HAEMATOLOGY

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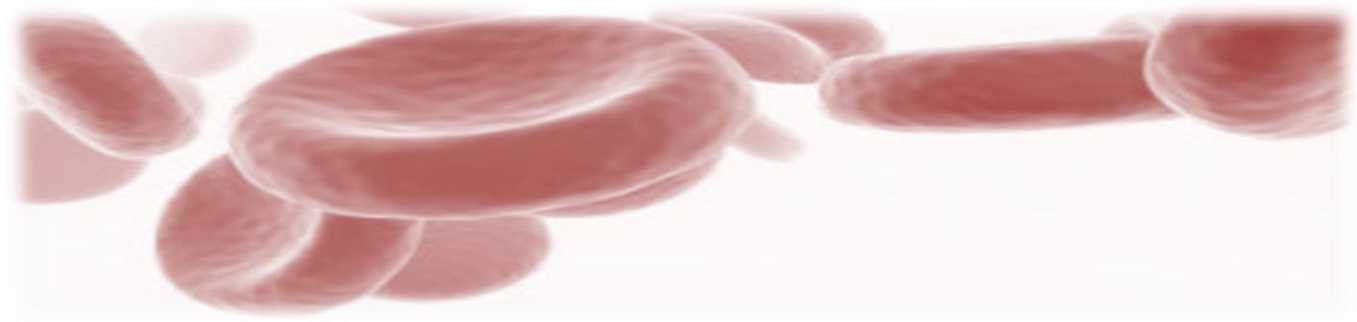

The main difference between Chronic and Acute leukemia is the presence of blasts in the peripheral blood, which happens, only in Acute Leukemia.

Acute Leukemia

- **Definition:** accumulation of abnormal (blasts/stem cells/ Immature precursors) of WBC in bone marrow and blood leading to:
  1. Bone marrow failure (anemia, neutropenia & thrombocytopenia).
  2. Organ infiltration (hepatosplenomegy, lymphadenopathy).
- **Pathogenesis:**
  1. Genetic predisposition.
  2. Infection.
  3. Previous therapy (It's called SECONDARY AL, usually in patients who had previous treatment CHEMOTHERAPY).
  4. Environmental factors (Radiation).

  2 or more will combine in an uncertain mechanism → Genetic alteration in the immature precursors →Mutations Block differentiation + Enhance proliferation + Decrease apoptosis.
- **Types of Acute Leukemia:**

  1. Acute myeloid Leukemia. (AML) 2. Acute lymphoid Leukemia. (ALL)
  3. **AMBIGUOUS lineage**,(which has stem cells markers with NO myeloid or lymphoid markers) .
- **Basis of Classification & Diagnosis:**

  1. Clinical **history** of previous chemotherapy (i.e. from adenocarcinoma)
  2. **Morphology** →Light microscopy

     A. Blasts should count **20% or more** of total cells.
     B. Blasts morphology (Important).

AML (Myeloblasts)	ALL (Lymphoblasts)
-Size: medium-Large.	-Size: small- medium
-Cytoplasm: abundant, granular.	-Cytoplasm: scanty, a granular
-Auer rods are characteristic.	-May be vacuolated

3. **Flow cytometry:** A laser based technology that detects amounts of malignant cells, and surface and cytoplasmic markers.
   - Stem Cell Markers: (CD34& TDT) → (If these two markers are absent, it is NOT ACUTE LEUKEMIA).
   - Lineage Specific markers :

Myeloid	**MPO**
B-Lymphoid	CD19
T-Lymphoid	CD3

4. Chromosomal Karyotyping
   - AML Karyotype: t(8;21), t (16;16) or inv(16), t (15;17) and t (9;11).
   - ALL Karyotype: t (9;22), t (4;11), t (12;21) and t (5;14).
5. Molecular study.

Acute myeloid Leukemia (AML)

- Proliferation of **malignant myeloid blasts** in bone marrow and blood.
- More in Adults.

➢ **Types & Classifications:**

a. According to morphology + flow cytometry → by FAB

b. According to Immunophenotype (Genetic) → by WHO

WHO Classification

AML with recurrent genetic abnormalities	Myelodysplasia related AML	Therapy related AML	AML, not otherwise specified (FAB)
1- t(8;21) 2- t(16;16) 3- t(15;17) Prognosis: Good	-Blasts≥ 20% -Significant dysplasia Prognosis: poor	-Blasts≥ 20% -Previous chemotherapy Prognosis: poor	-Blasts≥ 20% -Genetic: Normal -No dysplasia Prognosis: Standard

➢ **Clinical Features:**

1) Pancytopenia	Organ infiltration:
a. ↓WBC→infection b. ↓Hb→anemia c. ↓Platelets →bleeding	a. Hepatosplenomegally. b. Myeloid sarcoma c. Gum hypertrophy d. CNS disease e. Disseminated Intravascular Coagulation (DIC): Widespread activation of coagulation system → More with Acute Promyelocytic leukemia (M3).

*More with Acute Monoblastic Leukemia.

➢ **Prognosis:** (Better prognosis)

-Genetics: t(8;21), inv(16;16) or t(15;17)
-Age: < 60 years
-Primary better than secondary

Acute lymphoid Leukemia (ALL)

- Proliferation of malignant **lymphoid blasts** in bone marrow and blood.
- B and T lineages are affected.
- More common in Children.

➢ **Classification:**

FAB	L1	L2	L3 "Burkitt's " *
Cell Morphology	Homogenous (same shape & size)	Heterogenous	Homogenous
Cytoplasm	Little	More	Vacculated

*L3 (Burkitt's) represents mature lymphoid neoplasm so it is a type of lymphoma not acute lymphoblastic leukemia.

WHO	B-ALL	T-ALL
Markers	CD19, CD10	CD3
Age	Young	Older
WBC count	Less	Higher
Prognosis	Better	Worse
Genetics	t(9,22), t(4,11), t(12,21)	--------

- **Clinical Features:**

1. Pancytopenia	2. Organ Infiltration
- **↓WBC→ infection** - **↓ Hb → anemia** - **↓ Platelets → bleeding (Thrombocytopenia is a sign of ADVANCED ALL).**	- Lymphadenopathy - Testicles involvement - CNS disease - Mediastinal mass (thymus enlargement) → T-ALL characteristic.

- **Prognosis:** (Important)

	Better	Worse
Age	2 - 10 yrs	<2 - >10 yrs
Gender	F	M
WBC count	Low	High
Cell type	B cell	T cell
B-ALL phenotype	Common	Others
B-ALL genetics	Hyperdiploidy t(12;21)	Hypodiploidy t(9;22)
CNS involvement	No	Yes

Remember!

- 20% or more blasts = Acute leukaemia.
- Auer rods = AML
- AML M3 = DIC &target therapy
- Gum hypertrophy = mostly M4 or M5,
- Mediastinal = T-ALL
- Subtypes of AML (M0-M8) + cytogenetic abnormalities
- Subtypes of ALL (T or B cell)
- Main lineages markers are MPO, CD19(B-cell) and CD3(T-cell)
- Stem cell markers are CD34,TDT
- FAB classification based mainly on morphology
- WHO classification focused more on genetics

Myeloproliferative Neoplasms

- **MPN:** Malignant proliferation of myeloid cells (maturing cells) which are mainly granulocytes, in blood and bone marrow, it Occur mainly in adults Has Slow onset and long course
- **MPN features**:Cytosis + Organomegaly (mainly splenomgaly)+ High uric acid +Hypercellular bone marrow +Progression to acute leukaemia (mainly AML)
- **Polycythemia:** Absolute increase in total body red cell volume (or mass)

1- Manifests itself as a raised Hb or packed cell volume (PCV)
2- Hb is >16.5 in women or 18.5 g/dl in men

- **Classification of Polycythemia**

Relative Polycythemia	Polycythemia vera	2nd Polycythemia
Decreased plasma volume due to severe dehydration	Increased RBC mass due to malignant proliferation	Increased RBC mass due to high EPO: 1-COPD, Sleep apnea, smoking.. 2-High altitude &High affinity HB 4-Renal disease &Epo secreating tumor (Parathyroid adenoma)

1- Polycythemia Vera: MPN characterized by increased red blood cell production independent of the mechanisms that normally regulate erythropoiesis.

Diagnostic Features:	Clinical features	Investigations	Complication
1-HB >18.5g/dl in men ,16.5g/dl in women 2-Hypercellular bone marrow 3-JAK2 mutation in >95% of cases 4-Low Serum erythropoietin level	1-Increased blood viscosity(Hypertension,Headache, dizziness, visual disturbances & paresthesia) 2-Thrombosis(Deep vein thrombosis,Myocardial infarction ,Mesenteric, portal or splenic vein thrombosis) 3-Splenomegaly in 70% & Hepatomegaly in 40%	**1-CBC** RBC& Hb: increased WBC & PLT:mildly increased 2-**Blood smear:** Excess of normocytic normochromic RBC±Leukocytosis &thrombocytosis 3-**Bone marrow** Hypercellular+Predominant erythroid precursors increased megakaryocytes &Myeloid precursors.	1-**Myelofibrosis** **2-Acute leukemia**

2- Primary Myelofibrosis: Clonal MPN characterized by a proliferation of megakaryocytes & granulocytes in the bone marrow that associated with deposition of fibrous connective tissue and extramedullary haematopoiesis

Clinical features	Stages of PMF
• Anemia • Leukoerythroblastic blood picture. • Massive splenomegaly • Fibrotic bone marrow • JAK2 mutation (50%) • Risk of AML transformation (20%)	**1- Prefibrotic stage** Proliferation of megakaryocytes &Granulocytes then Leukocytosis Thrombocytosis **2-Fibrotic stage** Anemia Leukopenia Thrombocytopenia Extramedullary hematopoesis **3-postFibrotic stage** AML transformation

3- Essential Thrombocythemia: ET is MPN that involves primarily the megakaryocytic lineage & characterized by sustained thrombocytosis

Diagnostic Features	Clinical Presentation
1-Sustained thrombocytosis ≥450×10^9. 2-Hypercellular BM with megakaryocytic proliferation 3-Exclusion of: CML, MDS,PV &Primary Myelofibrosis 4-JAK2 mutation (60%),If negative ;no evidence of reactive thrombocytosis like Iron def. ,splenoctomy, surgery, infection ,autoimmune disease	Asymptomatic (50%) Thrombosis Bleeding Mild splenomegaly (50%) Mild hepatomegaly (20%)

- **JAK2:** Non receptor protein tyrosine kinase involved in signal transduction pathway
  - **JAK2 mutation :** Point mutation (at codon 617 in JH2) leads to loss of auto inhibitory control over JAK2. The mutated JAK2 is in a constitutively active state= Increased proliferation& Decreased apoptosis

Chronic Leukaemias

➢ **Chronic Leukaemias:** Heterogeneous group of hematopoietic neoplasms , Uncontrolled proliferation and decreased apoptotic activity with variable degrees of differentiation

1. Composed of relatively mature cells
2. Indolent. (If untreated, the course is in months or years)
3. Occurs mainly in adults

➢ Main Types of Leukemia

	Acute	Chronic
Lymphoid	ALL	LPN(CLL)
Myeloid	AML	MPN/MDS (CML)
Mixed	Acute Biphenotypic	
Non	Acute Undifferentiated	

➢ **Chronic Myeloid Leukemia (CML):** Stem cell MPN+Predominant proliferation of granulocytic cells

Consistently associated with the *BCR-ABL1* fusion gene located in the Philadelphia (Ph) chromosome which results from t(9;22)

➢

➢ Clinical features	➢ CML Phases
-Asymptomatic presentation(20-40%): **-Routine CBC : marked leukocytosis** **-Common symptoms : Fatigue ,weight loss or night sweating** **-Abdominal discomfort due to splenomegaly** **-Splenomegaly (Massive)**	**1-Chronic phase** • Leukocytosis (12-1000×10^9/L) • Mainly neutrophils & myelocytes • Blasts ≤10% ,Basophils≤ 20% • Stable course (years) **2-Accelerated phase** • Increasing counts • 10-19% blasts (basophils ≥20%) • Unstable course (months) **3-Blastic phase** • ≥20% blasts = Acute Leukemia • 80% AML & 20% ALL • (coarse: Weeks)

- **Pathogenesis of CML:** Tyrosine Kinase : is an enzyme involved in the activation of all steps of the transduction pathway of cellular proliferation.TK activity is increased abnormally when BCR gene and ABL1 gene are fused in Philadelphia chromosome (Uncontrolled proliferation).

Main Differential Diagnosis:

1-Chronic myelomonocytic leukemia (monocytosis ,BCR-ABL –ve)

2-Leukemoid reaction: Leukocytosis due to physiological response to stress or infection

	CML	Leukaemoid
Age	Adult	Any age
WBC count	High	High but <100,000
Differential	Mainly myelocytes and segmented	Mainly Bands
Morphology	Hypogranular	Toxic
Splenomegaly	+	-/+
NAP score	Low	High
BCR/ABL	+ve	-ve
Onset	Chronic	Acute

- **Neutrophil Alkaline Phosphatase (NAP)score:** Cytochemical stain that estimate the amount of alkaline phosphatase enzyme in neutrophilis , Low : CML&High: Leukemoid

Myelodysplastic Syndromes MDS

- **MDS: Group of myeloid neoplasms characterized by**

1. Peripheral cytopenia (Low HB ± Low WBC & Low PLT)
2. Dysplasia (abnormal morphology)
3. Ineffective hematopoiesis (hypercellular marrow)
4. Progression to AML (preleukaemic disease)
5. Enhanced apoptosis

Diagnostic Features	Genetic abnormalities
1-<u>Blood:</u> Pancytopenia with dysplasia **2- <u>Bone marrow</u>** Hypercellular with dysplasia	• In **-5, -7**

- **Chronic Myelomonocytic Leukemia (CMML):**
  Clonal Hematopoietic malignancy characterized by proliferation of both monocytes and neutrophils

1. Philadelphia chromosome must be negative
2. Blast must be less than 20%.

- **MDS/MPN disease:**

1. Features of MDS (dysplasia& enhanced apoptosis)
2. Features of MPN (marked proliferation)

- **MPN vs. MDS vs. MPN/MDS:**

1. **MPN= Cytosis**
2. **MDS= Cytopenia**
3. **MPN/MDS= Cytosis& Cytopenia**

Haemolysis

Features of increased red cell breakdown: **" v.imp "**

- ↑ Serum bilirubin is raised (unconjugated)
- ↑ Urine urobilinogen.
- ↑ Faecal stercobilinogen.
- Absent serum Haptoglobins.
- ↑ Lactate Dehydrogenase (LDH).

Features of increased red cells production: 1) Reticulocytosis 2) Bone marrow Erythroid Hyperplasia.

Damaged red cells:

(criteria) Morphology (e.g. Microspherocytes, Elliptocytes, red cells fragmentation).

Increased osmotic fragility

The main laboratory features of intravascular haemolysis are as follows:

1- Haemoglobinaemia and haemoglobinuria. 2- Haemosiderinuria

Causes of intravascular haemolysis: " imp

- Mismatched blood transfusion (usually ABO)
- G6PD deficiency with oxidant stress
- Red cell fragmentation syndromes
- Some autoimmune Haemolytic Anaemia.
- Paroxysmal Nocturnal Haemoglobinuria
- March haemoglobinuria
- Unstable haemoglobin
- Some drug-and infection-induced haemolytic anaemias

Question :

Which one of the following consider as a cause of intravascular hemolysis ?

Haemolytic Anaemia :

Congenital

Sickle cell disease & other Haemoglobin disorders (Hb genetic abnormalities: HbS,HbC,unstable). Thalassaemias

Acquired

Red cell fragmentation syndrome

cardiac valves

Molecular pathology of sickle cell anemia: " the doctor said u need to know just these 2 and we will ask u about it "

- Sickle cell anemia **" HbS"** α2 β2 6-GLU → VAL
- **"HbC"** α2 β2 6-GLU → LYS

Clinical manifestations of Sickle cell disease:

Hemolytic anemia
Tissue infarction

Clinical Manifestations in Sickle Anaemia:

- Hand-Foot Syndrome
- Bones, Joints Pain very severe
- Recurrent Infections & Chest Symptoms (Acute Chest Syndrome)
- CNS Presentations (from the infarctions)
- Leg Ulceration common " because of infarction"
- Skeletal Deformity
- Splenic & Hepatic Sequestration

Laboratory Diagnosis:

CBC
Blood Film
Sickle Solubility Test (+)
Hb Electrophoresis
Genetic Study

On X-RAY
they always have a fracture in the head of femur

Factors precipitating crises: " v.imp"
Infection (especially malaria)
Pyrexia
Exposure to cold
Dehydration
Pregnancy

Crises in sickle cell disease:
Hyperhaemolytic
aregenerative or aplastic
Small vessel occlusion

Doctor mentioned that:
Hereditary pattern in sickle cell disease " imp"

Higher acidity worsens the disease

Lymphoproliferative disorders

Important notes:

Lymphoma : Malignant lymphoid mass involving the lymphoid tissues

Lymphoid leukemia: Malignant proliferation of lymphoid cells in Bone marrow and peripheral blood

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Infectious mononucleosis : caused by Epstein-Barr virus

EBV implicated in the development of Burkitt's lymphoma and Hodgkin's disease.

Lab Investigation :

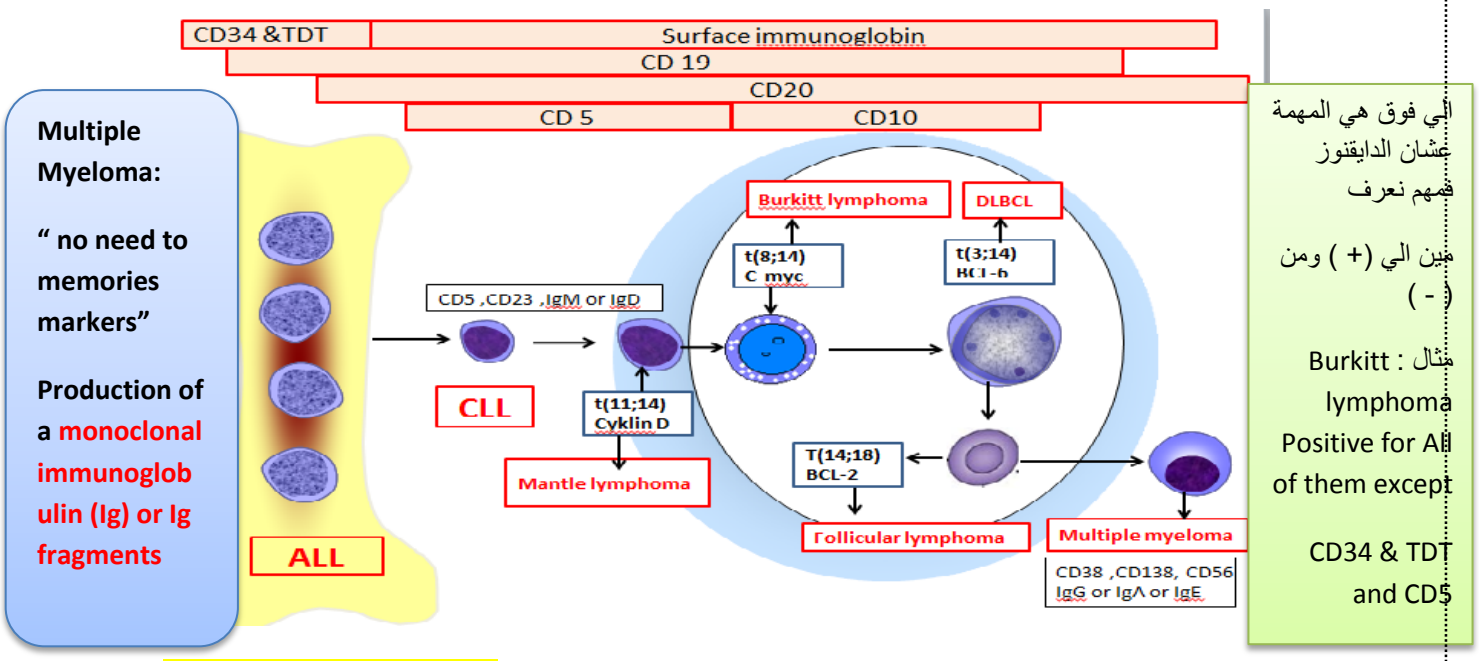
1-Virus specific antibodies IgM and IgG

2- Heterophile antibodies

Treatment: غير مطلوب

Malignant Lymphoproliferative disorders

the doctor said it's a very imp slide" "



CLL Staging: غير مطلوب

management : Follicular lymphoma غير مطلوب

Hodgkin Lymphoma : Pathogenesis of غير مطلوب

Bleeding disorders 1& 2

- ❖ Platlets defects and vessel wall defects causes:

1. Purpura : superficial bleeding into the skin
2. Mucocutaneous: bleeding of the epithelial surface of the organs

- ❖ Clotting factors defects causes:

1. Hematomas: deep tissue and muscles bleeding
2. Hemarthrosis: musculoskeletal bleeding.

- ❖ Tissue factors => stimulates blood coagulation cascade
- ❖ Thromboxane A2 => platlets aggeagation
- ❖ Serotonin => vasoconstriction => stop blood flow

1. Hereditary vascular disorders:

- Hereditary Hemorrhagic Telangiectasia
- Kasabach-merritt syndrome

2. Acquired Vascular Diseases:
   - Allergic purpura
   - Paraproteinemia and amyloidosis
   - Senile purpura
   - Vitamin c deficiency
   - Purpura associated with infections as in DIC.

- ❖ Important storage area in the platelets:

- Dense body
- Alpha granule
- lysosomes

- ❖ Dense body contains:
  - ATP/ADP
  - Ca^{+2}
  - Serotonin
- ❖ LYSOSOMES: for platelets metaboloism.
- ❖ Platelets glucoprotiens:

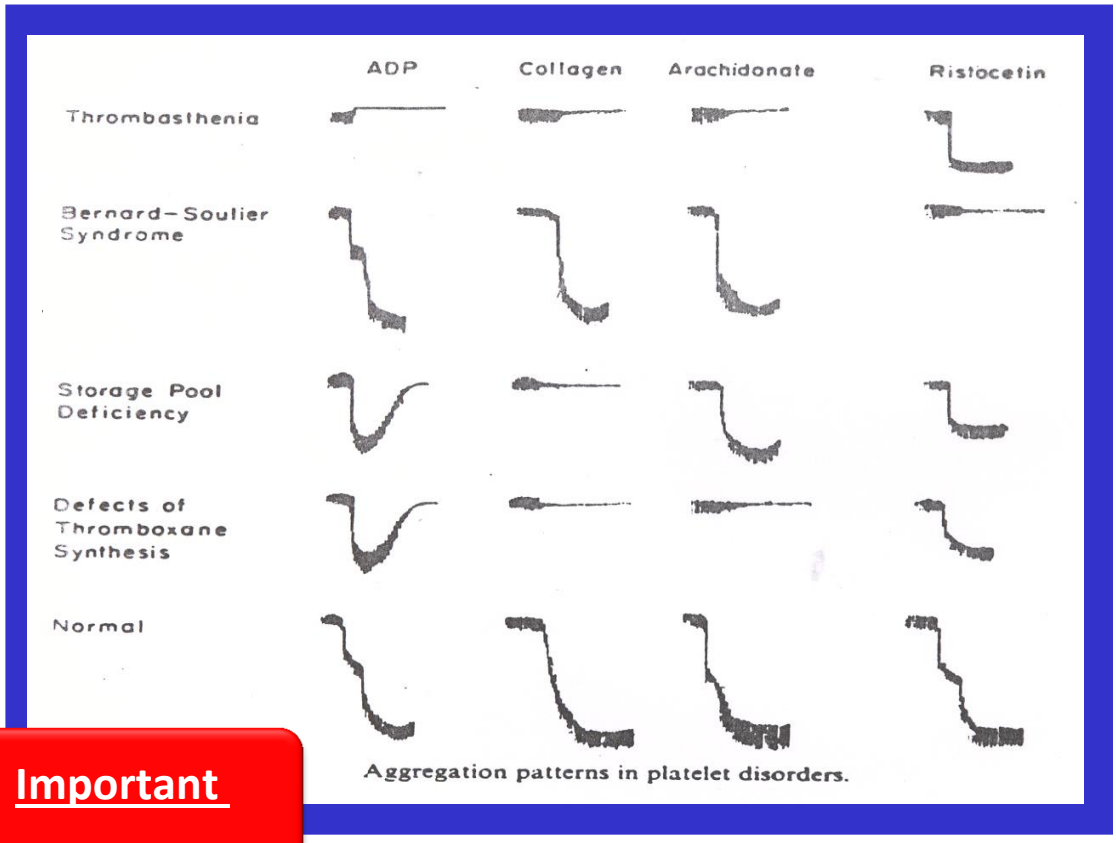
- Gp1b
- Gp-G11b-Gp111a

- ❖ When there is a vessel injury Gp1a will cause directly adhering of platelets with collagen in the subendothelial microfibrils while the other glycoproteins stimulate more platelets via binding with vW factor (aggregation).

Gb1a.

- ❖ **Gp1b** deficiency is associated with **Bernard- Soluier syndrome**.
- ❖ **GpIIb/IIIa** deficiency is associated with **Glanzmann's disease.**

- ❖ Normal bleeding time = 2.5-9.5 minutes
- ❖ Platelet aggregation test: performed by adding some substances (e.g. collagen, ADP, Arachidonate, Ristocetin)



Aggregation patterns in platelet disorders.

Important

A\ Inherited disorders of platelet function:

- ❖ **Membrane abnormalities:**

- Bernard soluir syndrome.
- Thrombasthenia

- ❖ Alpha granule deficiency:
- Gray platelet syndrome

- ❖ Miscellaneous
- May-Hegglin anomaly

- ❖ **Storage-pool (alpha granule- dense body) deficiency**
- Hermansky – Pudlak syndrome
- Wiskott – Aldrich syndrome

B\Causes of Acquired Platelet Dysfunction:

- Uremia
- Drugs

❖ **Clinical features of immune thrombocytopenia (ITB):**
  - **TB is associated with mucous membrane bleeding**
  - Marked ITB : spontaneous bleeding

❖ **Laboratory features of immune thrombocytopenia:**

- Thrombocytopenia with increased numbers of large platelets
- increased numbers and size of megakaryocytes.
- Reduced intravascular platelet survival
- Elevated levels of platelet-associated IgG

❖ **Thrombotic thrombocytopenic purpura(TTP) => CNS SYMPTOMES**
❖ **Hemolytic-uremic syndrome (HUS) => renal dysfunction**
❖ **Causes of (TTP) & (HUS):**
  - Infections (e.coli , shigella)

❖ When patient comes for the first time and needs an immediate operation, doctor must do CBC and Coagulation profile which indicate:

- Prothrombin time (10-14s) → covers the extrinsic pathway, mainly factor VII.
- Activated Partial Thromboplastin Time (30-40s) → covers the intrinsic pathway, measure factors XII, XI, IX and VIII.

❖ **Hemophilia types** :

1. Hemophilia A in factor VIII deficiency.
2. Hemophilia B in factor IX deficiency.

❖ **Classification of vW disease:**
Type 1: Partial quantitative deficiency
Type 2: Qualitative deficiency (functional abnormality)
Type 3: Complete quantitative deficiency

Correlation of coagulation factor activity and disease severity in haemophilia A or hemophilia B

Coagulation factor activity (percentage of normal)	Clinical manifestations
<1	Severe disease Frequent spontaneous bleeding episodes from early life Joint deformity and crippling if not adequately teated
1 – 5	Moderate disease Post-traumatic bleeding occasional spontaneous episodes
5 – 20	Mild diesease Post-traumatic bleeding

Diagnosis of Haemophilia A & Von – Willebrand's

Haemophilia A	VW Disease
Bleeding time normal	Bleeding time abnormal
PT normal	PT normal
PTT abnormal	PTT abnormal
Factor VIII C ↓	Factor VIII C ↓
VWf : normal	vWf ↓
Factor VIII related antigen vMF antigen: normal	vMF antigen ↓
Ristocetin co-factor normal	Ristocetin co-factor low
Platelets aggregation normal	Platelets aggregation abnormal

- **Disseminated intravascular coagulation (DIC)**
  - **Everything in the plasma will be consumed (abnormal) .**
  - There is Abnormal increased of fibrin in the circulation
- Causes od DIC:
  - Infections (septicemia , viremia , snake venom)
  - Malignancy **(AML-M3)**