i) Endothelial cells of BV have Weibel-Palladе bodies which consists of von-Willebr and factor (vWF) & P-selectins

ii) Platelets → α granules [vWF, PDGF, TGFβ, Factor 5 & 8, Fibrinogen]
→ Dense granules [ADP, Ca^{2+}, Serotonin, Epinephrine & Histamine]

iii) But M/Cly the Circulating multimeric form of vWF is released by Endotheli al cells of BV & this vWF carries ∴ it Factor 8 & prolongs its $t_{1/2} \approx 12$ hrs

iv) So in absence/↓ of vWF the $t_{1/2}$ of Factor 8 reduces to 2·5 hrs ⇒ This condition mimicks Haemophilia A (Factor 8↓) ∴ aka Pseudohaemophilia

v) ADAMTS synthesized by liver & is activated ∵ ↑shear flow in the Blood & Proteolysis of vWF [by ADAMTS-13] thereby inactivating it

vi) Platelet has 3 antigens on its surface namely GPIb [Affinity for vWF], GPIIbIIIa [Affinity for fibrinogen] & GP VI [Affinity for subendo-Collagen]

vii) Endothelial injury exposes the subendothelial collagen & vWF is released both gathering platelets via GP VI & GPIb respectively to seal the gap & this is called ADHESION which is followed by Platelet Activation/Secretion & then Aggregation] → Forming 1° Haemostatic Plug

viii) Activation of Platelets occurs by change in their shape & Degranulation [releasing Thromboxane A_2 & ADP] → Recruits more platelets

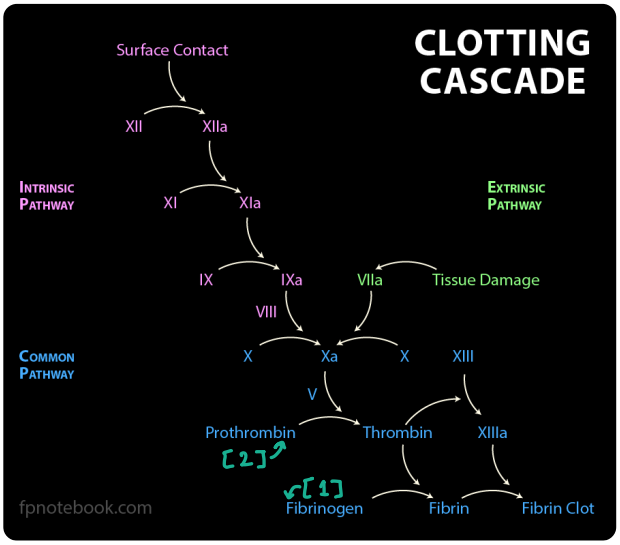
Aggregation of platelets occurs via GPIIbIIIa binding the Fibrinogen

Phosphatidyl serine flips outside the cells giving them a -ve charge which inturn leads to :-

a) activation of the Intrinsic coagulation pathway

b) activates Tenase which further activates Factor 8 & 9

c) activates Prothrombinase which further activates Factor 10 & 5



10) Intrinsic → activated by -ve charge

Extrinsic → activated by Tissue factor

Common pathway follows law of half as 10 → 5 → 2 → 1

11) The Fibrin Clot ⇒ 2° Haemostatic Plug & it is a weak clot, it is made strong by Factor 13 which causes Cross-Linking → Strong Clot

*] Assessment of various Pathways :-

1] Intrinsic pathway :- Glass test tube is used which already has -ve charge if not available, take Plastic test tube & add Kaolin/ Silica to provide -ve charge [Reports now read as aPTT K → for Kaolin]

Always test results are compared ∵ Control ⇒ Test aPTT is Ⓝ if ∵ in ± 7 sec of Control

∴ aPTT measures Factor 12, 11, 9, 8, 10, 5, 2 & 1 ⇒ Def. of >15-20% is detected

2] Extrinsic pathway :- Tissue factor is req. for clot formation obtained from Brain, but the quality differed depending upon the tissue factor used by various labs ∴ to standardise things WHO said that labs tissue factor should be compared ∵ that of WHO's [Human – Thromboplastin] ⇒ More towards 1 better is the tissue factor ⇒ ISI value [International Sensitivity Index], [International Normalized Ratio]

But now INR is used ⇒ $INR = \left[\frac{\text{Prothrombin time of test}}{\text{Prothrombin time of control}}\right]^{ISI}$

∴ Normal value of INR is <2 & PT test is Ⓝ if ± in <2sec of controls PT

Prothrombin time can pick up a fall of >10% ⇒∴ More sensitive than aPTT

3) Thrombin Time :- Thrombin is added to the test tube & it converts Fibrinogen into fibrin forming clot ∴ It's only for checking Fibrinogen

Clotting Factor Deficiency	Inheritance	Laboratory Abnormality			Treatment
		aPTT	PT	TT	
Fibrinogen	AR	+	+	+	Cryoprecipitate
Prothrombin	AR	+	+	–	FFP/PCC
Factor V	AR	+/–	+/–	–	FFP
Factor VII	AR	–	+	–	FFP/PCC
Factor VIII	X-linked	+	–	–	FVIII concentrates
Factor IX	X-linked	+	–	–	FIX concentrates
Factor X	AR	+/–	+/–	–	FFP/PCC
Factor XI	AR	+	–	–	FFP
Factor XII	AR	+	–	–	**No** risk of bleeding; treatment not indicated

*FFP: Fresh frozen plasma

PCC: Prothrombin complex concentrate

Parameters of the coagulation pathway and its uses

Parameter	Uses
Prothrombin Time (PT)	It is used to monitor the functioning of the extrinsic and the common coagulation pathways. Normal PT is 12–16 seconds.
Activated partial thromboplastin time (aPTT)	It is used to monitor the functioning of the intrinsic and the common coagulation pathways. Normal aPTT is 26–34 seconds. A relatively rare cause of prolonged aPTT is the presence of antibodies against coagulation plasma proteins called inhibitors. It can be seen due to the following reasons: Hemophilia A and B patients receiving clotting factors to control their bleeding episodes, Pregnancy, Autoimmune diseases, Malignancies (lymphoma, prostate cancer), and Dermatologic conditions.
Thrombin time (TT)	It is used for testing the conversion of fibrinogen into fibrin and depends on adequate fibrinogen levels.
Bleeding time (BT)	It is the time taken for a standardized skin puncture to stop bleeding. It tests the ability of blood vessels to constrict and platelets to form a hemostatic plug.
Fibrin degradation products (FDPs)	They are used to assess the fibrinolytic activity, and they are increased in disseminated intravascular coagulation (DIC).

*] Case study :-

1] PT is normal ie. Factor 10, 5, 2, 7 & 1 are Normal

TT is Normal ie. Fibrinogen is Normal

aPTT↑ ie. ↓12 :- No Bleeding [↑Thrombosis] aka Hagenan factor

↓ 11 :- Mild Bleeding [Haemophilia C → AR ie. M = F]

↓ 9 :- Severe Bleeding [Haemophilia B → X linked recessive ie. F>M)

↓ 8 → Severe Bleeding [Haemophilia B → X linked recessive ie. F>M)

→ Severe Bleeding [Pseudohaemophilia / Von Willebrand disease]

*] Don't confuse Pseudohaemophilia & Parahaemophilia [Factor V deficiency aka Owren disease]

*] ↑aPTT ∴ No bleeding also seen in

i) High molecular wt. Kininogen deficiency

ii) Prekallikrein deficiency

iii) Lupus anticoagulant [Platelet count is low]

2] BT, PT are Normal

aPTT↑ :- a) 8↓ } To differentiate, add Cryoprecipitate [8, vWF, Fibrinogen, 13, Fibronectin], improvement seen in 8↓ & Not in 9↓

b) 9↓ }

3] aPTT is Normal

PT↑ :- 7↓ but isolated 7↓ is rare & ∴ Think of Drugs eg. Warfarin [Vit K antag]

∴ ↓ in Vit K activated factors [via γ Carboxylation of their Glutamic acid residues] 2, 7, 9, 10 but ∵ $t_{1/2}$ of 7 is shortest (6 hrs), it is the first to ↓ or it can be due to Vit K deficiency as well

4] aPTT↑, PT↑
- i) 10↓ [TT is Ⓝ] :- Pt. has Amyloidosis & gives h/o Pinch purpura
- ii) 5↓ [TT is Ⓝ] :- Parahaemophilia/Owren disease
- iii] 2↓ [TT is Ⓝ] :- rare
- iv] Fibrinogen↓ [TT is ↑] :- Afibrinogenemia

*] Assessment of Factor 13 deficiency :-

i) It presents as Cephalhematoma or Excessive bleeding on separating the umbilical cord

ii) <u>Urea Clot Solubility Test</u> :- Two test tubes which have clot are taken & urea is added to both, if clot solubilizes ie 13↓ & if doesn't then 13 is Ⓝ because 13↓ produces a weak clot which is easily broken by urea

*] All these above tests were to check 2° Hemostatic plug formation

*] To check 1° Hemostatic plug formation do Bleeding time [BT] → Ⓝ 2-8 min via Ivy method [Forearm nick] or Duke method [Earlobe Nick]

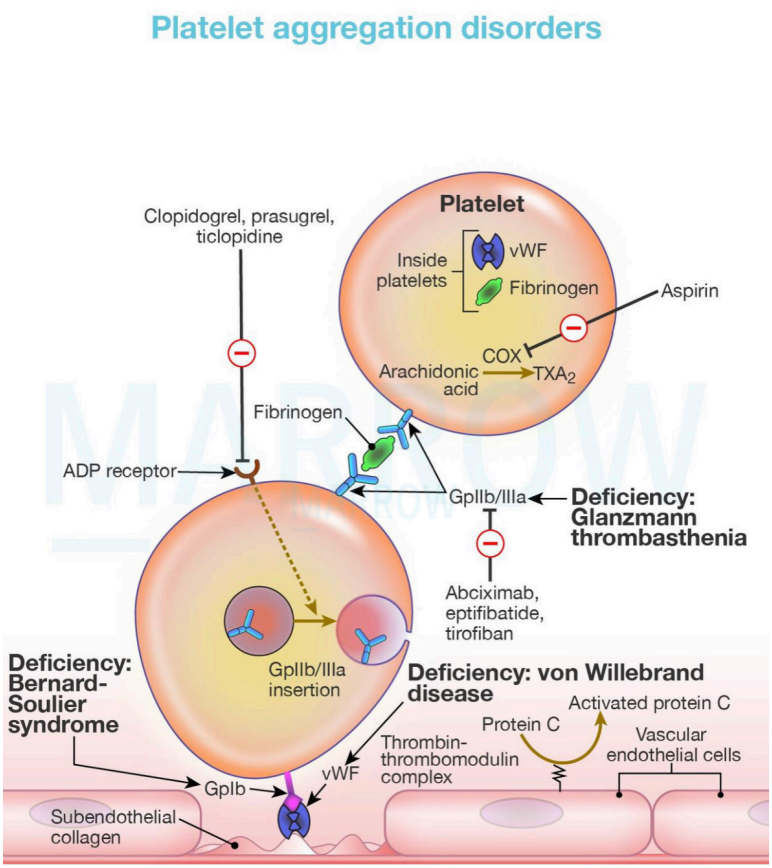
If BT↑ do Platelet count [Ⓝ 1·5 to 4 lakh], if this is also Ⓝ that means defect can be in :-

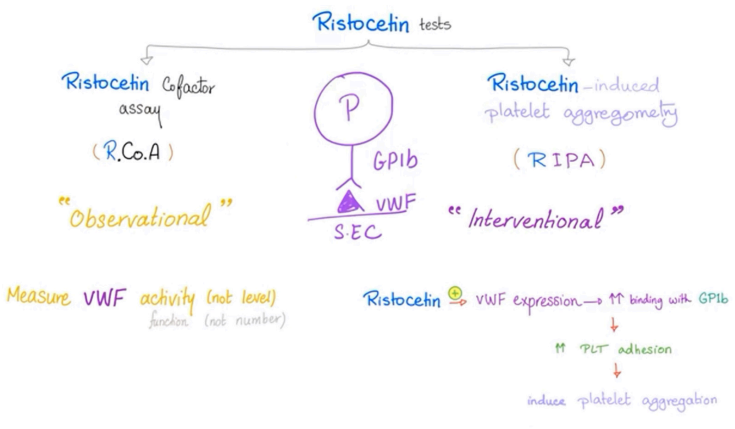
1] Adhesion → vWF ↓ :- vWF disease
→ GPIb ↓ :- Bernald Soulier syndrome

2] Secretion → α granule absent :- Gray Platelet syndrome
→ Dense granules absent :- Hermansky Pudlak syndrome

*] Secretion disorders are aka storage pool defects

3] Aggregation → GP IIb IIIa ↓ :- Glanzman Thromboasthenia
→ Fibrinogen ↓ :- Afibrinogenemia





Finding	Bernard–Soulier syndrome	Glanzmann's thrombasthenia	von Willebrand Disease
Inheritance	Autosomal **recessive**	Autosomal **recessive**	Most common (Type 1): Autosomal **dominant**
Defect	Absence of **Gp Ib-IX-V** receptor	Absence **Gp IIb/IIIa** receptor	**Type 1: Partial quantitative deficiency of von Willebrand factor (VWF)** **Type 2: Qualitative defect of VWF** **Type 3: Complete quantitative deficiency of VWF**
Mechanism	**Adhesion defect**: Platelets cannot adhere to subendothelium because of the lack of receptors (Gp Ib-IX-V) for VWF, which mediates platelet adhesion.	**Aggregation defect:** Platelets cannot aggregate because of lack of receptors (Gp IIb/IIIa) for fibrinogen that form the bridges between platelets during aggregation.	1. **Platelet adhesion defect:** Normally VWF binds to platelet GPIB-IX receptor and collagen in the subendothelium 2. **Factor VIII deficiency**- Normally VWF binds to factor VIII in circulation and prevents its degradation
Platelet count	Normal platelet count/ **mild Thrombocytopenia**	**Normal**	**Normal** (Except in type 2B-thrombocytopenia)
Platelet morphology	Large platelets/ **megathrombocytes with dense granules**	Normal	Normal/ large
Bleeding time	Prolonged	Prolonged	Prolonged
Platelet aggregation tests (collagen, ADP, thrombin)	Normal	**Abnormal**	Normal
Ristocetin aggregation test	**Abnormal (platelets does not aggregate)** Does not normalize after addition of normal serum	Normal (aggregation of platelets)	**Abnormal**, Normalizes after addition of normal serum
VWF factor levels	Normal	Normal	**Decreased**
PT/PTT	Normal	Normal	PT normal, **aPTT prolonged**

Differences between Bleeding Disorders and Coagulation disorders

Finding	Bleeding disorders	Coagulation disorders
Cause	Quantitative/Qualitative platelet abnormality	Deficiency of clotting factors
Sex	More common in females (commonly autoimmune)	More common in males (commonly X-linked)
Family history	Rare (except in VWD)	Common
Petechiae	Characteristic	Rare
Superficial ecchymosis	Small and Multiple	Large and Solitary
Bleeding from superficial cuts	Profuse	Minimal persistent
Hemarthrosis	Rare	Characteristic (most common-knee)
Deep dissecting hematomas	Rare	Characteristic (most common-iliopsoas)
Laboratory tests	Bleeding time is prolonged	PT and/or PTT are prolonged
Most common	Inherited: VWD	Inherited: Hemophilia A Acquired: Hemorrhagic diathesis of liver disease

VWD: von Willebrand disease

PT: Prothrombin time

PTT: Partial thromboplastin time