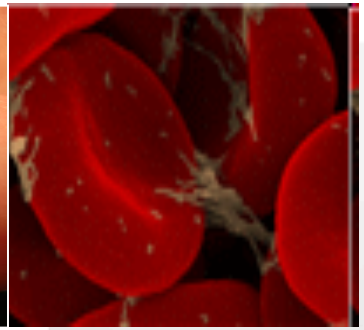
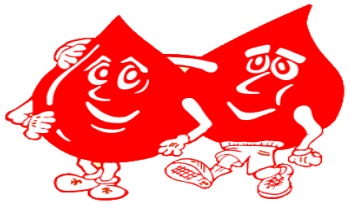
Sudan Symex Days II





COAGULATION DISORDERS

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sysmex

Hippocrates Medical Enterprises

Inherited Coagulation Disorders

Introduction

- *The existence of life long bleeding disorders and their familial occurrence was noted in the medical literature as early as the 16th century.*
- *A full understanding of the pathophysiology and genetics of these disorders was long delayed because of the complexities of the clotting mechanism.*

Hemophilia A

Definition :

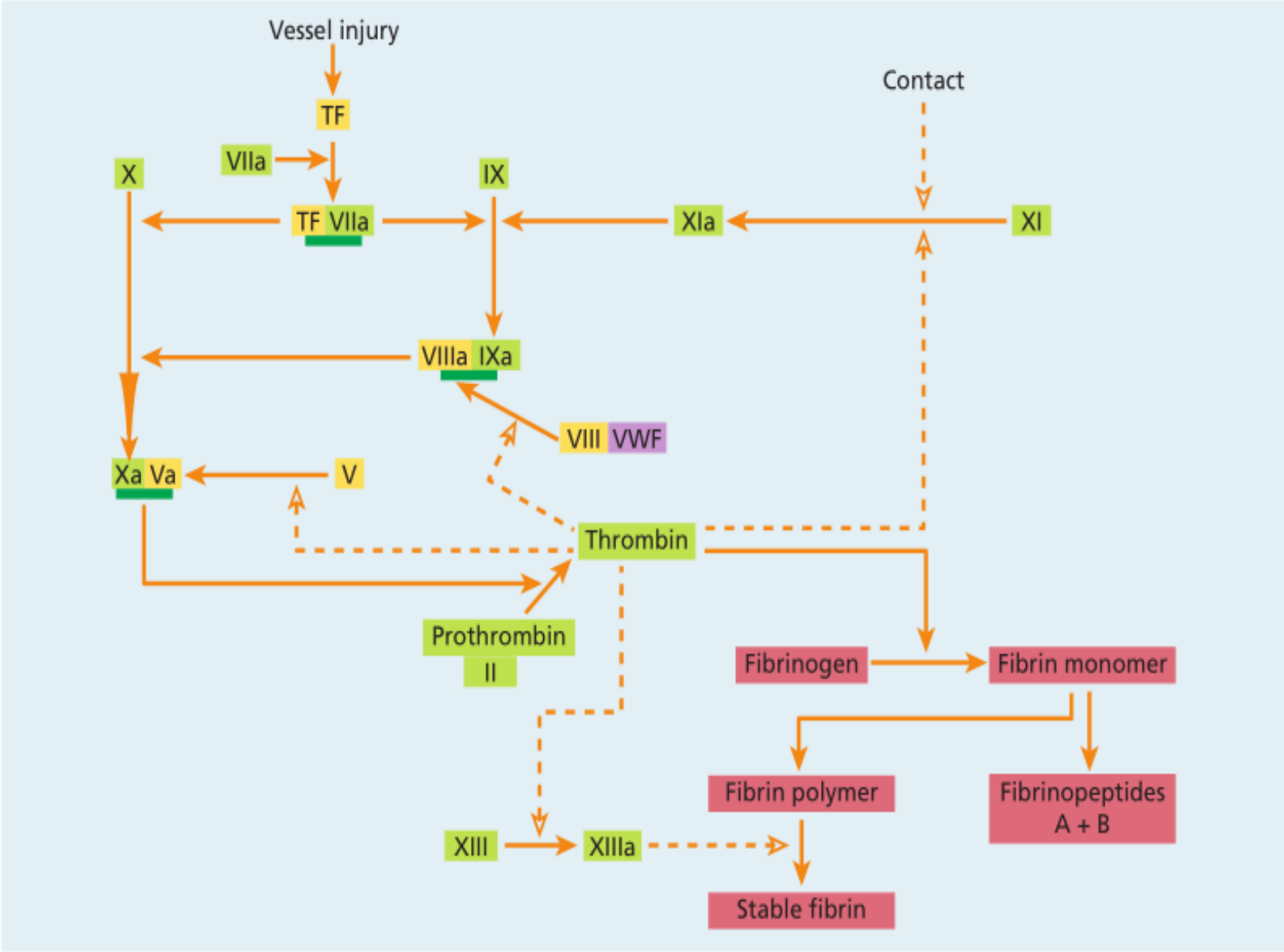
- ✓ *Hemophilia A is an X-linked hereditary disorder caused by defective synthesis of factor VIII.*

Incidence

- *Hemophilia A is the most common of the hereditary clotting factor deficiencies.*
- *Hemophilia A affects approximately 1 in 10,000 live births and is equally common in all ethnic groups.*
- *The vast majority of patients are males, but hemophilia can occur very rarely in females.*

Etiology and Pathogenesis

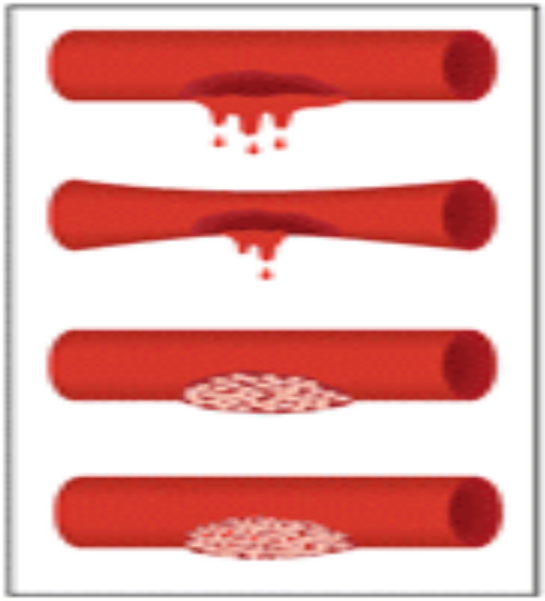
- *Hemophilia A is a heterogeneous disorder resulting from defects in the factor VIII gene that leads to absent or reduced circulating levels of functional factor VIII.*


Vessel injury
TF
VIIa
X
IX
TF VIIa
XIa
XI
Contact
VIIIa IXa
VIII VWF
Xa Va
V
Thrombin
Prothrombin
II
Fibrinogen
Fibrin monomer
Fibrin polymer
Fibrinopeptides
A + B
XIII
XIIIa
Stable fibrin

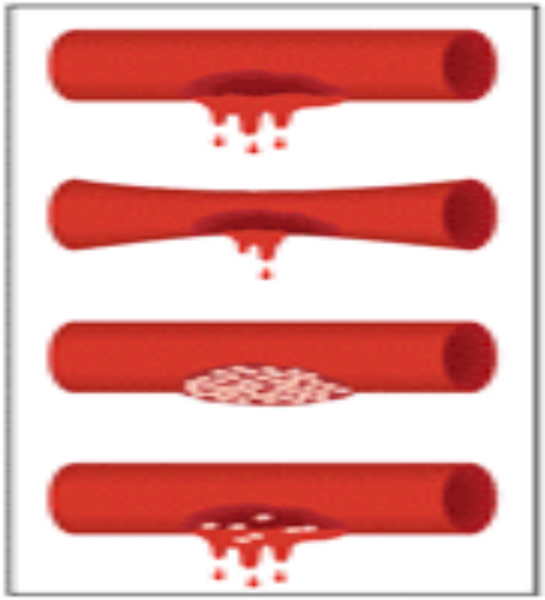
- *For factor VIII to be an effective cofactor for factor IXa, it must first be activated by thrombin.*
- *(VIIIa , IXa ,PL,Ca) form a functional factor X-activating complex "tenase".In the presence of activated factor VIIIa, the rate of factor X activation by factor IXa is dramatically enhanced → FXa ,FVa, PL,Ca → explosive generation of thrombin.*

The lack of factor VIIIa → failure to form the intrinsic tenase complex → failure to produce the thrombin burst characteristic of normal coagulation → a loose friable fibrin mesh is produced that is mechanically easily dislodged and which has increase susceptibility to fibrinolysis.

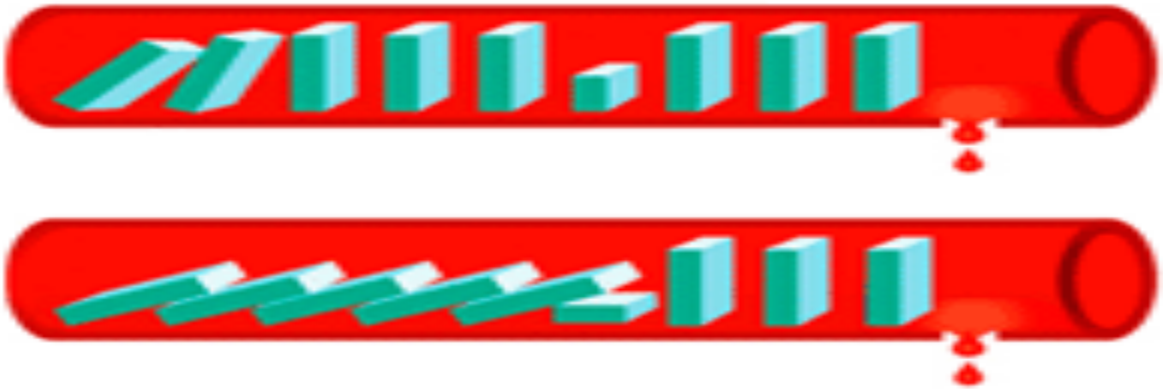
- ✓ *Fibrinolytic breakdown of the clot is also favoured by the failure of the weak thrombin burst to activate the thrombin – activatable fibrinolysis inhibitor (TAFI).*
- ✓ *The consequent failure to consolidate the primary haemostatic (platelet) plug results in the characteristic bleeding pattern of hemophilia, which is delayed after trauma but much prolonged.*



Normal Clotting Process



Clotting in Hemophilia



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Genetics

- *The inheritance is sex-linked but up to 33% of patients have no family history and result from spontaneous mutation.*

- *Approximately half of the patients have missense or frame shift mutations or deletions in the factor VIII gene.*
- *In others a characteristic flip-tip inversion is seen in which the factor VIII gene is broken by an inversion at the end of the X-chromosome.*

Clinical features

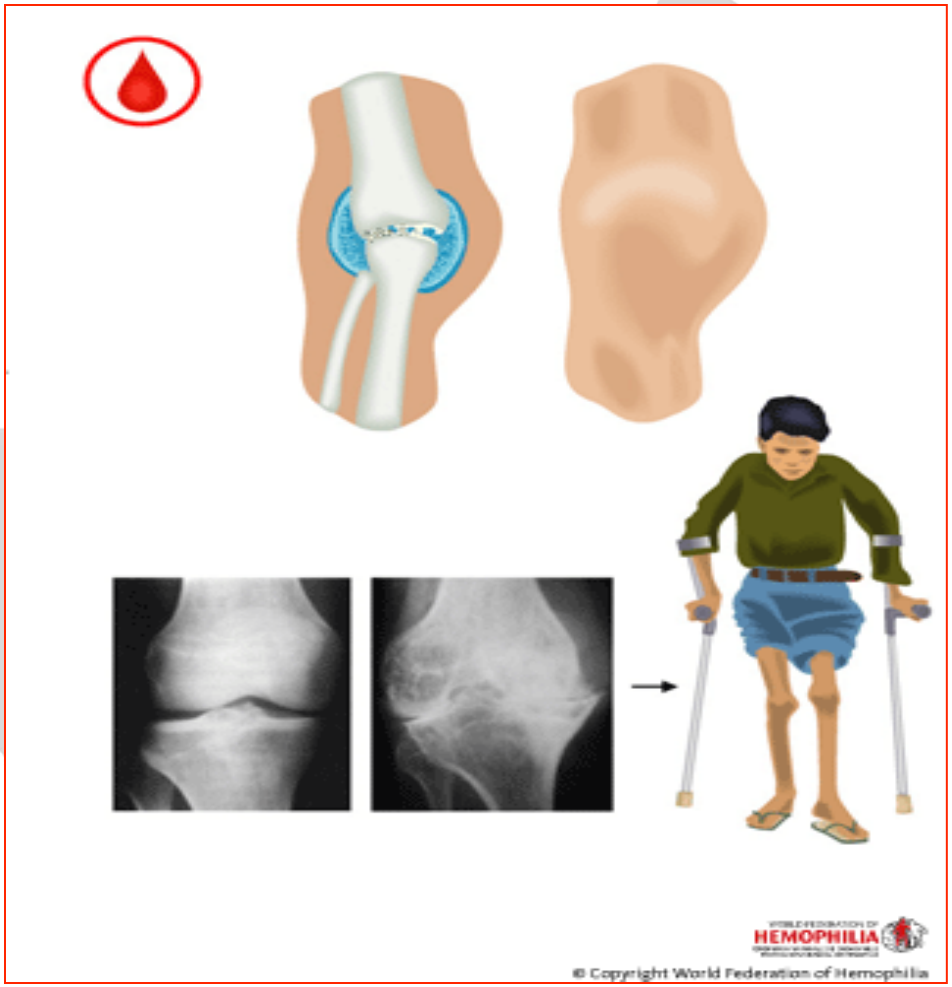
- *Infants may suffer from profuse post-circumcision haemorrhage .*
- *Joint and soft tissue bleeding and excessive bruising when they start to be active.*
- *Blood is highly irritating to the synovium and causes an immediate inflammatory reaction in the joint.*

arthritis

The synovium stops producing the slippery, oily fluid that helps the joint move

The joint becomes stiff, painful to move, and unstable

hemophilic arthritis



- *Hemorrhage into subcutaneous connective tissues or into muscles may occur with or without a known trauma.*
- *Prolonged bleeding occurs after dental extractions.*

Clinical Classification of Hemophilia

- ✓ *The clinical severity of the disease correlates with the extent of the factor VIII deficiency .*

Table 124-2. Clinical Classification of Hemophilia		
Classification	**Factor VIII Level**	**Clinical Features**
Severe	≤1% of normal (≤0.01 U/mL)	1. Spontaneous hemorrhage from early infancy
		2. Frequent spontaneous hemarthroses and other hemorrhages, requiring clotting factor replacement
Moderate	1–5% of normal (0.01–0.05 U/mL)	1. Hemorrhage secondary to trauma or surgery
		2. Occasional spontaneous hemarthroses
Mild	6–30% of normal (0.06–0.30 U/mL)	1. Hemorrhage secondary to trauma or surgery
		2. Rare spontaneous hemorrhage

laboratory findings

- *The following tests are abnormal:*

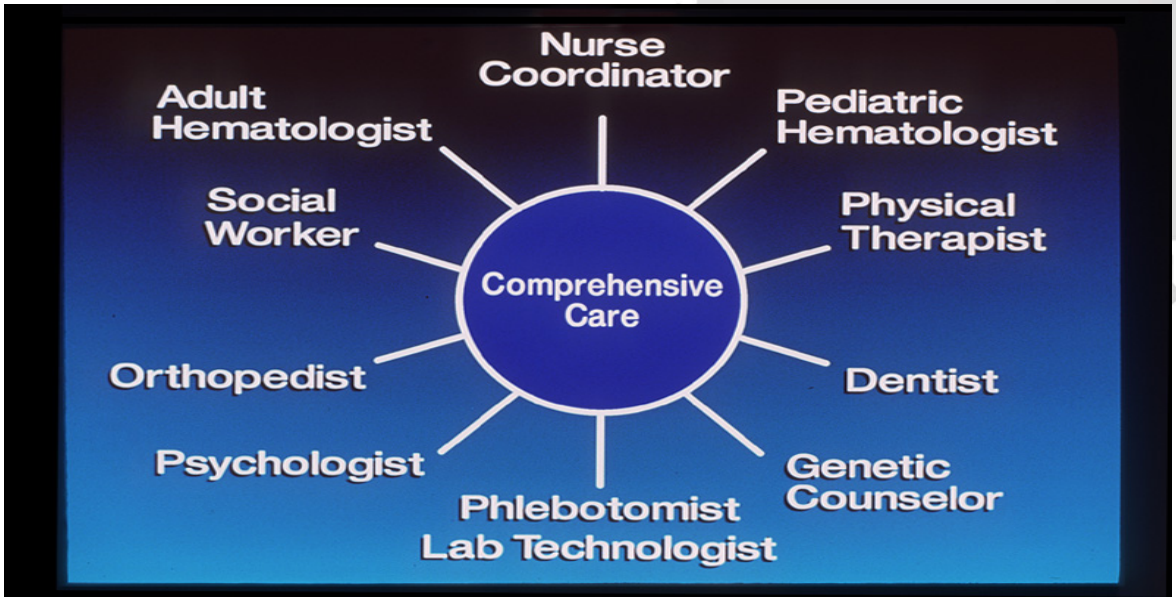
1 -Activated partial thromboplastin time (APTT).

2- Factor VIII clotting assay.

- *The bleeding time and prothrombin time (PT) tests are normal.*

Management

- *Most patients attend specialized haemophilia centres where there is a multidisciplinary team dedicated to their care.*



- ✓ *Local supportive measures used in treating haemarthroses and haematomas include resting the affected part and the prevention of further trauma.*
- ✓ *avoidance of aspirin and other agents that interfere with platelet aggregation.*
- ✓ *Hemophilics are advised to have regular conservative dental care.*
- ✓ *certain activities such as body contact sports are to be avoided.*

Factor VIII Replacement Therapy

✓ ***Cryoprecipitate:***

❖ *It contains approximately 80 U of factor VIII in 10 mL of solution.*

❖ *Many uses of the product have been replaced by factor concentrates, but it is still routinely stocked by many hospital blood banks.*

Dr. Graham Pool discovered factor VIII-rich cryoprecipitate

✓ *Factor VIII replacement therapy:*

- *FVIII concentrated from donated blood plasma or alternatively recombinant FVIII (made possible by genetic engineering advances). are now available for clinical use. .*

- ✓ *Guidelines exist for the plasma level to be achieved for different types of haemorrhage.*
- ✓ *For major surgery, serious post-traumatic bleeding or when haemorrhage is occurring at a dangerous site, the factor VIII level should be elevated to 100% and then maintained above 50% when acute bleeding has stopped, until healing has occurred.*

Table 41.2 Indications and guidelines for factor replacement in haemophilia A

Site of haemorrhage	*Optimal factor level (%)*	*Dose (units/kg body weight)*	*Duration (days)*
		FVIII	
Joint	30–50	20–30	1–2
Muscle	30–50	20–30	1–2
Gastrointestinal tract	40–60	30–40	7–10
Oral mucosa	30–50	20–30	Until healing
Epistaxis	30–50	20–30	Until healing
Haematuria	30–100	25–50	Until healing
Central nervous system	60–100	50	7–10
Retroperitoneal	50–100	30–50	7–10
Trauma or surgery	50–100	30–50	Until healing

✓ Prophylactic treatment:

❖ *After the first spontaneous joint bleed most boys with severe hemophilia are started on prophylactic factor VIII three times a week, aiming to keep their factor VIII levels above 1%.*

✓Inhibitors treatment :

One of the most serious complications of hemophilia is the development of antibodies (inhibitors) to infused factor VIII which occurs in 30 – 40% of (mainly severe) patients, especially children.

Thisrenders the patient refractory to further replacement therapy.

- ✓ *Recombinant activated factor VII (VIIa) and Activated prothrombin complex concentrates (FEIBA-factor VIII inhibitor bypassing activity) can be useful in the treatment of bleeding episodes.*
- ✓ *In the longer term, immunosuppression with cyclophosphamide, intravenous Immunoglobulin and high-dose factor VIII has also been successful.*

Factor IX deficiency

Definition and *History:*

- ✓ *Haemophilia B is an X - linked deficiency of FIX.*
- ✓ *In 1952 researchers describe a new type of hemophilia, It is called Christmas Disease after the first patient, Stephen Christmas, of Toronto.*

Incidence

The incidence is one-fifth that of haemophilia A

Hemophilia B occurs in 1 of every 30,000 live male births. As with hemophilia A, hemophilia B is found in all ethnic groups and has no geographic predilection.

Pathogenesis

- *Factor IX is a vitamin K-dependent, single-chain glycoprotein. It is activated by the factor VII-TF complex, or factor XIa, forming the active enzyme factor IXa. Once activated, factor IXa activates factor X in the presence of factor VIIIa, phospholipid and calcium .*

Factor Xa converts prothrombin to thrombin in the presence of factor Va, PL, and calcium. Thus, deficiency of factor IX results in delayed conversion of prothrombin to thrombin →

a loose friable fibrin mesh is produced that is mechanically easily dislodged and which has increase susceptibility to fibrinolysis.

Genetics

- *Factor IX is coded by a gene close to the gene for factor VIII near the tip of the long arm of the X chromosome.*
- *The vast majority of mutations being missense.*

Clinical Features

- *Bleeding episodes in patients with hemophilia B are clinically indistinguishable to episodes in patients with hemophilia A.*

Laboratory findings

The following tests are abnormal:

1- APTT

2 -Factor IX clotting assay

As in haemophilia A, the bleeding time and PT tests are normal.

Treatment

- ✓ *Factor IX Replacement:*

Dosage calculation in the treatment of hemophilia B follows the same principles as set out for FVIII deficiency, except that a higher initial dosage is required .

WORLD HEMOPHILIA DAY
APRIL 17

Hereditary disorders of other coagulation factors

- *Deficiency of fibrinogen, prothrombin, factors V, VII, combined V and VIII, factors X, XI, XIII are rare. In most the inheritance is autosomal recessive.*

Factor XI deficiency

- ✓ *is seen mainly in Ashkenazi Jews and occurs in either sex.*
- ✓ *factor XI deficiency causes excess bleeding only after trauma such as surgery, and is treated by factor XI concentrate or fresh frozen plasma.*

Factor XIII deficiency

- ✓ *This rare autosomal recessive disorder usually presents with delayed bleeding from the umbilical stump during the first 3 weeks of life.*
- ✓ *The diagnosis is made by measuring clot solubility in 5 mol/L urea solution as a screening test followed by a specific FXIII assay.*
- ✓ *Bleeding is treated with FXIII concentrate; cryoprecipitate (10 mL/kg) can also be used.*

Von Willebrand disease

- *VWD is the most common inherited bleeding disorder*
- *The basic defect is a deficiency of one or more aspects of VWF functional activity.*

The abnormality may be quantitative or qualitative.

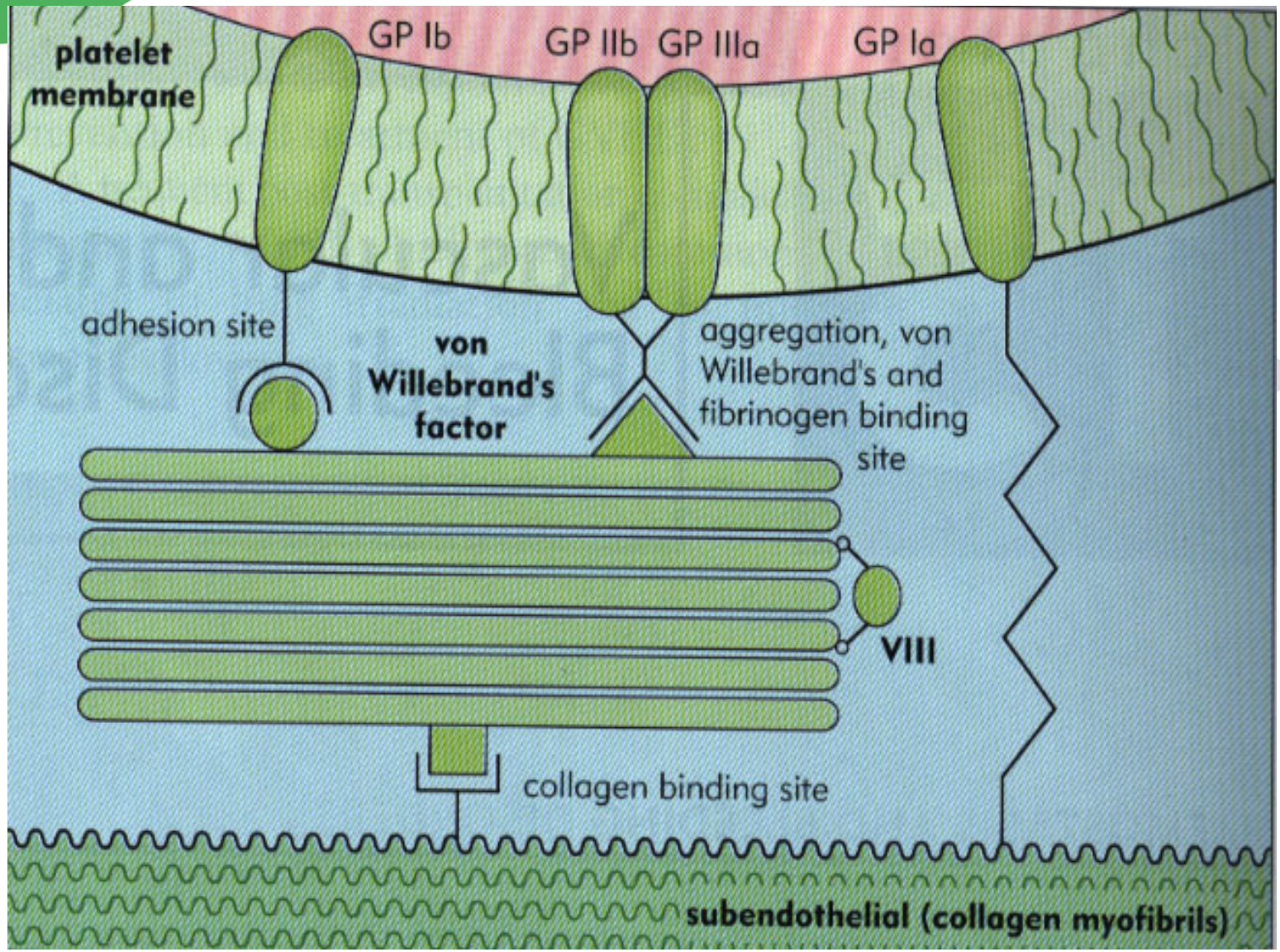
Historical Background

- ✓ *In 1926, Eric von Willebrand described a bleeding disorder in 24 of 66 members of a family from the Åland Islands. Both sexes were affected, and the bleeding time was prolonged despite normal platelet counts .*

Pathogenesis

VWF is produced in endothelial cells and megakaryocytes. It has two roles :

- ✓ *It promotes platelet adhesion to damaged endothelium .*
- ✓ *carrier molecule for factor VIII, protecting it from premature destruction.*


platelet membrane
GP Ib
GP IIb
GP IIIa
GP Ia
adhesion site
von Willebrand's factor
aggregation, von Willebrand's and fibrinogen binding site
VIII
collagen binding site
subendothelial (collagen myofibrils)

Deficiency of VWF

- *deficiency of VWF can give rise to a dual haemostatic defect:*
- ✓ *reduced plasma levels of FVIII (due to its shorter half - life in the absence of VWF).*
- ✓ *a defect in primary haemostasis because of the failure in assisting platelets to adhere to the cut edges of small blood vessels .*

Classification of VWD

Three types of VWD have been described:

- ✓ *Type1 VWD: Quantitative partial deficiency*
- ✓ *Type2 VWD: Functional abnormality*
- ✓ *Type3 VWD: Complete deficiency*

❖Type 1 VWD:

- ✓ *The most common variant accounts for 75% of cases. is characterized by VWF that is normal in structure and function but decreased in quantity (in the range of 20–50% of normal).*
- ✓ *Inherited as an autosomal dominant.*

❖ *Type2 VWD*

❑ Type 2A :

- ✓ *This results from a lack of high - and intermediate - size VWF multimers.*
- ✓ *Arise from failures of intracellular processing or from accelerated proteolysis in the circulation due to mutations close to the ADAMTS -13 cleavage site.*

- Type 2B:

"gain of function" mutation of VWF GPIb - binding site resulting in spontaneous VWF binding to platelets and clearance of the resulting platelet complexes, leading to thrombocytopenia and loss of the most active (large) VWF multimers.

❑ Type 2M :

- *loss of platelet - binding activity is seen despite the presence of normal VWF multimeric composition and is caused*

by mutations in the VWF GPIb - binding site.

❑ Type 2N:

This is characterized by reduced VWF affinity for FVIII but it is normal in other respects. Thus, laboratory investigations reveal only a reduced FVIII , which is easily mistaken for mild hemophilia.

- *Type3 VWD*:

✓ *Much less common.*

✓ *VWF is completely absent.*

✓ *Inherited as an autosomal recessive disorder.*

Clinical Features

- ✓ *The clinical features vary according to the severity of the deficiency.*
- ✓ *Patients suffer from bruising, epistaxes, prolonged bleeding from minor cuts, and excessive, but not often life - threatening, bleeding after trauma or surgery .*
- ✓ *Menorrhagia, unexplained by local or hormonal factors, can also be the presenting symptom: an estimated 20% of women with menorrhagia have VWD.*

- ✓ *Much less common(type 3) VWD, where VWF is completely absent and FVIII levels are usually around 1 or 2 units/dL. These patients have a bleeding tendency that clinically resembles severe haemophilia A.*

Diagnosis

- *The diagnosis of VWD is based on the recognition that for normal VWF function it must be:*

(i) present in adequate amounts,

(ii) have a normal multimeric structure

(iii) have intact functional domains (binding sites).

❖ *Preliminary diagnosis :*

After obtaining a suggestive personal history and a family history, the preliminary tests required are:

- ✓ *full blood count: the platelet Count is normal except for type 2B disease (where it is low).*
- ✓ *The bleeding time roughly explores the VWF–Gp-Ib interaction and is usually prolonged, but may be normal in patients with mild forms of VWD.*

- ✓ *coagulation screen: the APTT may be prolonged (von Willebrand disease cannot be excluded by a normal activated partial thromboplastin time (APTT).*
- ✓ *Factor VIII assay*

Factor VIII is measured using an APTT-based one stage clotting assay or chromogenic assay.

- *VWF level :*

  Measurement of VWF antigen (VWF:Ag) is useful to demonstrate quantitative deficiencies of VWF, and is nowadays available as latex immunoassay for most automated coagulometers.

✓ *Von Willebrand factor activity:*

❑ *Platelet - dependent function:*

the standard assessment of VWF adhesive functional activity remains the ristocetin cofactor assay (VWF:RCo). In this assay, dilutions of patient plasma are tested for their ability to promote platelet agglutination in the presence of the antibiotic ristocetin .

- *Binding of VWF to collagen (collagen binding activity):*

  ELISAs are available to measure binding of patient VWF to immobilized collagen. VWF:CB is dependent on the presence of HMW multimers and an intact collagen binding site.

❖ *Secondary tests for classification of VWD :*

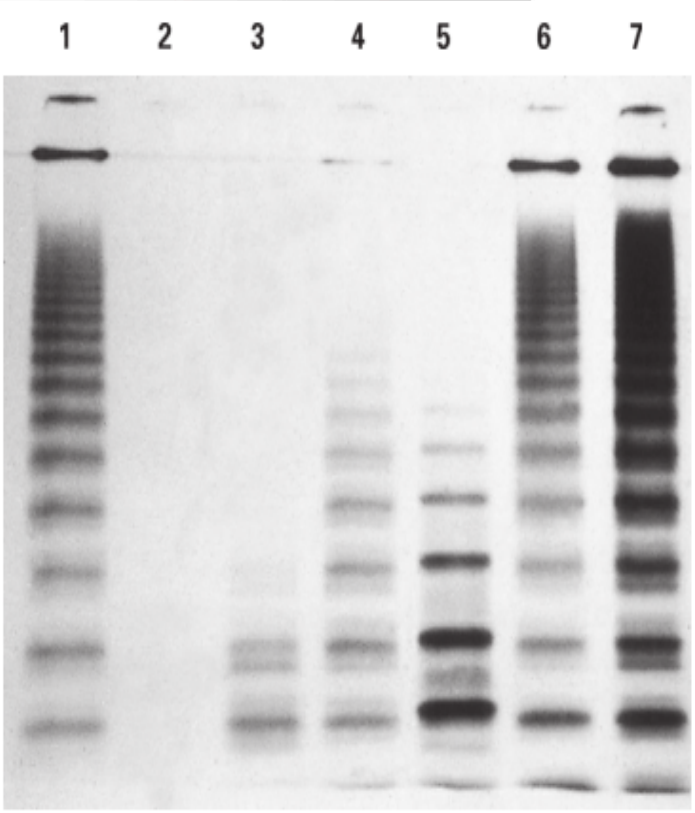
If a deficiency suggestive of VWD is detected then further tests, in particular multimeric analysis and ristocetin - induced platelet aggregation (RIPA) are recommended to allow accurate sub-typing of the VWD.

- ✓ *Multimer Analysis:*

This is generally accomplished by agarose gel electrophoresis of plasma VWF to separate VWF multimers on the basis of molecular size, with the largest multimers migrating more slowly than the intermediate or smaller multimers.

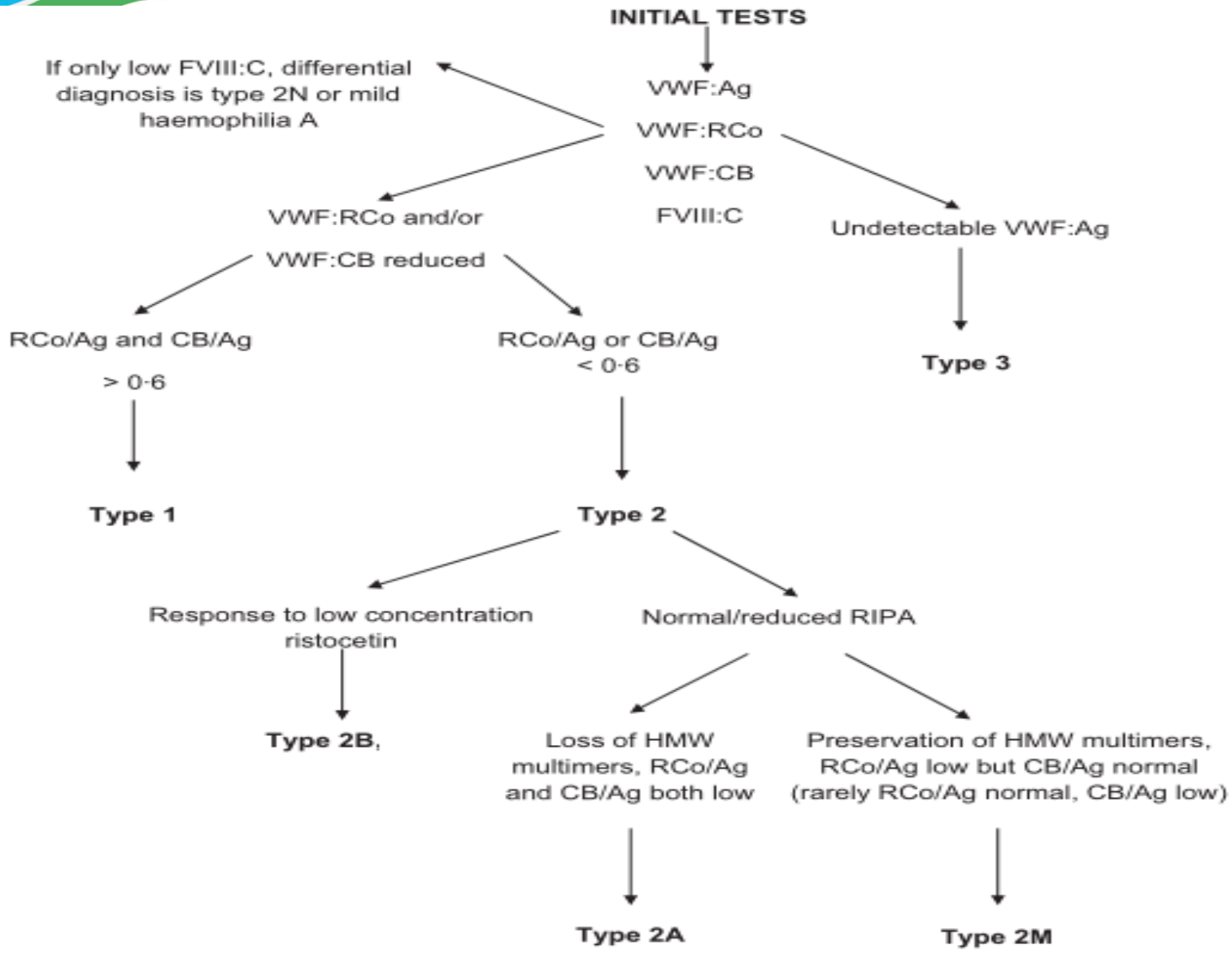
Electrophoretic analysis of von Willebrand factor (VWD) multimer patterns.

- *The largest multimer appear at the top of the gel.*
- *The normal pattern with numerous large multimers and a triplet pattern visible in the smaller multimers are shown in lane 7.*
- *Lanes 1 and 6 are compatible with type 1 VWD in which there is a generalized decrease in multimer numbers but the normal triplet pattern is retained.*
- *In lane 2 there is virtually no VWF detected, indicating type 3 VWD.*
- *Lanes 3 and 5 show both abnormality of VWF amount and multimer pattern, indicating type 2A(Large+intermediate multimers).*
- *In lane 4 there is a selective loss of the large multimers typical of type 2B VWD.*



- ✓ Ristocetin-Induced Platelet Agglutination:

also measures platelet agglutination caused by ristocetin-mediated VWF binding to platelet. In the case of RIPA, ristocetin is added directly to patient platelet-rich plasma. This activity is generally reduced in most VWD patients. Hyper responsiveness to RIPA results from type 2B VWD mutation.


INITIAL TESTS
VWF:Ag
VWF:RCo
VWF:CB
FVIII:C
If only low FVIII:C, differential diagnosis is type 2N or mild haemophilia A
VWF:RCo and/or VWF:CB reduced
Undetectable VWF:Ag
Type 3
RCo/Ag and CB/Ag > 0·6
RCo/Ag or CB/Ag < 0·6
Type 1
Type 2
Response to low concentration ristocetin
Normal/reduced RIPA
Type 2B,
Loss of HMW multimers, RCo/Ag and CB/Ag both low
Preservation of HMW multimers, RCo/Ag low but CB/Ag normal (rarely RCo/Ag normal, CB/Ag low)
Type 2A
Type 2M

Management

Options are as follows:

- ✓ *Local measures and antifibrinolytic agent (e.g. tranexamic acid for mild bleeding).*
- ✓ *Desmopressin infusion for those with type 1VWD. This releases VWF from endothelial stage sites 30 min after intravenous infusion.*
- ✓ *High-purity factor VWF concentrates for patients with very low VWF levels.*

- ✓ *Cryoprecipitate has been successfully used in the past, but since it is not currently treated to inactivate viruses, it is less desirable.*

Acquired *Coagulation disorders*

Acquired coagulation disorders

- ✓ *The acquired coagulation disorders are more common than the inherited disorders. Unlike the inherited disorders, multiple clotting factor deficiencies are usual.*

Causes of Acquired coagulation disorders

- *Deficiency of vitamin K-dependent factors:*
- ✓ *Haemorrhagic disease of the newborn.*
- ✓ *Biliary obstruction.*
- ✓ *Malabsorption of vitamin K(e.g. Tropical sprue, gluten induced enteropathy).*
- ✓ *VitaminK-antagonist therapy (e.g. Coumarin).*
- ✓ *Liver disease.*
- ✓ *Disseminated intravascular coagulation.*

- ❖ *Inhibition of coagulation*
- ✓ *Specific inhibitors (e.g. antibodies against factor VIII).*
- ✓ *Non-specific inhibitors (e.g. antibodies found in systemic lupus erythematosus, rheumatoid arthritis).*
- ❖ *Miscellaneous:*
- ✓ *Diseases with M-protein production.*
- ✓ *Therapy with heparin, defibrinating agents or thrombolytics.*
- ✓ *Massive transfusion syndrome.*

Disseminated intravascular coagulation

Definition:

- ✓ *Widespread inappropriate intravascular deposition of fibrin with consumption of coagulation factors and platelets.*
- ✓ *occurs as a consequence of many disorders which release procoagulant material into the circulation or cause widespread endothelial damage or platelet aggregation.*

- ✓ *It may be associated with a fulminant haemorrhagic or thrombotic syndrome with organ dysfunction or run a less severe and more chronic course.*
- ✓ *The main clinical presentation is with bleeding but 5-10% of patients manifest microthrombotic lesions (e.g. with gangrene of limbs).*

Table 130–1. Clinical Conditions That May Be Complicated by DIC
Sepsis/severe infection
Trauma
Malignancy
Solid tumors
Acute leukemia
Obstetrical conditions
Amniotic fluid embolism
Abruptio placentae
HELLP syndrome
Vascular abnormalities
Kasabach-Merritt syndrome
Other vascular malformations
Aortic aneurysms
Severe allergic/toxic reactions
Severe immunologic reactions (e.g., transfusion reaction)
Heatstroke

HELLP, hemolysis, elevated liver enzymes, and low platelet count.

Pathogenesis

- ✓ *The key event underlying DIC is increased activity of tissue factor. This can come from its release into the circulation from damaged tissues or from up-regulation of tissue factor on circulating monocytes or endothelial cells in response to pro-inflammatory cytokines (e.g.interleukin-l, tumour necrosis factor, endotoxin).*

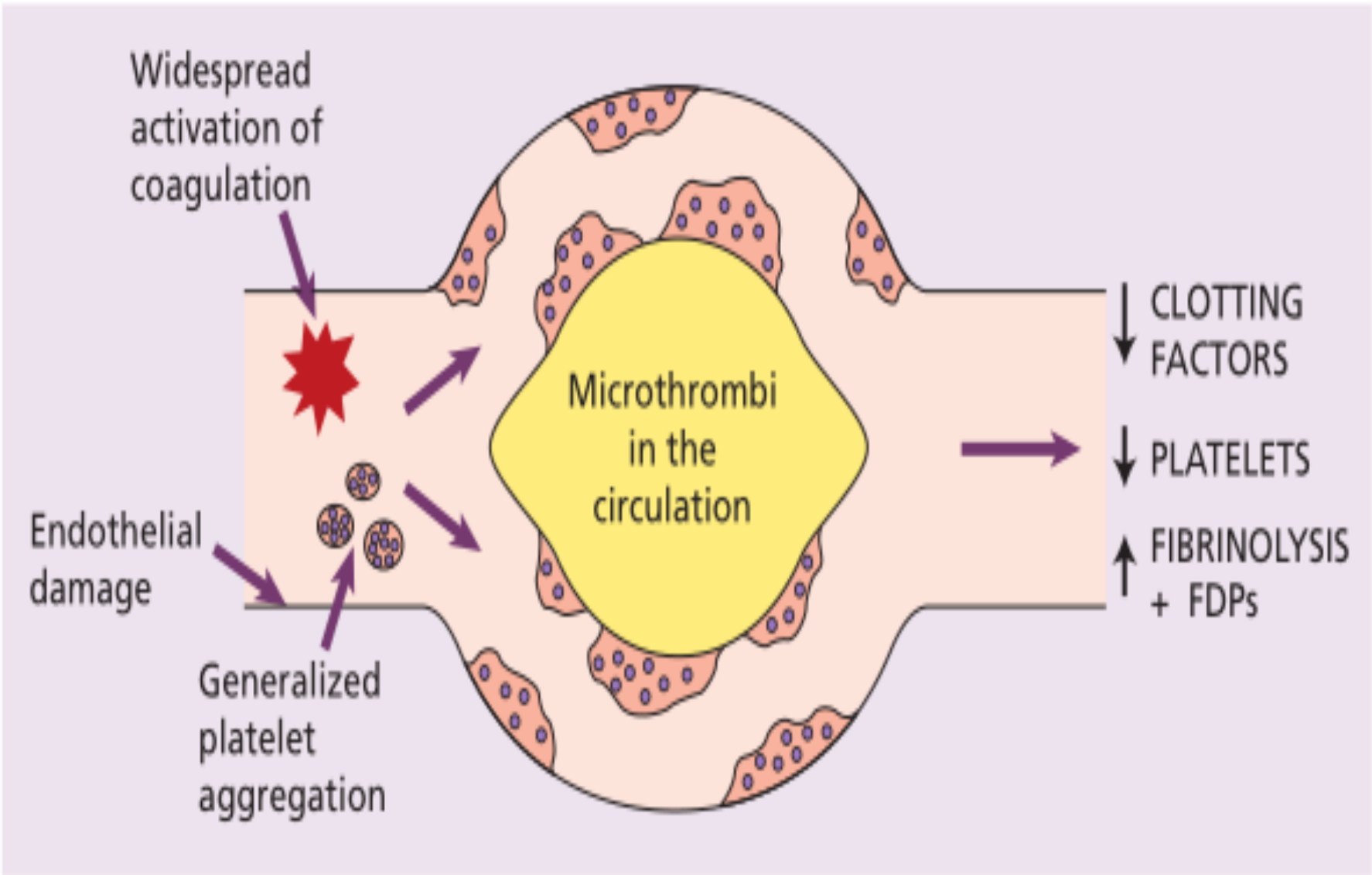
The complex of TF–factor VIIa catalyzes the conversion of factor X to Xa and factor Xa

generation of thrombin

- *Thrombin generated by the TF pathway amplifies clotting through the following activities:*
- ✓ *It activates platelets, giving rise to platelet aggregation.*
- ✓ *It activates factors VIII, V, and XI, yielding further thrombin generation.*
- ✓ *It activates factor XIII , which crosslinks fibrin clots.*
- ✓ *It activates thrombin-activatable fibrinolysis inhibitor (TAFI), making clots resistant to fibrinolysis.*

- *The widespread generation of thrombin in DIC induces deposition of fibrin, which leads to the consumption of substantial amounts of platelets, fibrinogen, factors V and VIII, protein C, antithrombin , and components of the fibrinolytic system.*
- *This situation results in massive depletion of these components that is further aggravated because of their decreased synthesis by the liver, which frequently is affected in DIC.*

- *Depending on the magnitude and nature of component depletion, bleeding, enhanced thrombosis, or both can result.*
- *Microangiopathic hemolytic anemia also occurs as a result of blood cells passing through vessels that are partially occluded by thrombi.*


Widespread activation of coagulation
Endothelial damage
Generalized platelet aggregation
Microthrombi in the circulation
CLOTTING FACTORS
PLATELETS
FIBRINOLYSIS + FDPs

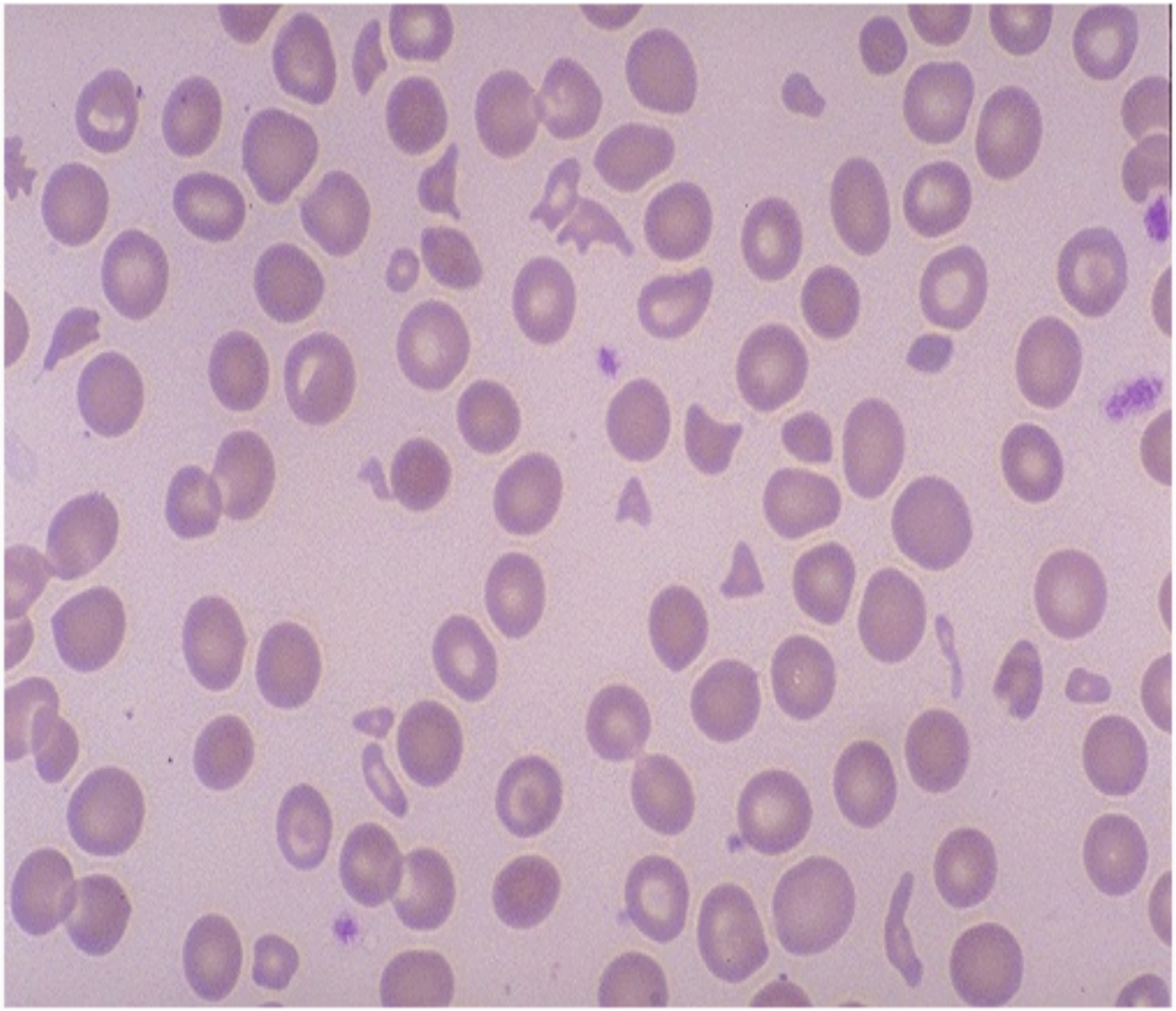
Clinical features

- ✓ These are dominated by bleeding, particularly from venepuncture sites or recent wounds.
- ✓ There may be generalized bleeding in the gastro-intestinal tract, into the lungs, uro-genital tract and in obstetric cases vaginal bleeding may be particularly severe.
- ✓ Less frequently, microthrombi may cause skin lesions, renal failure ,cerebral ischaemia.

Laboratory findings

- ✓ *Blood film examination:*

In many patients there is a haemolytic anaemia ('microangiopathic') and the red cells show prominent fragmentation because of damage caused when Passing through fibrin strands in small vessels.



Tests of haemostasis:

1 -The platelet count is low.

2 -Fibrinogen concentration is low.

3 -The thrombin time is prolonged.

4 -High levels of fibrin degradation products such as D-dimers are found in serum and urine.

5 -The PT and APTT are prolonged.

Treatment

- ✓ *Treatment of the underlying cause is most important. The management of patients who are bleeding differs from that of patients with thrombotic problems.*

Bleeding:

- ✓ *Supportive therapy with fresh frozen plasma and platelet concentrates is indicated in patients with dangerous or extensive bleeding*

Thrombosis:

- ✓ *The use of heparin or antiplatelet drugs to inhibit the coagulation process is considered in those with thrombotic problems such as dermal ischaemia.*

Thank
You!