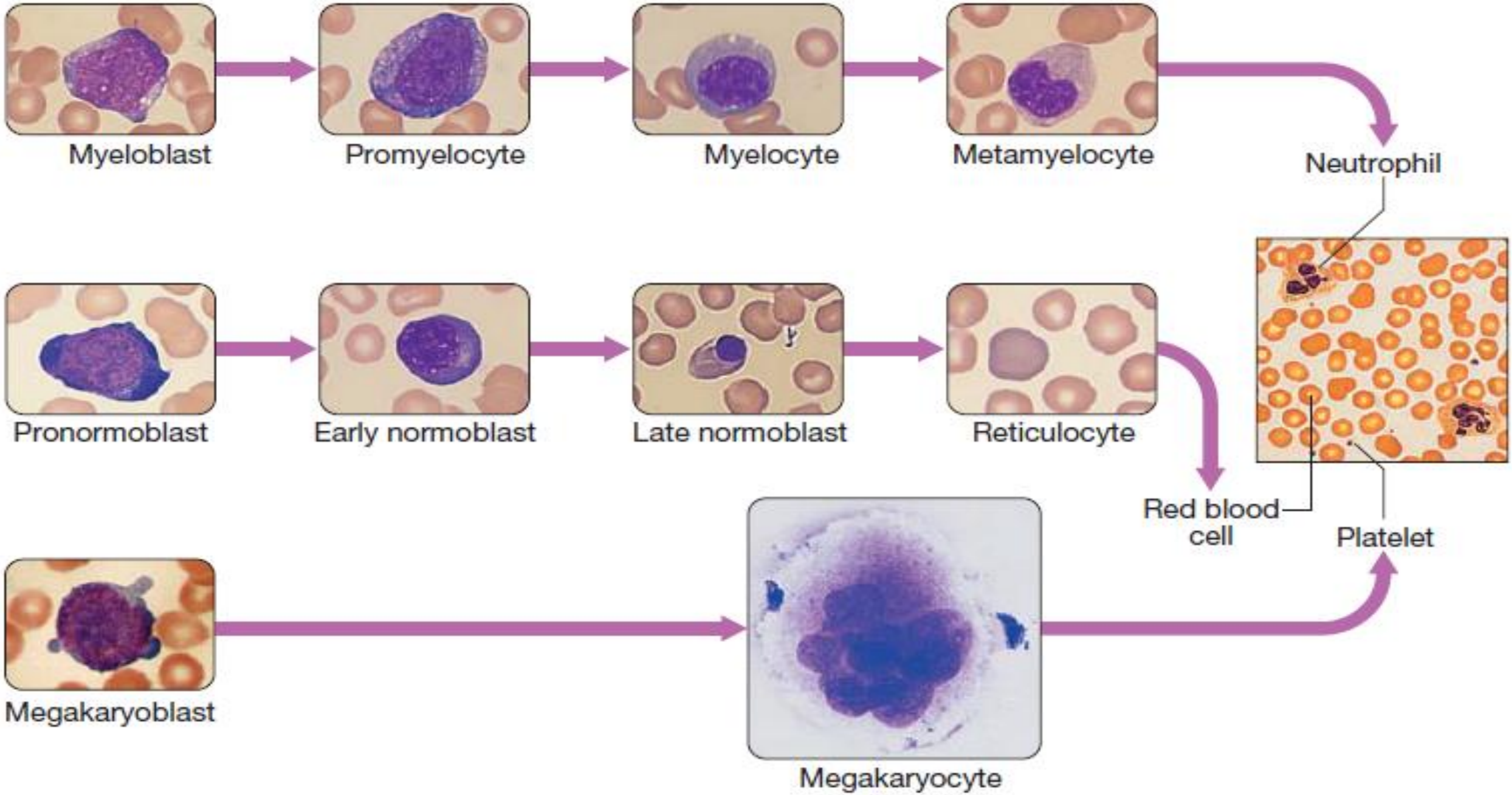
- Mesenchymal stem cells differentiate into
    - skeletal muscle
    - cartilage
    - cardiac muscle, and fat cel
- while others differentiate into nerves, liver, and blood vessel endothelium.


Vascular sinusoid
Fat cell
Megakaryocyte
Myelocyte
Bony trabecula
Blast cells and progenitor cells
Neutrophil
Lymphocyte
Erythroid 'nest'

Blood cells and their functions

1) RBCs

- RBCs precursors formed in the BM from the erythroid (CFU–E) progenitor cells are called erythroblasts or normoblasts, which will divide and acquire Hb, later these cells will lose the nucleus to form the reticulocyte. which still contains ribosomal material in the cytoplasm, giving these large cells a faint blue tinge (‘polychromasia’).


Myeloblast
Promyelocyte
Myelocyte
Metamyelocyte
Neutrophil
Pronormoblast
Early normoblast
Late normoblast
Reticulocyte
Red blood cell
Platelet
Megakaryoblast
Megakaryocyte

- **Reticulocytes** lose their ribosomal material and mature over 3 days, then released into the circulation.
- (reticulocytosis) reflect increased erythropoiesis
- **Erythropoietin** is a polypeptide hormone produced by renal interstitial peritubular cells in response to hypoxia. It stimulates the proliferation and differentiation of red cell precursors. Failure of erythropoietin production in patients with renal failure causes anemia, which can be treated with exogenous recombinant erythropoietin or similar pharmacological agents called erythropoiesis-stimulating agents, e.g. darbepoetin.

- The normal RBC life span in circulation is only 120 days.
- Unnucleated, **8 µm** biconcave discs, filled with Hb, which delivers O2 to the tissues.
- It has a deformable membrane so it can pass through the smallest capillaries, it consists of a lipid bilayer with a ‘skeleton’ of proteins
- Inherited abnormalities of any of these proteins can affect the cells membrane when they pass through the spleen or can lead to abnormally shaped red cells ex: spherocytes, or elliptocytes

- RBCs are exposed to osmotic stress in the pulmonary and renal circulation
- in order to maintain homeostasis, the membrane contains ion pumps, which control intracellular levels of sodium, potassium, chloride and bicarbonate.
- In the absence of mitochondria, RBCS get their energy anaerobic glycolysis and the pentose phosphate pathway in the cytosol.
- Membrane glycoproteins also form the antigens recognised by blood grouping as ABO and Rhesus systems.

Hemoglobin

is a protein specially adapted for oxygen transport. It is composed of four globin chains, each surrounding an iron-containing porphyrin pigment molecule termed haem.

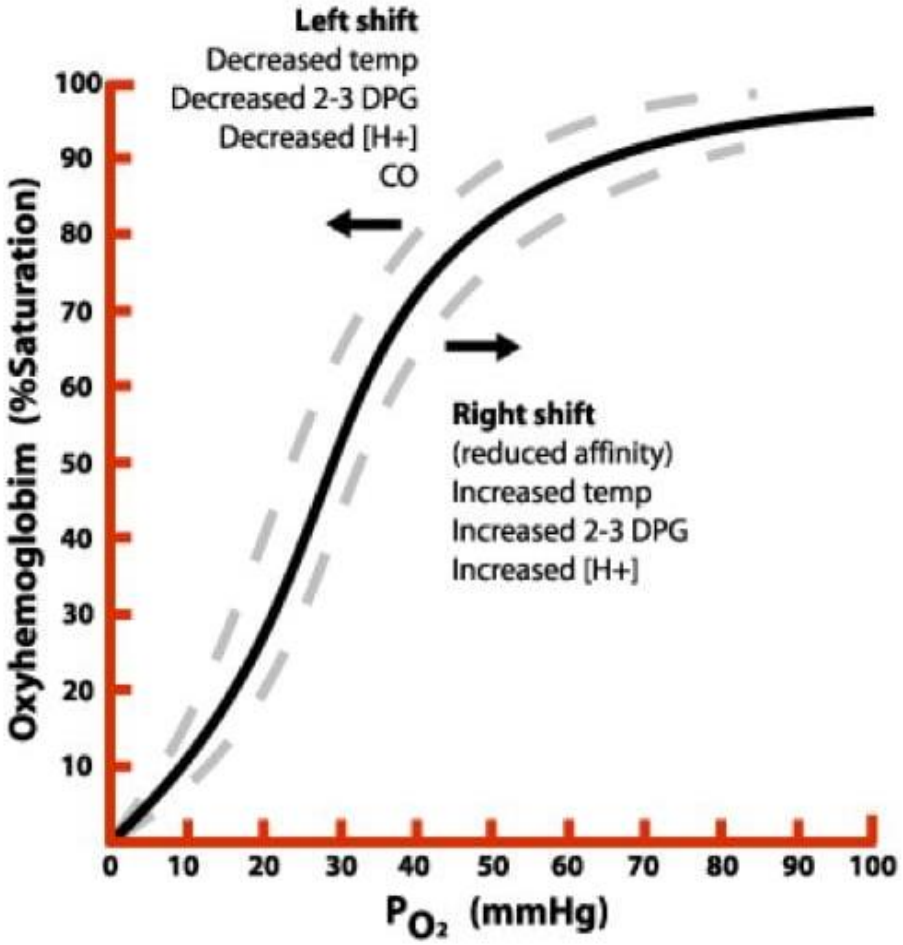
Globin chains are a combination of two alpha and two non-alpha chains;

- Hb A (αα/ββ) ≥ 90% of adult Hb
- Hb F (αα/γγ) is the predominant Hb in the fetus

Each haem molecule contains a ferrous ion (Fe2+), to which oxygen reversibly binds; the affinity for O2 increases as successive oxygen molecules bind.

When 2,3-bisphosphoglycerate (2,3-BPG), a product of red cell metabolism, binds to the Hb it lowers oxygen affinity (more oxygen released from blood)

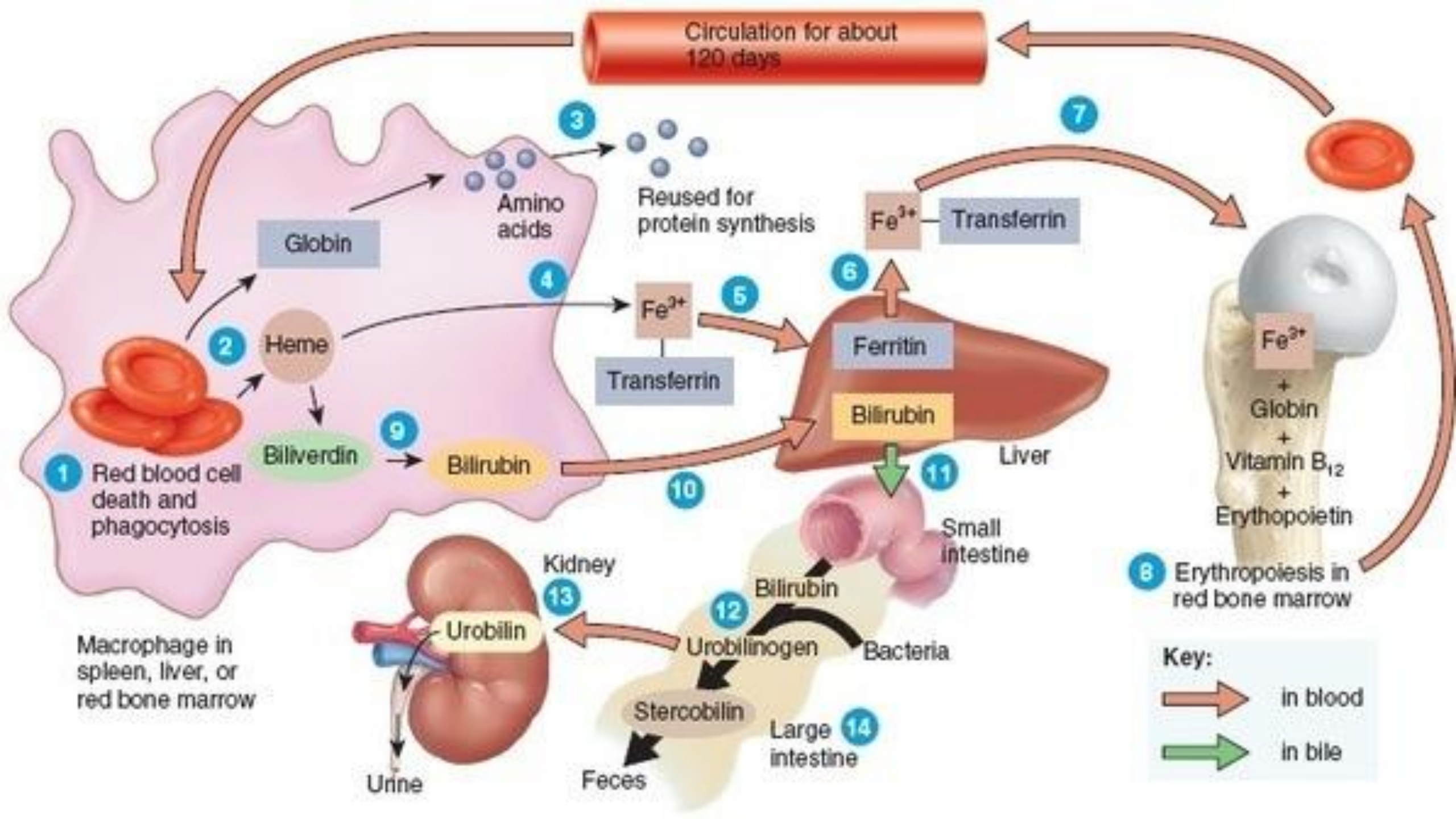
- HbF is unable to bind 2,3-BPG and has a left-shifted oxygen dissociation(less oxygen released), which, together with the low pH of foetal blood, ensures foetal O2
- Strong oxidising agents, such as dapsone, can convert ferrous iron in haemoglobin to its ferric state (Fe3+).
- The resultant methaemoglobin also has a left-shifted oxygen dissociation curve, which can result in tissue hypoxia



- Genetic mutations affecting the haem-binding pockets of globin result in haemoglobinopathies or unstable haemoglobins.
- Alpha globin chains are produced by two genes on chromosome 16, and beta globin chains by a single gene on chromosome 11;
- imbalance in the production of globin chains results in the thalassaemia
- Defects in haem synthesis cause the porphyria

Destruction

- At the end of RBCs lifespan the will be phagocytosed by the reticulo-endothelial system (RES)
- Amino acids from globin chains are recycled and iron is removed from haem for reuse in Hb synthesis.
- The remnant haem structure is degraded to bilirubin and conjugated with glucuronic acid and excreted in bile.
- In the small bowel, bilirubin is converted to stercobilin and excreted in feces, but a small amount is excreted by the kidney as urobilinogen.
- haemolysis or ineffective haematopoiesis results in jaundice and increased urobilinogen.
- Free intravascular Hb is toxic and is normally bound by haptoglobins, a protein produced by the liver


Circulation for about 120 days
Amino acids
Globin
Reused for protein synthesis
Fe3+
Transferrin
Heme
Fe3+
Transferrin
Ferritin
Bilirubin
Liver
Biliverdin
Bilirubin
1 Red blood cell death and phagocytosis
Macrophage in spleen, liver, or red bone marrow
Small intestine
Kidney
Urobilin
Urine
Bilirubin
Urobilinogen
Bacteria
Stercobilin
Feces
Large intestine
Fe3+ + Globin + Vitamin B12 + Erythopoietin
8 Erythropoiesis in red bone marrow
Key:
in blood
in bile

lifespan of mature cells in the blood

- erythrocytes (90-120 d)
- neutrophils (~1 d)
- platelets (7-10 d)
- lymphocytes (varies – memory cells persist for years)

Role of lymphoid organs

_ spleen: part of the reticuloendothelial system, sequesters aged RBCs, removes opsonized cells, and site

of antibody production

_ thymus: site of T-cell maturation and involutes with age

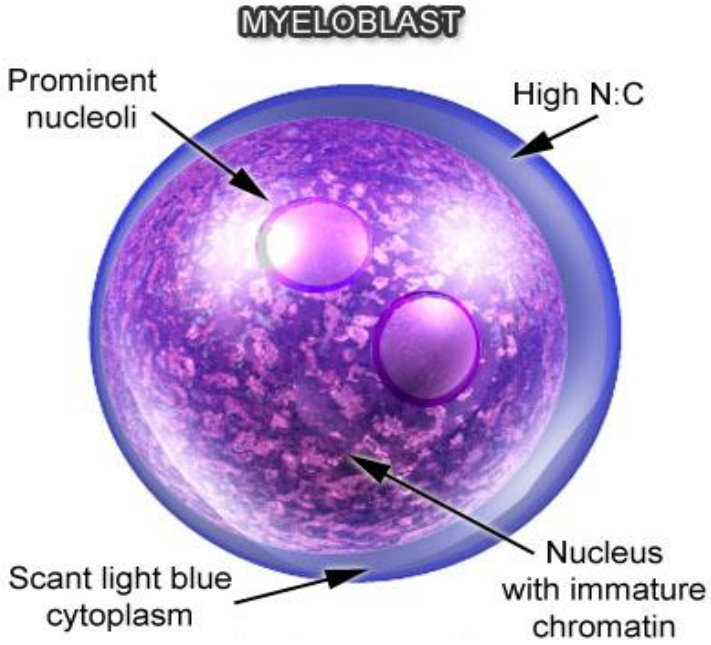
_ lymph nodes: sites of B and T-cell activation (adaptive immune response)

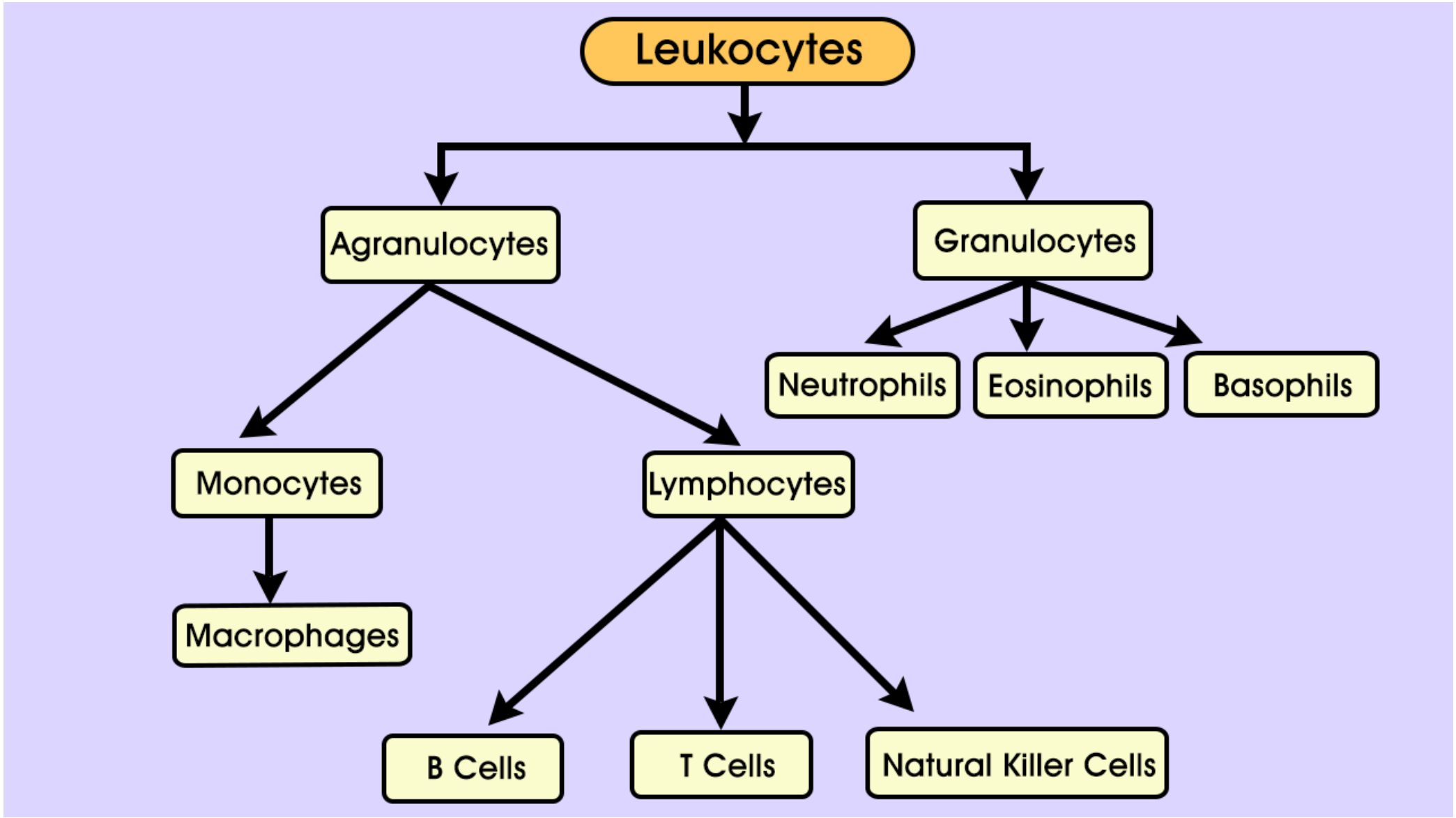
White cells (leucocytes)

- are the cells of the immune system, involved in protecting the body against both infectious disease and foreign invaders.
- It consist of :
  - granulocytes (neutrophils, eosinophils and basophils)
  - agranulocytes (monocytes and lymphocytes)
- Granulocytes and monocytes are formed from CFU–GM cells during myelopoiesis.

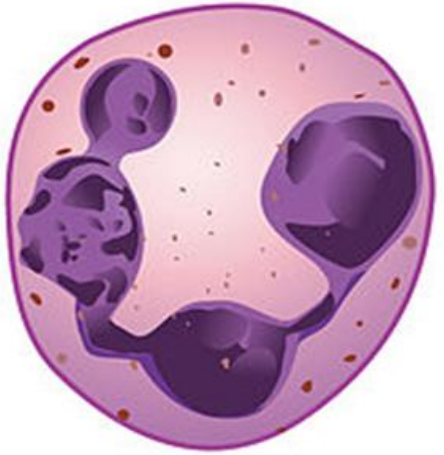
- The first recognisable granulocyte in the marrow is the **myeloblast**, a large cell with a small amount of basophilic cytoplasm and a primitive nucleus with open chromatin and nucleoli.
- As the cells divide and mature, the nucleus segments and the cytoplasm acquires specific neutrophilic, eosinophilic or basophilic granules

  This takes about 14 days.




Leukocytes
Agranulocytes
Granulocytes
Neutrophils
Eosinophils
Basophils
Monocytes
Lymphocytes
Macrophages
B Cells
T Cells
Natural Killer Cells

- The cytokines G–CSF, GM–CSF and M–CSF are involved in the production of myeloid cells
- G–CSF can be used clinically to hasten recovery of blood neutrophil counts after chemotherapy
- Myelocytes or metamyelocytes are normally found only in the BM but may appear in the circulation in infection or toxic states.
- The appearance of more primitive myeloid precursors in the blood is often associated with the presence of nucleated red cells and is termed a 'leucoerythroblastic' picture; this indicates a serious disturbance of BM function.



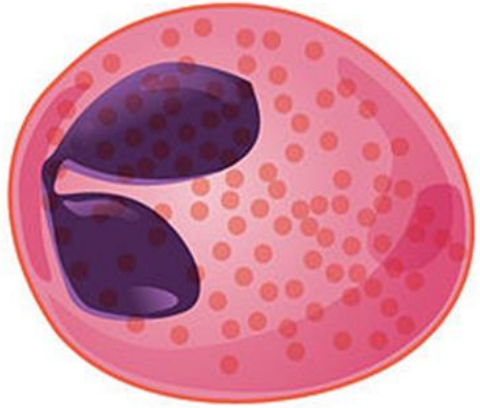
Neutrophils

- The predominant WBCs in the blood of adults
- **10–14 µm** in diameter, with a multilobular nucleus containing **2–5** segments and granules in their cytoplasm
- Function: recognise, ingest and destroy foreign particles and microorganisms
- Daily ~ $\mathbf{10^{11}}$ neutrophils enter the circulation, where cells may be circulating freely or attached to endothelium in the marginating pool.
- These two pools are equal in size; factors such as exercise or catecholamines increase the number of cells flowing in the blood

- Neutrophils spend **6–10** hours in the circulation before being removed, principally by the spleen
- Alternatively, they pass into the tissues and either are consumed in the inflammatory process or undergo apoptotic cell death and phagocytosis by macrophages

Eosinophils

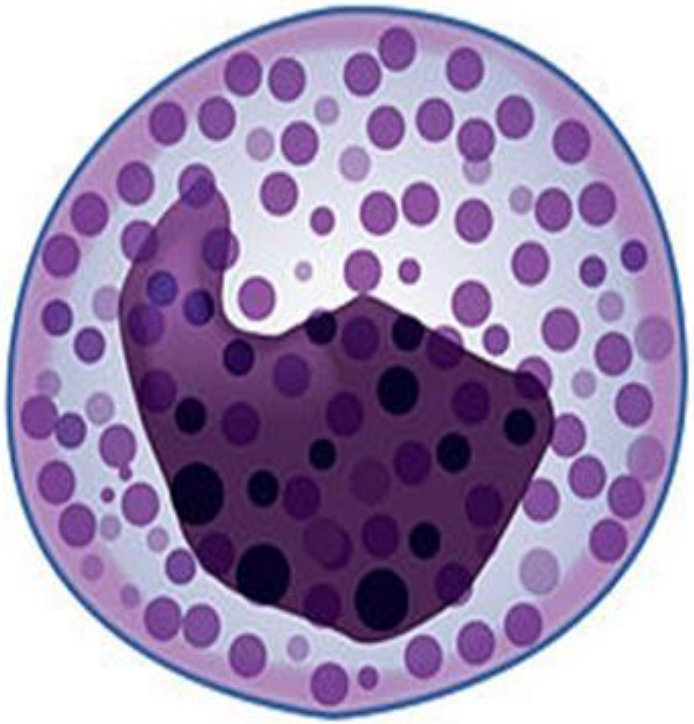
- represent **1–6%** of the circulating WBC.
- similar size to neutrophils but have a bilobed nucleus and prominent orange granules on Romanowsky staining
- Eosinophils are phagocytic and their granules contain a peroxidase capable of generating reactive oxygen species and proteins involved in the intracellular killing of protozoa and helminths
- They are also involved in allergic reactions (e.g. atopic asthma,)



Eosinophil

Basophils

- Representing **<1**% of circulating WBCs.
- With dense black granules that obscure the nucleus.
- Mast cells resemble basophils but are found only in the tissues.
- These cells are involved in hypersensitivity reactions



Basophil

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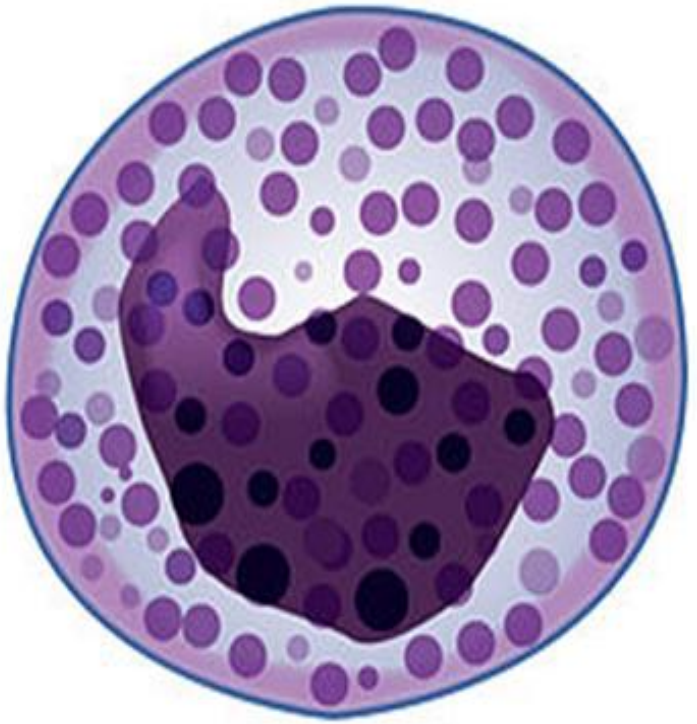


Basophil

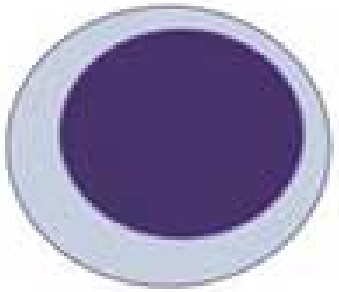
Monocytes

- Monocytes are the largest of the white cells, with a diameter of **12–20 µm** and an irregular nucleus in abundant pale blue cytoplasm containing occasional cytoplasmic vacuoles
- These cells circulate for a few hours and then migrate into tissue, where they become macrophages, Kupffer cells or antigen-presenting dendritic cells
- The former phagocytose debris, apoptotic cells and microorganisms

Lymphocytes

- There are two main types: T cells (which mediate cellular immunity) and B cells (which mediate humoral immunity)
- Lymphoid cells that migrate to the thymus develop into T cells, whereas B cells develop in the bone marrow.
- **~ 80%** of lymphocytes in the circulation are T cells
- Size heterogeneous, could be as small as RBCs as large and as neutrophils
- Small lymphocytes are circular with scanty cytoplasm but the larger cells are more irregular with abundant blue cytoplasm.
- Lymphocyte lifespan (a few days to many years).

- Cell surface antigens (‘cluster of differentiation (CD) antigens), which appear at different points of lymphocyte maturation and indicate the lineage and maturity of the cell, are used to classify lymphomas and lymphoid leukemias.



lymphocyte

Hemostasis

Is the process of clot formation in case of vascular injury in order to prevent excessive bleeding

Successful haemostasis can be achieved by complex interactions between the

- vascular endothelium
- platelets
- von-Willebrand factor
- coagulation factors
- natural anticoagulants
- fibrinolytic enzymes

Dysfunction of any of these components may result in haemorrhage or thrombosis

Platelets

- Platelets are derived from Megakaryocytic progenitor cells (CFU–Meg) divide to form megakaryoblasts then undergo maturation process to produce megakaryocytes, large numbers of platelets then fragment off from each megakaryocyte into the circulation.
- This process is stimulated by thrombopoietin produced in the liver. Platelets
- Circulate for **8–10** days then destroyed in the RES
- ~**30%** of peripheral platelets are normally pooled in the spleen and do not circulate.

- The platelets are discoid, with a diameter of **2–4 μm** The surface membrane invaginates to form a tubular network, the canalicular system, which provides a conduit for the discharge of the granule content following platelet activation.
- Drugs that inhibit platelet function and thrombosis ex: aspirin, clopidogrel, and ticagrelor, dipyridamole and the glycoprotein IIb/IIIa inhibitors abciximab, tirofiban and eptifibatide(which prevent fibrinogen binding;

Resting platelets Activated platelets

CBC: Complete blood count

Gender/Age	: COCUK , -1	Patient Number	: 167
Birthday	:	Doctor Name	:
Date of Application	: 2.10.2022 09:13	Requesting Unit	:

Hematology

Test Name	Result	Reference Value	Unit	Expl
RBC (Erythrocytes)	4.87	4.06 - 5.30	10^6/uL	
HCT(Haematocrit)	42.8	38 - 52	%	
HGB (Hemoglobin)	14.2	12.0 - 16.0	g/dL	
MCV	87.9	78 - 96	fL	
MCH	29.2	26 - 32	pg	
MCHC	33.2	32 - 36	g/dL	
RDW-SD	44.2	37 - 54	fL	
RDW-CV	13.8	11.5 - 14.5	%	
WBC (Leukocyte)	8.89	3.70 - 11.00	10^3/uL	
NEU%	66.3	39.3 - 73.7	%	
NEU#	5.89	1.63 - 6.96	10^3/uL	
LYM%	25.3	18 - 45.3	%	
LYM#	2.25	1.09 - 2.99	10^3/uL	
MON%	7.3	2 - 8	%	
MON#	0.65	0.240 - 0.79	10^3/uL	
EOS%	0.9	.7 - 6	%	
EOS#	0.08	.03 - .44	10^3/uL	
BASO%	0.2	0.0 - 1.70	%	
BASO#	0.02	0.0 - 1	10^3/uL	
PLT (Platelet Count)	189	155 - 450	10^3/uL	
MPV	10.6	6.9 - 10.6	fL	
PDW	12.4	9 - 17	fL	
PCT	0.20	0.17 - 0.35	%	
P-LCR	30.6	13 - 43	%	

***** Clinical Use of RDW (red cell distribution width)**

To distinguish the etiologies of microcytosis:

- **Iron deficiency: increased RDW (anisocytosis) as cells are of varying sizes in iron deficiency**
- **Thalassemia minor: normal RDW (also expect a high RBC count) as cells are of similar size due to genetic defect in Hb**

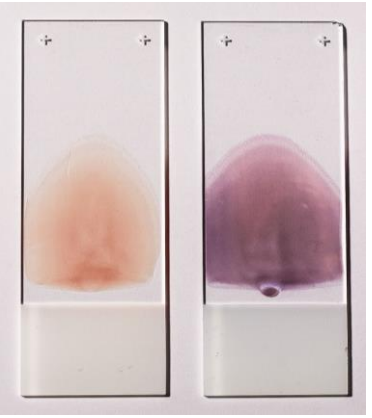
CBC: Complete blood count

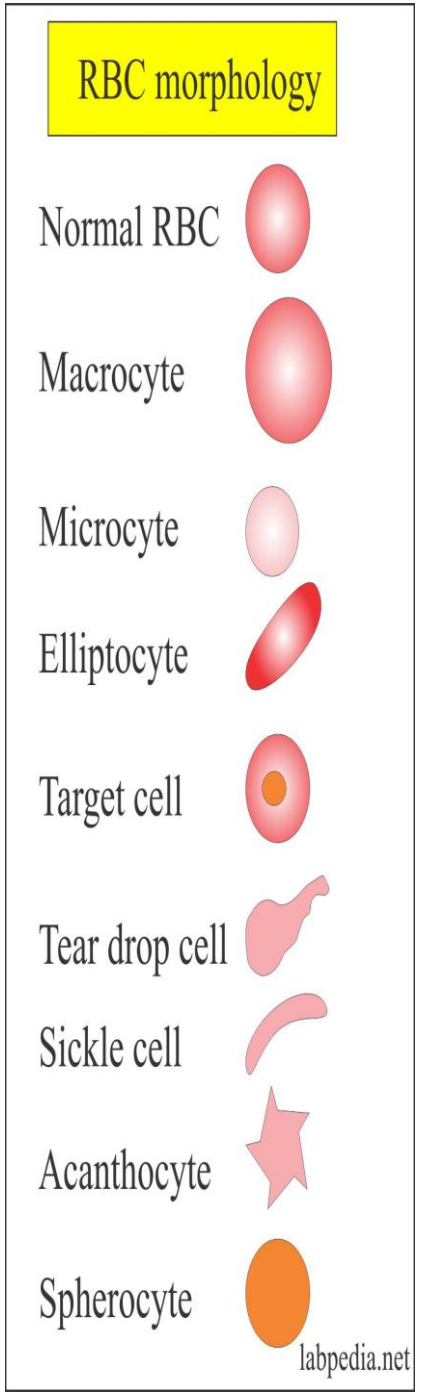
Hb can be estimated by multiplying The hct, by 3.3

Gender/Age	: FEMALE , 32		Patient Number :
Birthday	: 1990-01-01		Date of Application
Institution	:		Diagnostic
Laboratory Name	: Hematology		Barcod Date
Requesting Unit	: الاستشارية		Sampling Date
Barcod / Sample Type	: 152734 / Blood		Approval Date
Method	:		
Test Name	**Result**	**Unit**	**Reference Value**
RBC (Erythrocytes)	4.41	10^6/uL	4.06 - 5.30
HCT(Haematocrit)	27.0	%	38 - 52
HGB (Hemoglobin)	6.7	g/dL	12.0 - 16.0
MCV	61.2	fL	76 - 96
MCH	15.2	pg	26 - 32
MCHC	24.8	g/dL	32 - 36
RDW-SD	43.5	fL	37 - 54
RDW-CV	19.7	%	11.5 - 14.5
WBC (Leukocyte)	9.6	10^3/uL	3.70 - 11.00
NEU%	65.6	%	39.3 - 73.7
NEU#	6.3	10^3/uL	1.63 - 6.96
LYM%	26.1	%	18 - 45.3
LYM#	2.5	10^3/uL	1.09 - 2.99
PLT (Platelet Count)	425	10^3/uL	155 - 450
MPV	---.-	fL	6.9 - 10.6
PDW	---.-	fL	9 - 17
PCT	--.--	%	0.17 - 0.35
P-LCR	---.-	%	13 - 43
MXD#	0.8		
MXD%	8.3		

App

Blood Film Interpretation





RED BLOOD CELLS

Size

• microcytic (MCV <80 μm^3), normocytic (MCV = 80-100 μm^3), macrocytic (MCV >100 μm^3)

• anisocytosis: RBCs with increased variability in size (increased RDW)

_ iron de_ciency anemia, hemolytic anemias, myelo_brosis, blood transfusion, and MDS

Colour

• hypochromic: increase in the size of central pallor (normal = less than 1/3 of RBC diameter)

_ iron deficiency anemia, anemia of chronic disease, and sideroblastic anemia

• polychromasia: increased reticulocytes (pinkish-blue cells)

_ increased RBC production by bone marrow

Shape

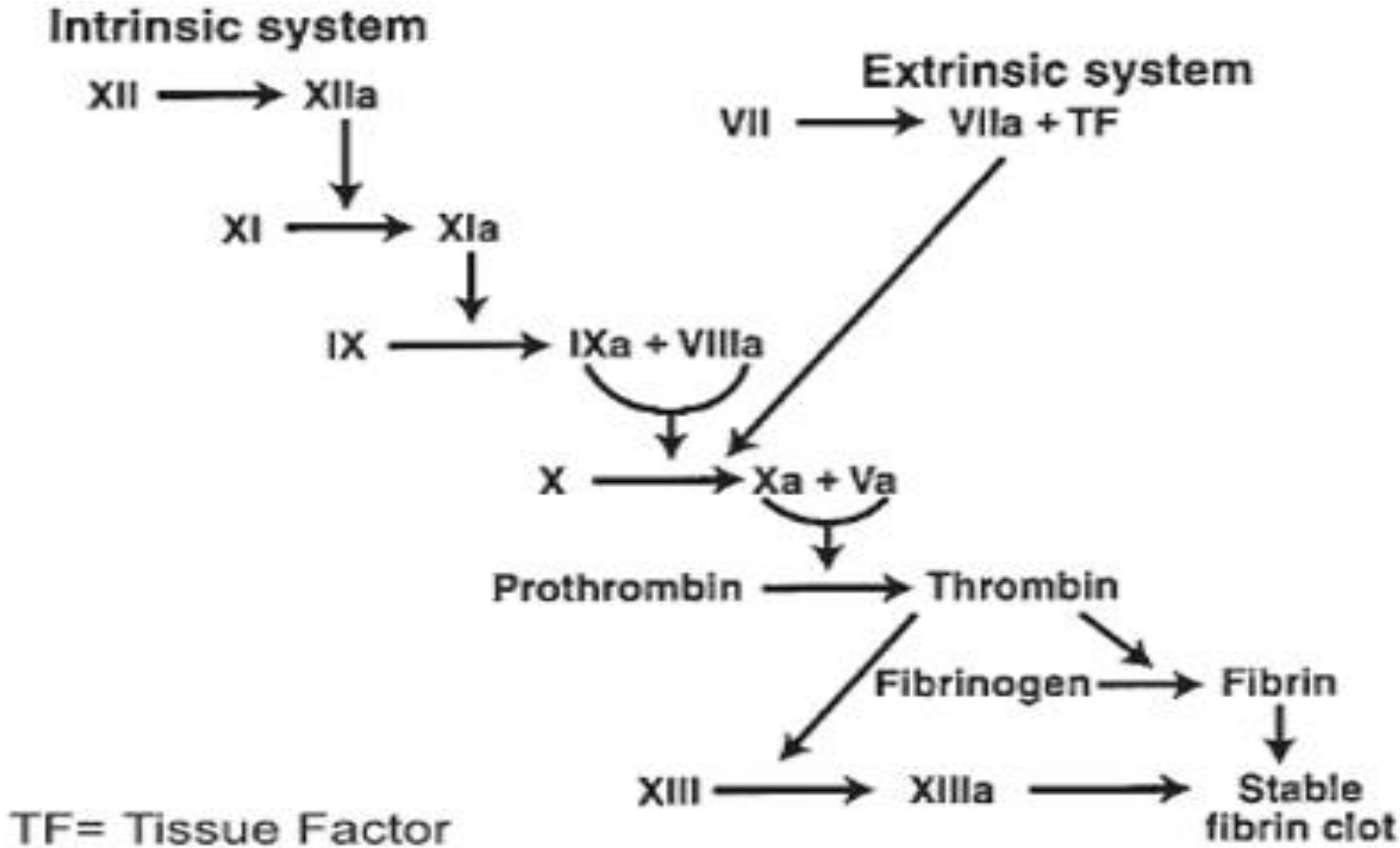
• poikilocytosis: increased proportion of RBCs of abnormal shape

_ iron deficiency anemia, myelofibrosis, severe B12 deficiency, MDS, and burns

Clotting factors

- The coagulation system consists of a cascade of soluble inactive zymogen proteins, When activated, each is capable of activating one or more components of the cascade.
- Activated factors are designated by the suffix ‘a’. Some of these reactions require phospholipid and calcium.
- Coagulation occurs by two pathways: it is initiated by the extrinsic (or tissue factor) pathway and amplified by the ‘intrinsic pathway’

- Clotting factors are synthesised by the liver, although factor V is also produced by platelets and endothelial cells.
- Factors II, VII, IX and X require post-translational carboxylation to allow them to participate in coagulation.
- The carboxylase enzyme responsible for this in the liver is vitamin K-dependent
- This production can be inhibited by warfarin, which is the basis of the anticoagulant effect of coumarins
- Congenital (e.g. haemophilia) and acquired (e.g. liver failure) causes of coagulation factor deficiency are associated with bleeding


Intrinsic system
XII
XIIa
XI
XIa
IX
IXa + VIIIa
Extrinsic system
VII
VIIa + TF
X
Xa + Va
Prothrombin
Thrombin
Fibrinogen
Fibrin
XIII
XIIIa
Stable fibrin clot
TF= Tissue Factor

23.3 Coagulation screening tests[1]

Investigation	Reference range[2]	Situations in which tests may be abnormal
Platelet count	150–400×10⁹/L	Thrombocytopenia
Prothrombin time (PT)	9–12 secs	Deficiencies of factors II, V, VII or X Severe fibrinogen deficiency
Activated partial thromboplastin time (APTT)	26–36 secs	Deficiencies of factors II, V, VIII, IX, X, XI, XII Severe fibrinogen deficiency Unfractionated heparin therapy Antibodies against clotting factors Lupus anticoagulant Multiple factor deficiency (e.g. DIC)
Fibrinogen concentration	1.5–4.0 g/L	Hypofibrinogenaemia, e.g. liver failure, DIC

[1]N.B. International normalised ratio (INR) is used only to monitor coumarin therapy and is not a coagulation screening test. [2]Ranges are approximate and may vary between laboratories.
(DIC — disseminated intravascular coagulation)

References

1. Davidsons Principles and Practice of Medicine 21ed

2. Toronto Notes for Medical Students, Inc. 2020

Thank you