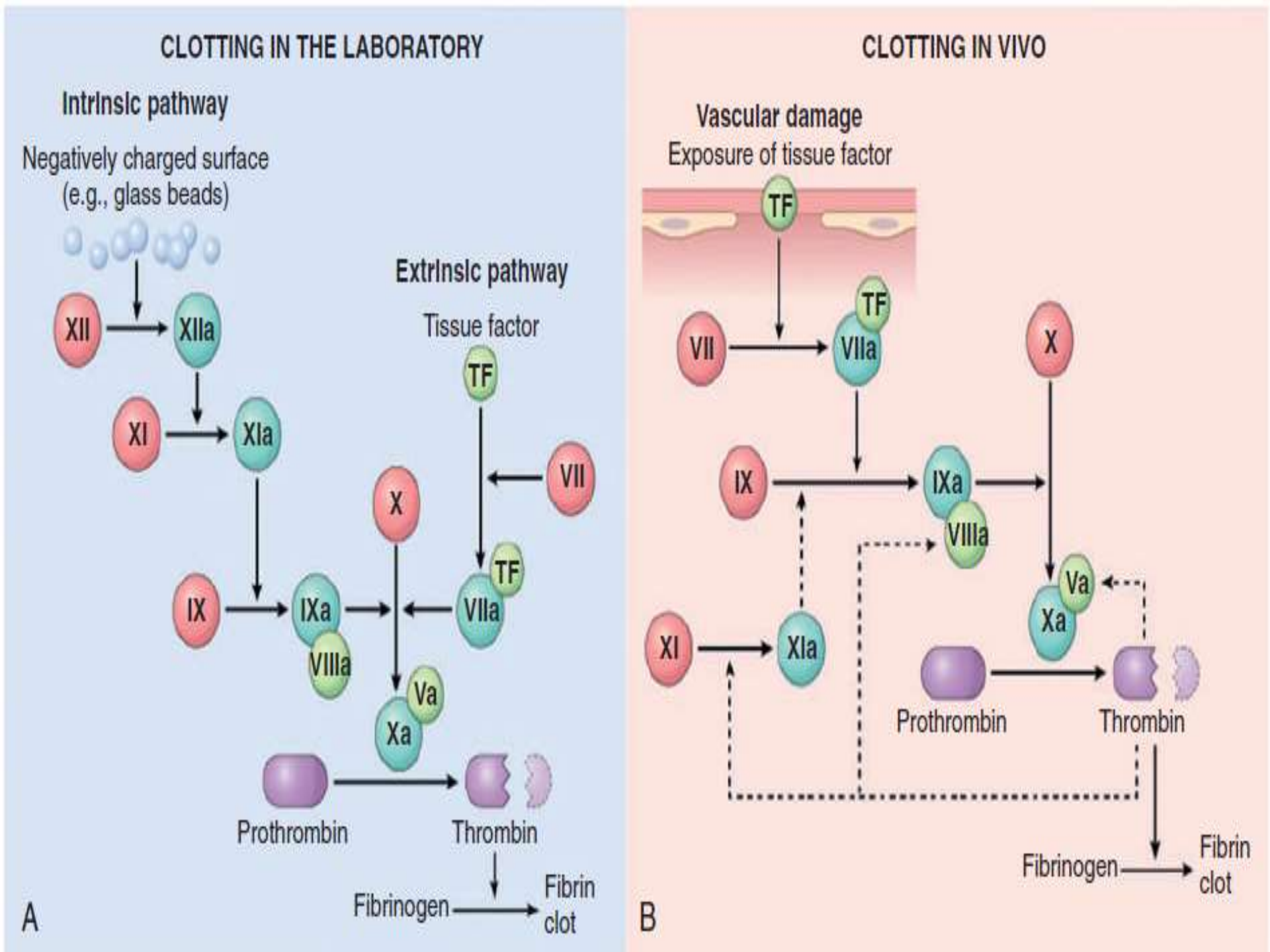
Hemostasis

- Hemostasis depends on the integrity of
  - Blood vessels
  - Platelets
  - Coagulation factors
  - Anticoagulation factors


CLOTTING IN THE LABORATORY
Intrinsic pathway
Negatively charged surface
(e.g., glass beads)
XII
XIIa
XI
XIa
IX
IXa
VIIIa
X
Va
Xa
Prothrombin
Thrombin
Fibrinogen
Fibrin clot
Extrinsic pathway
Tissue factor
TF
VII
VIIa
A
CLOTTING IN VIVO
Vascular damage
Exposure of tissue factor
TF
VII
VIIa
X
IX
IXa
VIIIa
Va
Xa
XI
XIa
Prothrombin
Thrombin
Fibrinogen
Fibrin clot
B

BLEEDING DISORDERS

Definition: Diseases characterized by a tendency to bleeding with deficient hemostasis.

** Bleeding occurs either → spontaneously or→ due to minor trauma

The most important tests for investigation of suspected coagulopathies include:

(1)Prothrombin time (PT). This test assesses the extrinsic and common coagulation pathways.

It measures the time (in seconds) needed for plasma to clot after addition of tissue thromboplastin (e.g., brain extract) and Ca2+ ions.

((factors VII, X, V,II (prothrombin), and fibrinogen)).

(2) Partial thromboplastin time (PTT). This test assesses the intrinsic and common coagulation pathways. It measures the time (in seconds) needed for the plasma to clot after the addition of kaolin, cephalin, and Ca2+

((factorsXII, XI, IX, VIII, X, V, II, and fibrinogen)).

Prolongation is seen in hemophiliacs and in patients with lupus anti-coagulant

The most important tests for investigation of suspected coagulopathies include:

- **(3)Platelet count.** The reference range is 150,000 to 450,000/μL. Counts outside this range must be confirmed by a visual inspection of a peripheral blood smear.

- **(4) Tests of platelet function**. Newer instrument-based assays that provide quantitative measures of platelet function show promise but are not yet available for routine use in the clinic.

Causes of Abnormal Bleeding

- Vascular disorders.
- Thrombocytopenia.
- Platelet function defects.
- Defective coagulation.

Coagulation disorders

- **Coagulation disorders** result from either **congenital** or **acquired** deficiencies of clotting factors. Acquired deficiencies are most common and often involve several factors simultaneously.
- **Vitamin K** is required for the synthesis of prothrombin and clotting factors VII, IX, and X, and its deficiency causes a severe coagulation defect.
- **The liver** synthesizes several coagulation factors and also removes many activated coagulation factors from the circulation; thus, hepatic parenchymal diseases are common causes of complex hemorrhagic diatheses.
- **DIC** also may lead to multiple concomitant factor deficiencies.
- Rarely, **autoantibodies** may cause acquired deficiencies limited to a single factor.

Inherited Deficiencies of Coagulation Factors

Deficiency	Incidence in Population	Gene on Chromosome	Mode of Inheritance
Fibrinogen	1:1 million	4	AR
Prothrombin	1:2 million	11	AR
Factor V	1:1 million	1	AR
Factor VII	1:500,000	13	AR
Factor VIII	1:10,000	X	XLR
Factor IX	1:600,000	X	XLR
Factor X	1:1 million	13	AR
Factor XI	1:1 million	4	AR
Factor XIII	1:2 million	6 & 1	AR

Coagulation disorders

- Hereditary deficiencies of many coagulation factors also have been identified.
- Hemophilia A (a deficiency of factor VIII) and Hemophilia B (Christmas disease, a deficiency of factor IX) are X-linked traits
- Other deficiencies are autosomal recessive disorders.
- Of the inherited deficiencies, only von Willebrand disease, hemophilia A, and hemophilia B are sufficiently common to warrant further consideration.

Nomenclature of Factor VIII

Factor VIII	Protein lacking or aberrant in hemophilia A
Factor $VIII_c$	Functional property of factor VIII missing in hemophilia A, measured by coagulation assays
Factor $VIII_{Ag}$	Antigenic property of factor VIII, measured by immunoassays

Hemophilia A

- Incidence: 1 in 10,000 (common).
- Sex-linked.
- 30% of the cases have no family history (recent mutation or generations of silent carriers).
- Caused by absolute reduction of factor VIII or normal amount but defective factor VIII (FVIII:C).
- Severity of disease depends on factor VIII level
  - Normal level 100 U/dl
  - Severe cases level <2 U/dl (<1%)
  - Moderate cases level 2-5 U/dl (2-5%)
  - Mild cases level 5-25 U/dl (5-30%)

Hemophilia A

- Affects male
- Less commonly excessive bleeding occurs in heterozygous females…preferential inactivation of the X chromosome carrying the normal factor VIII gene (unfavorable lyonization)
- C/O:

-Easy bruising and massive hemorrhage after trauma or operative procedure.

-Petechia are characteristically absent.

Hemophilia A

- Sites of bleeding:
  - Large joints and soft tissue
  - Urinary tract and GI tract
  - Brain
  - Nose
- Laboratory tests:
  - Prolonged PTT (corrected by mixing study). Normal PT and TT.
  - Low factor VIII assay.
- Treatment: replacement therapy (factor VIII concentrate or recombinant VIII).
- Inhibitor antibodies develop in 18-50% of patients

Hemophilia in Females

Exceedingly rare, seen in:

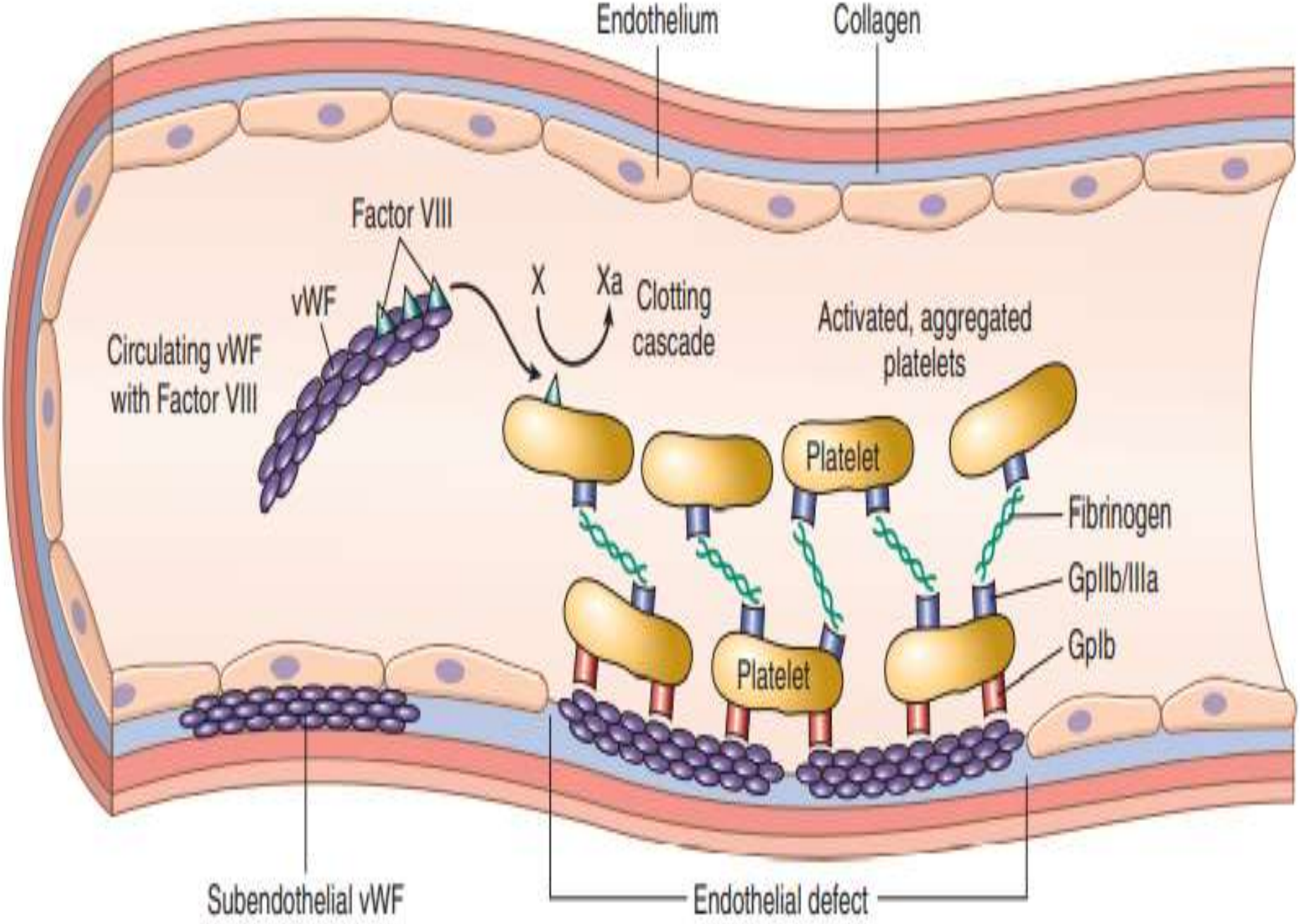
- Mating between a carrier mother and affected father
- Carriers with abnormalities of X-chromosome
  - Extreme lyonization
  - X mosaicism or deletion
  - Newly mutant gene

Hemophilia B

- Incidence: 1 in 60,000.
- Sex-linked.
- Severity of disease depends on factor IX level
  - Normal level 100 U/dl
  - Severe cases level <2 U/dl
  - Moderate cases level 2-5 U/dl
  - Mild cases level 5-25 U/dl
- Bleeding sites: similar to hemophilia A.
- Laboratory tests:
  - Prolonged PTT. Normal PT and TT.
  - normal factor VIII assay.

vWF

- Adhesive protein, bridges collagen to platelets receptor GPIb
- Carrier protein to factor VIII
- Ristocetin induces platelets agglutination in the presence of vWF (**RIPA**)
- Stored in Weibel-Palade bodies of endothelial cells and a granules of platelets


Endothelium
Collagen
Factor VIII
vWF
Circulating vWF
with Factor VIII
X
Xa
Clotting
cascade
Activated, aggregated
platelets
Platelet
Fibrinogen
GpIIb/IIIa
GpIb
Platelet
Subendothelial vWF
Endothelial defect

von Willebrand Disease

*The most common hereditary bleeding disorder (prevalence 0.1-1%).

- von Willebrand disease is transmitted as an autosomal
- dominant disorder. I
- Mild bleeding problems
  - Mucous membrane bleeding
  - Easy bruising
  - menorrhagia
  - Post-operative bleeding
- Most cases are inherited as Autosomal dominant disorder.
- ? When to suspect:

*Both sexes are affected, and presented with prolonged bleeding times (BT) despite normal platelet counts. (early historical observation)

von Willebrand Disease

*vWD differs from classic Hemophilia A in 3 cardinal manifestations:

1. Autosomal inheritance rather than sex linked
2. Consistently prolonged bleeding time (BT)
3. Mucocutaneous bleeding rather than hemarthroses and deep muscle hemorrhage.

von Willebrand Disease

*vWD : quantitative or qualitative defects of plasma vWF.

- Either Dominant or Recessive.
- Divided into 3 main type: 1,2, and 3.
- Type 2 vWD is further subdivided into 4 subtypes, designated as 2A,2B, 2M, and 2N.

- Additional disease related to platelet vWF receptor, *platelet type/pseudo-vWD* (similar presentation of vWD, but doesn't involve a mutation of the vWF gene).

von Willebrand Disease

*vWD : Severe forms generally present with

A normal PT

A prolonged PTT that corrects with mixing study

Normal platelet count

An abnormal BT

*Lab. Assessment should include assays for:

F VIII:C activity, vWF: Ag, RIPA, and vWF multimeric analysis.

Table 1. Classification of vWF[8]

Subtype	Description	Inheritance	Lab Findings	Multimer	Prevalence
Type 1	Quantitative deficiency of vWF	Autosomal dominant	Parallel reductions in vWF antigen, activity, and Factor VIII	Normal Distribution	70–80%
Type 2A	Abnormal platelet-dependent function of vWF; loss of large multimers	Autosomal dominant	Reduced vWF activity-to-antigen ratio (< 0.6)	Loss of mid-sized and highest molecular weight multimeters	10–15%
Type 2B	Increased platelet-dependent functions of vWF; loss of large multimers	Autosomal dominant	Reduced vWF activity-to-antigen ratio (< 0.6) Abnormal Ristocetin-induced platelet aggregation	Loss of highest molecular weight multimers secondary to platelet binding and clearance	Approx. 5%
Type 2M	Abnormal platelet-dependent function of vWF	Autosomal dominant	Reduced vWF activity-to-antigen ratio (< 0.6)	Normal distribution	Rare
Type 2N	Decreased affinity of vWF for Factor VIII	Autosomal dominant	Reduced Factor VIII level (2–10%)	Normal distribution	Rare
Type 3	Near complete deficiency of vWF	Autosomal recessive	Marked reductions or absence in vWF levels Low Factor VIII (5–10%)	Absent	Rare

DIC (Consumptive Coagulopathy)

- **DIC** occurs as a complication of a wide variety of disorders; it is caused by the **systemic activation of coagulation** and results in the formation of thrombi throughout the microcirculation.
- As a consequence, **platelets and coagulation factors** are consumed and, secondarily, fibrinolysis is activated.
- Thus, DIC can give rise either to tissue hypoxia and **microinfarcts** caused by myriad **microthrombi** or to a bleeding disorder related to pathologic activation of fibrinolysis and the depletion of the elements required for hemostasis (hence the term consumptive coagulopathy).
- This entity probably causes bleeding more commonly than all of the congenital coagulation disorders combined

DIC (Consumptive Coagulopathy)

Accelerated platelet destruction in combination with coagulation factor consumption

- Acute presentation: Anemia and thrombocytopenia
- Chronic: Thrombosis

Imbalance between the action of Thrombin and the action of Plasmin

Triggering mechanisms:

- Activation of extrinsic, intrinsic pathways or direct activation of factor X/II

DIC

- THROMBOSIS AND FIBRINOLYSIS
- TRIGGERS:

1. Release of Thromboplastin
   (adenocarcinoma, leukemia, inflammation)
2. Widespread endothelial injury (release of TF and exposure of VWF)

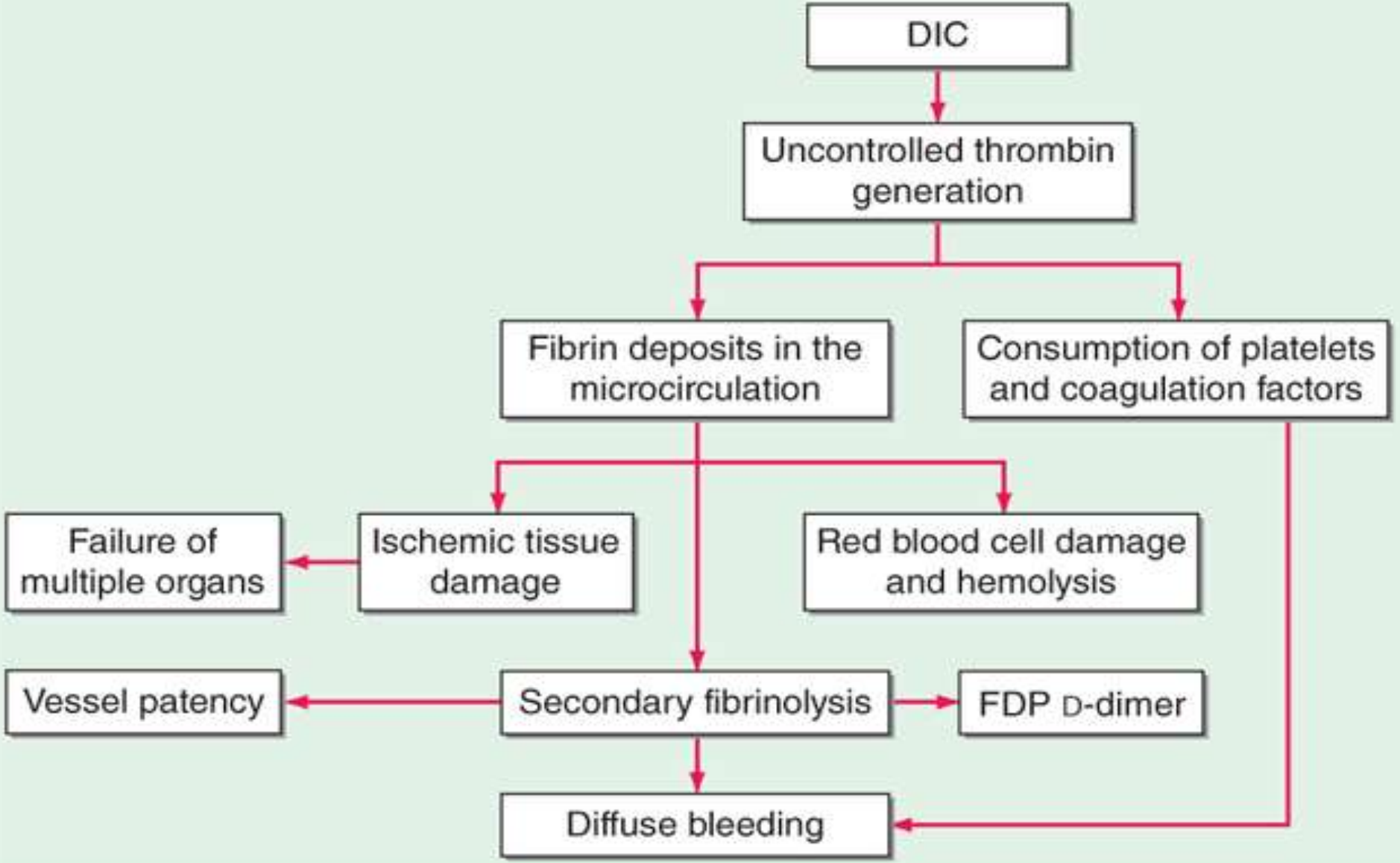
DIC

- TWO COMPONENTS:

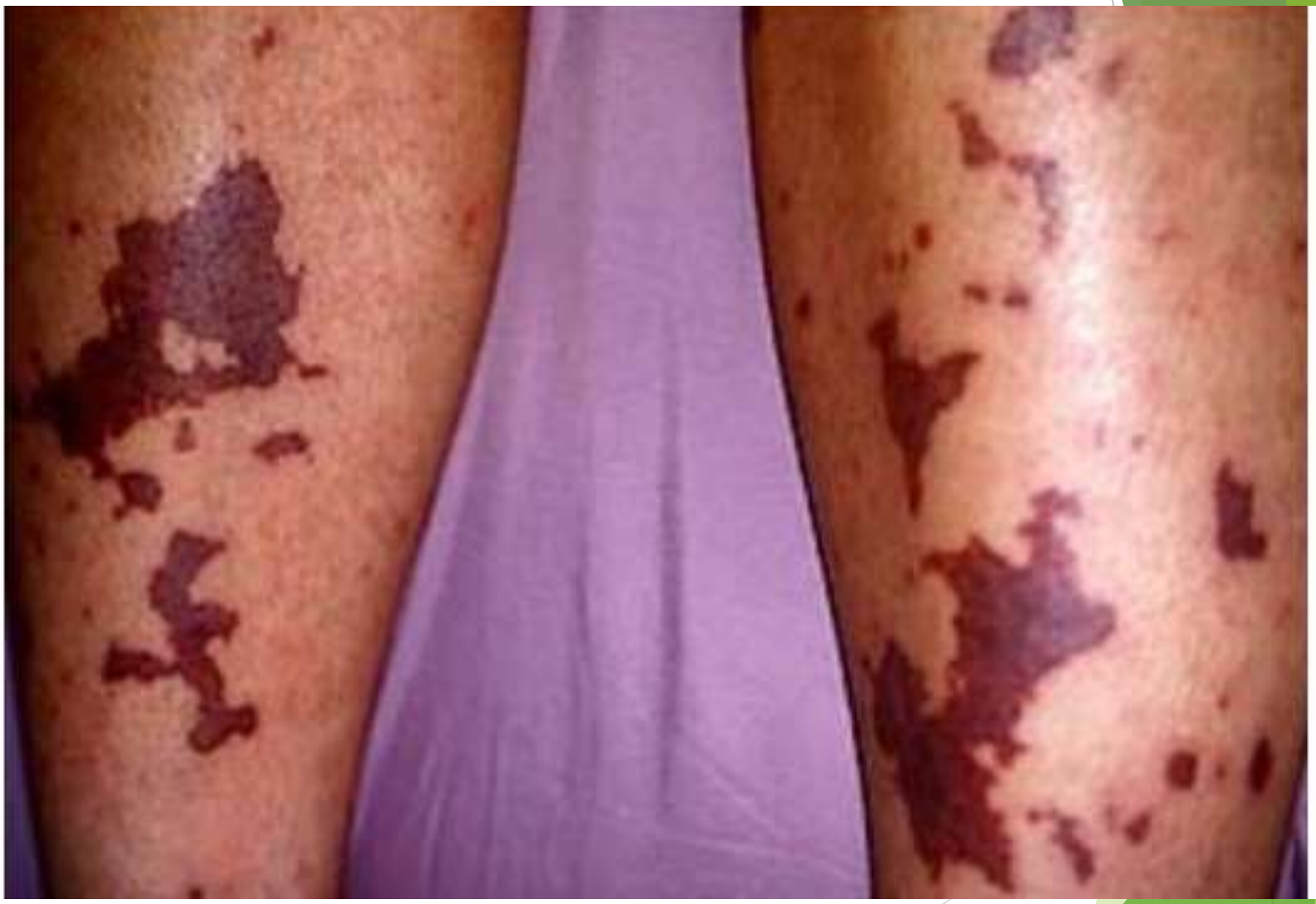
1. Microangiopathic hemolytic anemia
2. Fibrinolysis/FDPs:

a. **Inhibit platelet coagulation**
b. **Anti-thrombotic activity**

Disseminated Intravascular Coagulation Algorithm



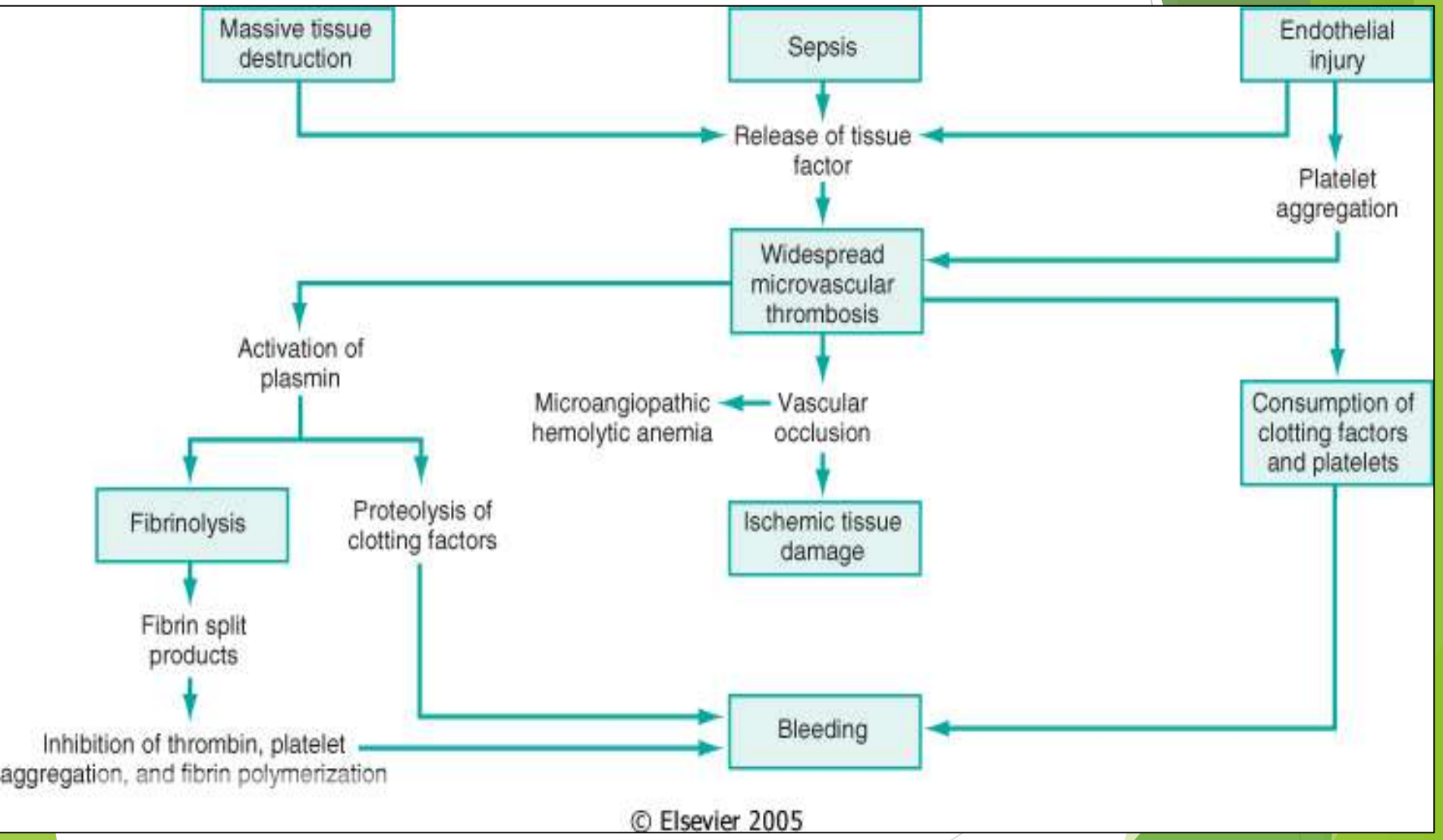
Source: Dan L. Longo
Harrison's Hematology and Oncology, Third Edition
www.hemonc.mhmedical.com



DIC (Consumptive Coagulopathy)

- Thrombosis, fibrinolysis, bleeding.
- Obstetric complications, infections, cancers (panc, lung, prost, stomach, APML), massive tissue injury (trauma, burn), others (shock, liver, heat stroke, vasculitis, intravascular hemolysis).
- Lab: Low plat,. High fibrinogen, PT, PTT and FDP.

DIC


Massive tissue destruction
Sepsis
Endothelial injury
Release of tissue factor
Platelet aggregation
Widespread microvascular thrombosis
Activation of plasmin
Microangiopathic hemolytic anemia
Vascular occlusion
Consumption of clotting factors and platelets
Fibrinolysis
Proteolysis of clotting factors
Ischemic tissue damage
Fibrin split products
Bleeding
Inhibition of thrombin, platelet aggregation, and fibrin polymerization
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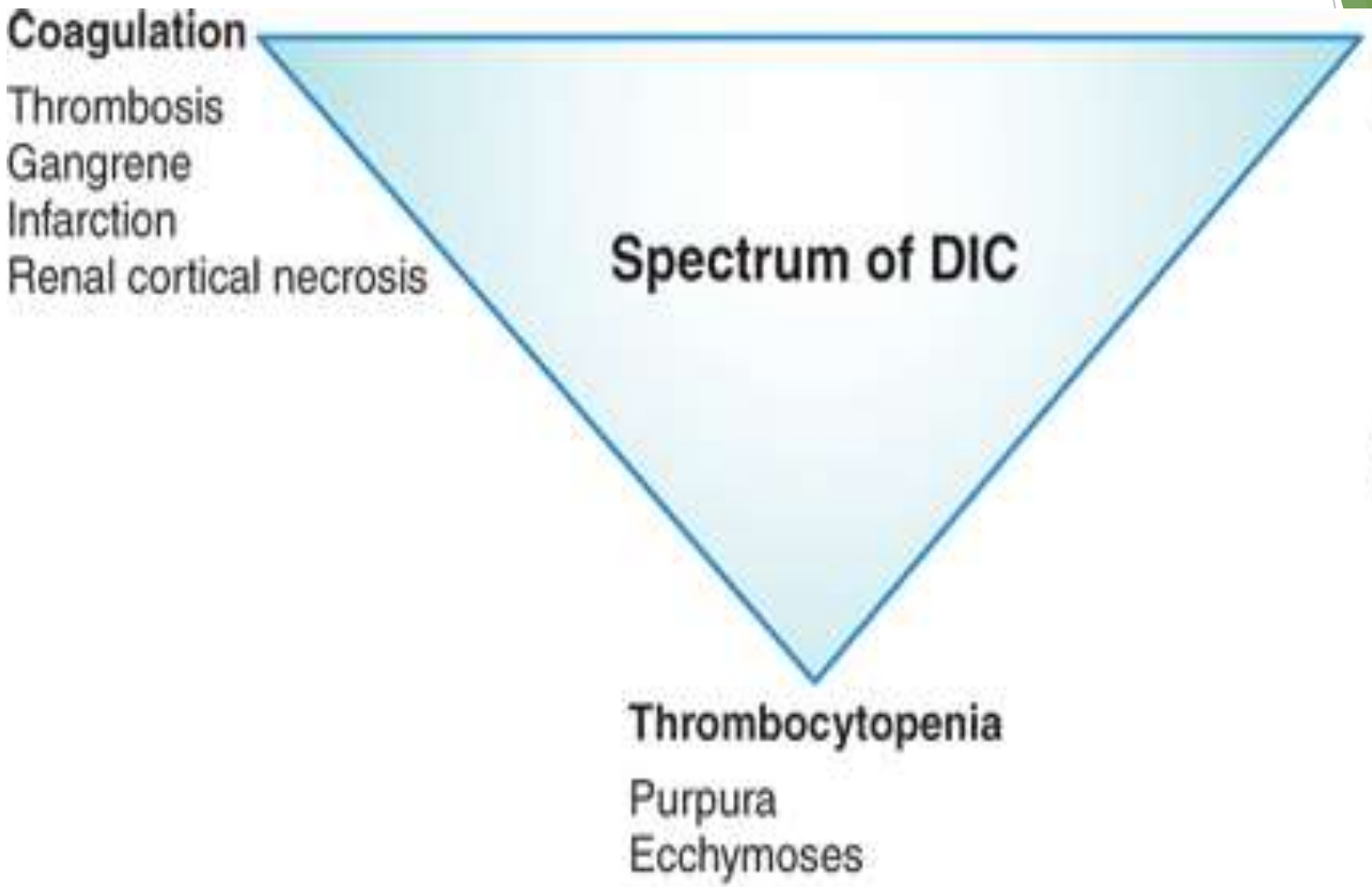

Source: H. Franklin Bunn, Jon C. Aster: Pathophysiology of Blood Disorders
www.accessmedicine.com

DIC

- Clinical Features
- Depending on the balance between clotting and bleeding tendencies, the range of possible clinical manifestations is enormous.
- In general, **acute DIC** (e.g., that associated with obstetric complications) is dominated by **bleeding**
- **Chronic DIC** (e.g., as occurs in those with cancer) tends to manifest with signs and symptoms related to **thrombosis**.
- The abnormal clotting usually is confined to the microcirculation, but large vessels are involved on occasion.
- The manifestations may be minimal, or there may be shock, acute renal failure, dyspnea, cyanosis, convulsions, and coma.

DIC

- **The prognosis** varies widely depending on the nature of the underlying disorder and the severity of the intravascular clotting and fibrinolysis. **Acute DIC** can be life threatening and must be treated aggressively with anticoagulants such as heparin or the coagulants contained in fresh frozen plasma.

- Conversely, **chronic DIC** is sometimes identified unexpectedly by laboratory testing.

- In either circumstance, definitive treatment must be directed at the underlying cause

Platelet disorders

- **Isolated thrombocytopenia is associated with a bleeding tendency and normal coagulation tests**

Relationship Between Platelet Count and Bleeding

- Normal range 150-450X10^3 per µl.
- Levels above 60x10^3/µl will not cause bleeding under normal conditions.
- Levels below 20x10^3/µl will cause:

  Petechiae, mucosal bleeding.

  Post-operative bleeding, CNS bleeding.
- Levels around 5x10^3/µl can lead to fatal CNS or GI hemorrhage.
- Levels between 20 and 60x10^3/µl may cause bleeding (depending on platelets functional status).

Classification of Thrombocytopenia

1-Failure of production (aplastic anemia, radiation, chemo.Rx)

2-Increased platelet destruction (ITP)

3-Abnormal distribution (Splenic sequestration)

Table 12.13 Causes of Thrombocytopenia

Decreased Production of Platelets
Generalized Bone Marrow Dysfunction
Aplastic anemia: congenital and acquired
Marrow infiltration: leukemia, disseminated cancer
Selective Impairment of Platelet Production
Drug-induced: alcohol, thiazides, cytotoxic drugs
Infections: measles, HIV infection
Ineffective Megakaryopoiesis
Megaloblastic anemia
Paroxysmal nocturnal hemoglobinuria
Decreased Platelet Survival
Immunologic Destruction
Autoimmune: ITP, systemic lupus erythematosus
Isoimmune: posttransfusion and neonatal
Drug-associated: quinidine, heparin, sulfa compounds
Infections: infectious mononucleosis, HIV infection, cytomegalovirus infection
Nonimmunologic Destruction
Disseminated intravascular coagulation
TTP
Giant hemangiomas
Microangiopathic hemolytic anemias
Sequestration
Hypersplenism
Dilutional
Multiple transfusions (e.g., for massive blood loss)

DIC, Disseminated intravascular coagulation; *HIV*, human immunodeficiency virus; *ITP*, immune thrombocytopenic purpura; *TTP*, thrombotic thrombocytopenic purpura.

Thrombocytopenia Due to Increased Platelets Destruction

- **Immune Thrombocytopenic Purpura (ITP)**
- Secondary immune thrombocytopenia as in SLE
- Drug related immune thrombocytopenia as in quinidine and heparin.
- Post transfusion thrombocytopenia
- Neonatal thrombocytopenia either due to autoantibodies or alloantibodies
- **DIC and microangiopathic hemolytic anemia**

ITP

- Primary (idiopathic) or secondary
- Acute (self limiting) or chronic.

Acute ITP (Idiopathic/Childhood)

- Affects children.
- Develops acutely with 1-2 week duration.
- Bruising and petechia
- Preceded by infection or vaccination in 75% of cases.
- Initial Plt.count <20,000
- Self limited, Spontaneous remission in >90% of cases.
- Severe cases benefit from steroids or IV immunoglobulins.

Chronic Immune Thrombocytopenic Purpura (ITP)

- High incidence in women of child bearing age (20-50).
- NO recent history of drug or recent infection.
- Mostly idiopathic, secondary causes include SLE, HIV, CLL, Hodgkin's disease, drugs (uncommon).
- Autoantibodies against GP IIb/IIIa, or Ib/IX (30% of cases).
- Platelets lifespan reduced to hours.
- Megakaryocytes increased.
- Petechial bleeding, easy bruising, menorrhagia.

ITP
Diagnosis

- Decreased platelet count (10-50x10^9/l).
- Hb. and WBCs are normal.
- PB: large platelet.
- BM: Increased Megakaryocytes numbers.
- BT: Mild prolongation; not as prolonged as the BT in other diseases ass/w same decrease in Plt. Count.
- Antiplatelet antibodies.

ITP
Treatment

- Steroids.
- Splenectomy (long term Rx.).
- High dose IV immunoglobulins.
- Immunosuppressive therapy.

Microangiopathic Thrombocytopenia TTP/HUS

- The term thrombotic microangiopathies encompasses a spectrum of clinical syndromes that include **thrombotic thrombocytopenic purpura** (TTP) and **hemolytic uremic syndrome** (HUS).

Thrombotic thrombocytopenic Purpura (TTP)

Thrombocytopenia and Microangiopathic hemolytic anemia characterize this disorder

Thrombotic thrombocytopenic Purpura (TTP)

- Usually affects adult
- Pathogenic mechanism:

Inherited and Sporadic:

- Deficiency of metalloprotease (ADAMTS 13) needed for cleaving very HMW_vWF (multimers).
- More common, non-familial acquired: autoantibody against ADAMTS 13
- Platelet micro-aggregate (Hyaline microthrombi) formation.
- Acute Thrombocytopenia, fever, microangiopathic hemolytic anemia, neurologic abnormalities, renal dysfunction.

* *Diagnosis should be suspected in any patient who presents acutely with thrombocytopenia.*

* *Female > males*

* *3^{rd}-4^{th} decade*

* *Hyaline (platelet rich) microthrombi are the characterstic pathologic feature (capillary of skin and gingiva).*

* *D.D: DIC.*

Laboratory Results:

Microangiopathic hemolytic anemia picture;
Schistocytes
Reticulocytosis
NRBC's in PB
Increase Mega in BM.

Signs of hemolysis:

Increase LDH
Increase indirect bilirubin
Decrease Haptoglobin

Normal PT, PTT, D-Dimer but elevated BT.

Rx: Plasma exchange.

Hemolytic Uremic Syndrome (HUS)

NON-IMMUNE THROMBOCYTOPENIA

- More commonly seen in pediatric population.
- E. coli O157:H7 (toxin induced endothelial damage)
- Bloody diarrhea followed by acute renal failure.
- Platelet microaggregate (Hyaline microthrombi) formation, usually limited to the glomerular capillaries.
- Acute Thrombocytopenia, Microangiopathic hemolytic anemia, Renal failure.
- Normal PT, PTT, D-Dimer but elevated BT.
- Rx: Conservative (dialysis, antihypertensive,...).

Hemolytic Uremic Syndrome (HUS)

* *Resemble TTP but:*

- More seen in pediatric population
- After viral/bacterial infection
- Pathologic thrombi almost always limited to glomerular capillaries

The End

Good luck