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# PHYSIOLOGY OF HEMOSTASIS
# DISORDERS OF PRIMARY HEMOSTASIS AND COAGULATION

**CHISINAU – 2020**

## PLATELET STRUCTURAL COMPONENTS IMPORTANT FOR HEMOSTASIS

(Williams M.E., Kahn M.J., American Society of Hematology Self-Assessment Program. Blackwell Publishing: 2005)

*Platelet membrane glycoproteins*

GP IIb-IIIa: fibrinogen receptor, critical for platelet aggregation

GP Ib-IX-V: von Willebrand factor receptor: critical for initial platelet adhesion to subendothelium

GP Ia-IIa, GP VI: collagen receptor, involved in initial platelet adhesion

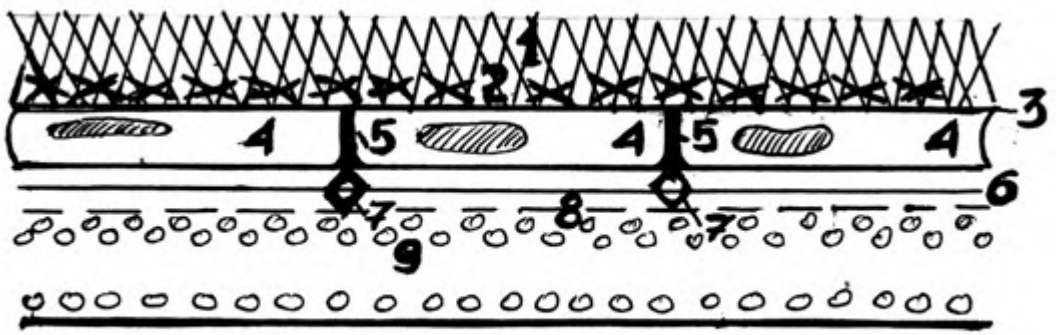
*Platelet granules*

Alpha granules: contain proteins that contribute to platelet adhesion, aggregation and coagulant activity

Dense granules: contain ADP and calcium, which contribute to platelet aggregation and coagulant activity

Lysosomes: contain acid hydrolases that may participate in thrombus degradation

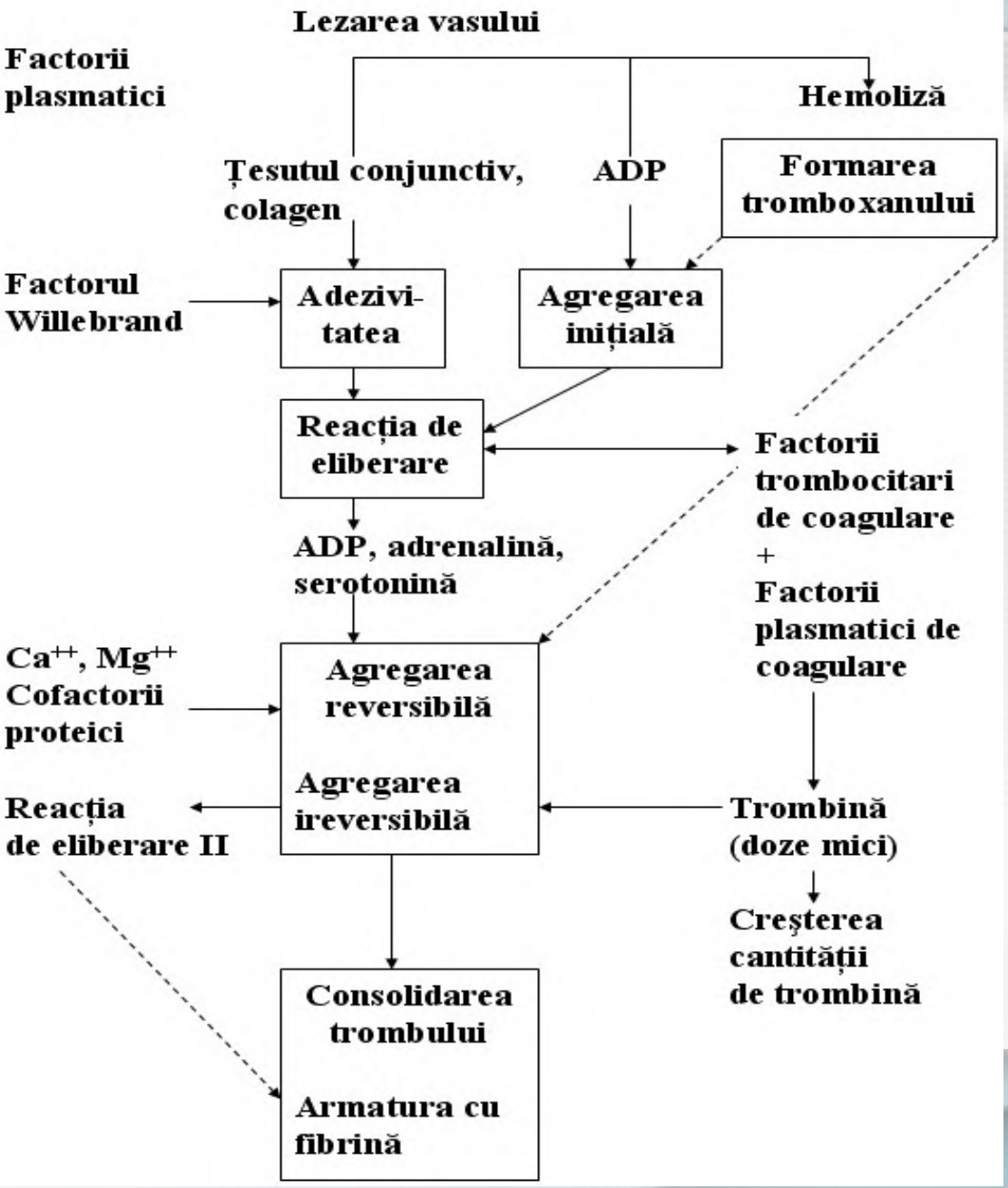
ADP = adenosine diphosphate.




# Structura capilarului

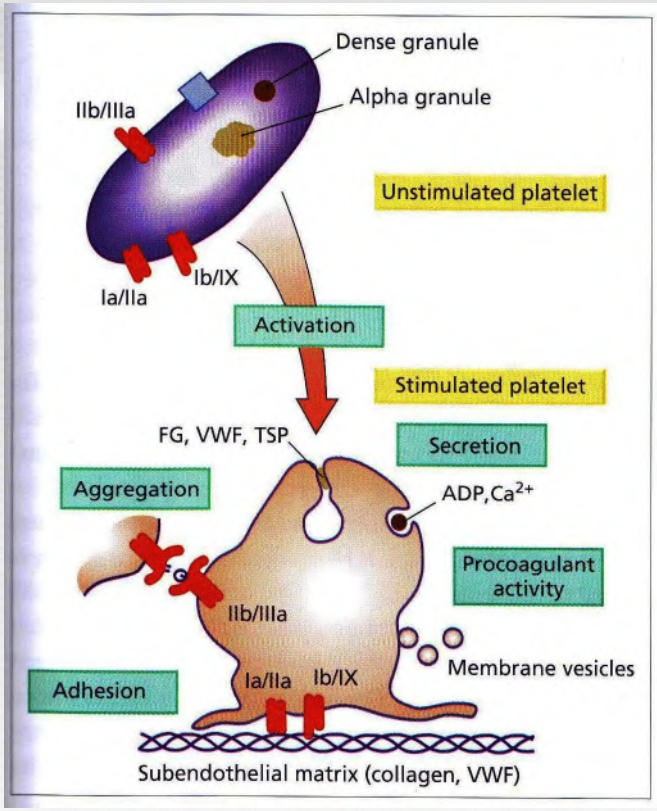
1. țesutul de sprijin pericapilar
2. fibrele de colgen
3. membrana bazală
4. celulă endotelială
5. spațiul interendotelial
6. membrana de fibrină
7. trombocitele
8. atmosfera endotelială plasmatică
9. trombocite în circulație

# Schema hemostazei vasculo-trombocitare



# MODEL OF NORMAL PRIMARY HEMOSTASIS

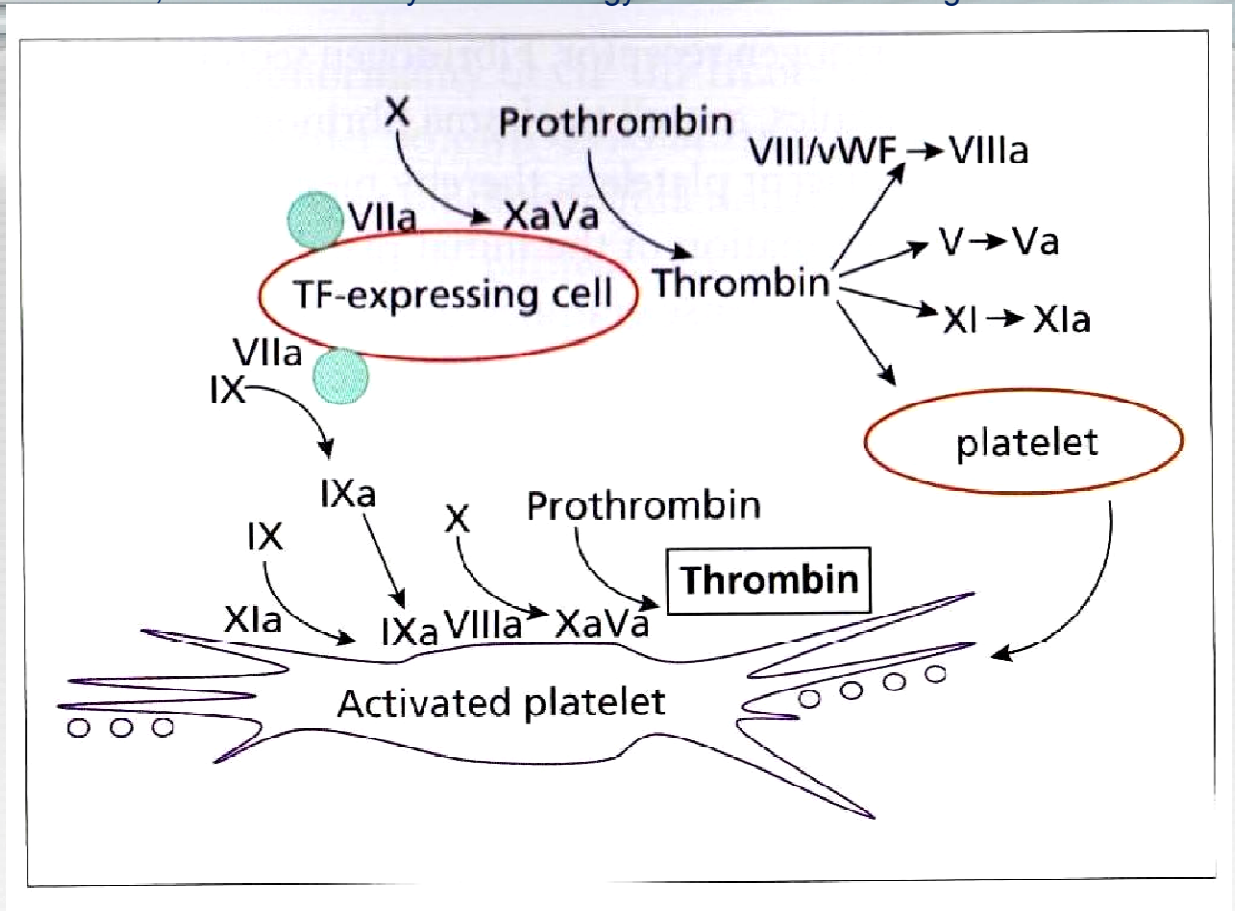
(Williams M.E., Kahn M.J., American Society of Hematology Self-Assessment Program. Blackwell Publishing: 2005)




Platelet activation. Platelets can undergo activation through stimulation by soluble agonists such as thrombin or by contact (adherence) to the subendothelial matrix. Activation changes the platelet from a disk to a sphere with pseudopods. Activation is followed by secretion of granular contents, aggregation and rearrangement of membrane phospholipids, resulting in potentiation of phospholipid-dependent procoagulant activity. Reproduced with permission from George JN, Shattil SJ. The clinical importance of acquired abnormalities of platelet function. N Engl J Med. 1991;324:27–39.

# MODEL OF NORMAL HEMOSTASIS

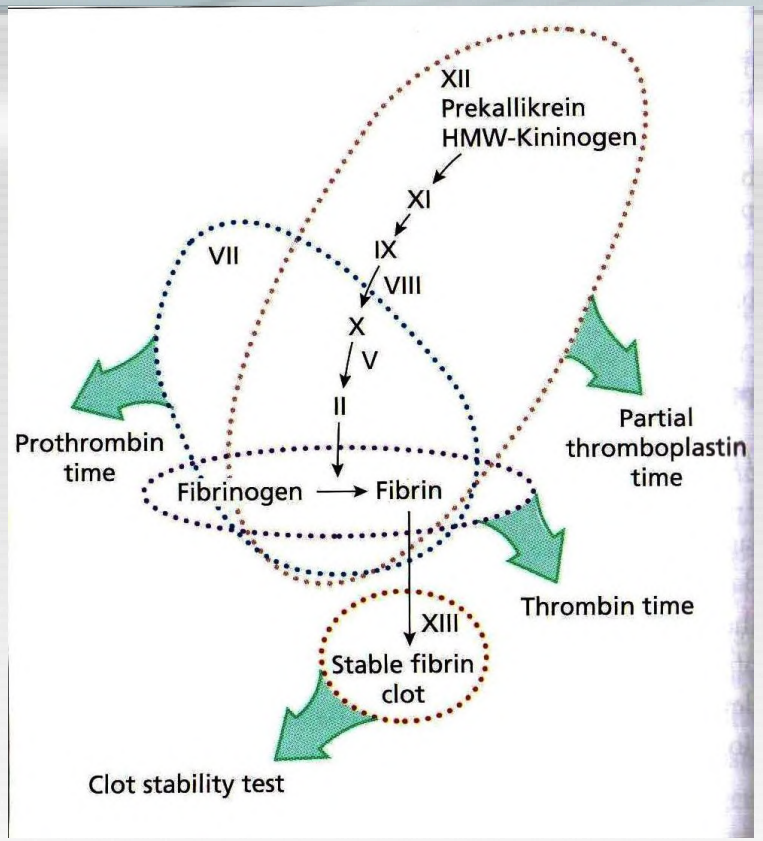
(Williams M.E., Kahn M.J., American Society of Hematology Self-Assessment Program. Blackwell Publishing: 2005)




Model of normal hemostasis depicting essential role of tissue factor–factor VIIa in initiating thrombin generation and subsequent key interaction of factors XI, IX and VIII on the surface of the platelet with resultant larger thrombin burst. Adapted from Hoffman M, Monroe DM, III, Roberts HR. Activated factor VII activates factors IX and X on the surface of activated platelets: thoughts on the mechanism of action of high-dose activated factor VII. Blood coagulation and fibrinolysis. 1998;9(51):561–564.

# *PLASMA COAGULATION REACTIONS*

(Williams M.E., Kahn M.J., American Society of Hematology Self-Assessment Program. Blackwell Publishing: 2005)




Plasma coagulation reactions in *in vitro* laboratory assays. Note the differences between this diagram and Figure 14-2. Factor XII, prekallikrein and high-molecular-weight kininogen are required for a normal partial thromboplastin time but not for normal *in vivo* hemostasis. Also, plasma factor XI may not always be required for normal *in vivo* hemostasis. Platelets and tissue factor are required for normal *in vivo* hemostasis but are supplied by exogenous reagents in the laboratory assays. This diagram outlines the coagulation factors required for each of four basic tests.

***SCREENING TESTS FOR EXAMINATION OF PRIMARY HEMOSTASIS:***

**bleeding time (Duke test) – up to 3 minutes,**

**thrombocyte count – 150.0 – 400.0 x $10^9$/l,**

**clot retraction – 0.3 – 0.4 / 44 – 66%,**

**thrombocyte adhesiveness – 40 – 50%,**

**ristocetin platelet aggregation – 10seconds.**

***SCREENING TESTS FOR EXAMINATION OF BLOOD COAGULATION*:**

**partial thromboplastin time (activated ) – 37 – 50 seconds,**

**plasma prothrombin time – 16 – 18 seconds,**

**plasma thrombin time – 28 – 32 seconds,**

**fibrinogen assay – 2 – 4 g/l,**

**coagulation time – 8 – 12 minutes,**

**fibrin degradation product assay – 0 – 5 µg/ml,**

**antithrombin III assay – 80 – 125%,**

**autocoagulation test – 9 – 11 seconds,**

**fibrinolytic activity – 30 – 40 minutes,**

**prothrombin assay – 95 – 100%.**

## *TYPES OF HEMORRHAGIC SYNDROME:*

1. Petechiae and ecchymoses occur in thrombocytopenias and some coagulation disorders (factor II,V, X deficiencies);
2. Hematomas, hemarthroses are usually seen in hemophilias;
3. Petechiae, ecchymoses and hematomas (mixed type) are characteristic for von Willebrand disease, factor II, V, X deficiencies, DIC, overdosage of anticoagulants;
4. Vascular purpura occurs in infectious and immune vasculitises;
5. Angiomatous type usually is registered in telangiectasias, hemangiomas.

## *CLASSIFICATION HEMOSTASIS DISORDERS*:

I. Disorders of primary hemostasis: thrombocytopenias, platelet functional disorders (platelet disfunctions), vascular diseases;

II. Disorders of secondary hemostasis (coagulation): coagulation factor deficiencies (hemophilias, etc.)

III. Mixed disorders: von Willebrand disease, DIC.

IV. Fibrinolytic disorders.

## *CLASSIFICATION OF THROMBOCYTOPENIAS*:

**1. Deficient platelet production** may result from any of a number of processes: aplastic anemia, marrow injury by myelosuppressive drugs or irradiation, bone marrow involvement in neoplastic diseases, vitamin $B_{12}$–deficiency anemia, folic acid deficiency anemia, etc.

**2. Accelerated platelet destruction** is the most common cause of thrombocytopenia. When the rate of platelet destruction exceeds the compensatory increase in platelet production thrombocytopenia develops. Platelet destruction may result from both intracorpuscular defects (hereditary thrombocytopenia, such as Wiskott-Aldrich syndrome) and extracorpuscular abnormalities (immunologic destruction, mechanical damage in patients with massive splenomegaly, prosthetic heart valves, hemangioendotheliomas, microangiopathies).

Trombocytopenias caused by immunologic platelet destruction include autoimmune, heteroimmune, alloimmune, and transimmune thrombocitopenic purpuras.

**3. Consumption of thrombocytes** occurs in disseminated intravascular coagulation, thrombotic thrombocitopenic purpura, massive thromboses.

**4. Platelet abnormal pooling** may result from spleen disorders, hypothermia, dilution of platelets with massive transfusions.

***AUTOIMMUNE THROMBOCYTOPENIC PURPURA (AITP)*** is believed to occur when platelets undergo premature destruction as a result of autoantibody or immune complex deposition on their membranes. The site of destruction is usually the reticuloendothelial system of the spleen and less commonly the liver.

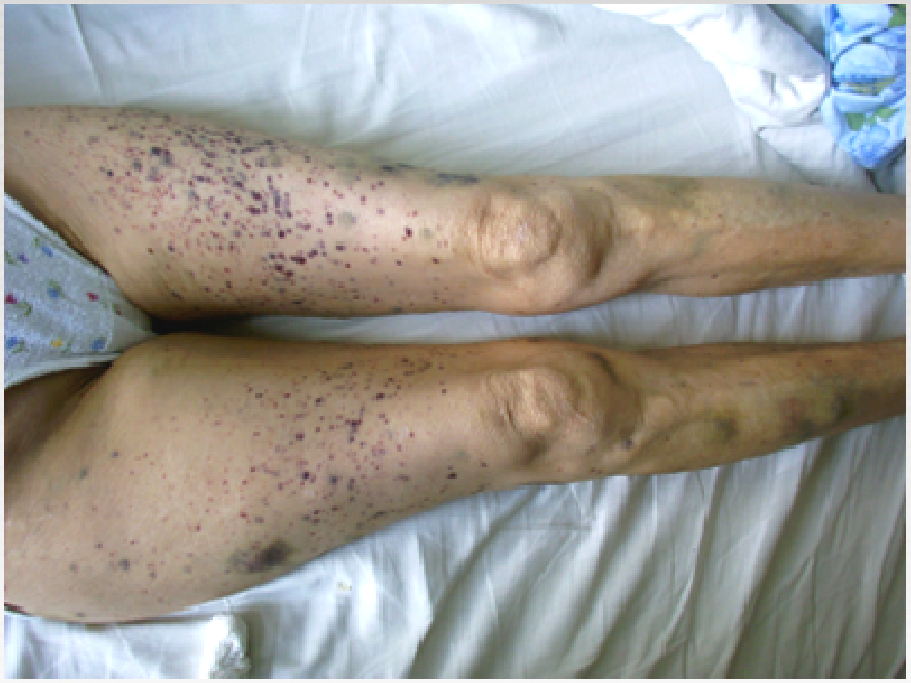
In many instances, the underlying disorder responsible for the formation of platelet antibodies is known (malignant lymphomas, chronic lymphocytic leukemia, liver cirrhosis, chronic hepatitis). These are commonly called secondary autoimmune thrombocytopenias. In other instances, there are no known etiologic factors and the disorder is called idiopathic thrombocytophenic purpura.

AITP mainly affects females. According to the data of Műller-Eckhardt (1976), the incidence of AITP is 4.5 males and 7.5 females per 100 000 population.

***PATHOGENESIS.*** AITP is caused by platelet-specific autoantibodies that bind to autologous platelets which are then rapidly cleared from the circulation by the mononuclear phagocyte system via macrophage Fc receptors. Antibodies may bind to the same antigens on megakaryocytes. In the majority of cases (85%) the increased quantities of IgG have been demonstrated on the platelet surface. Platelet membrane glycoprotein IIb/IIIa (CD 41) is a dominant antigen. Antiplatelet autoantibidies bind to many of the major platelet membrane glycoproteins through the Fab portion of the molecule.

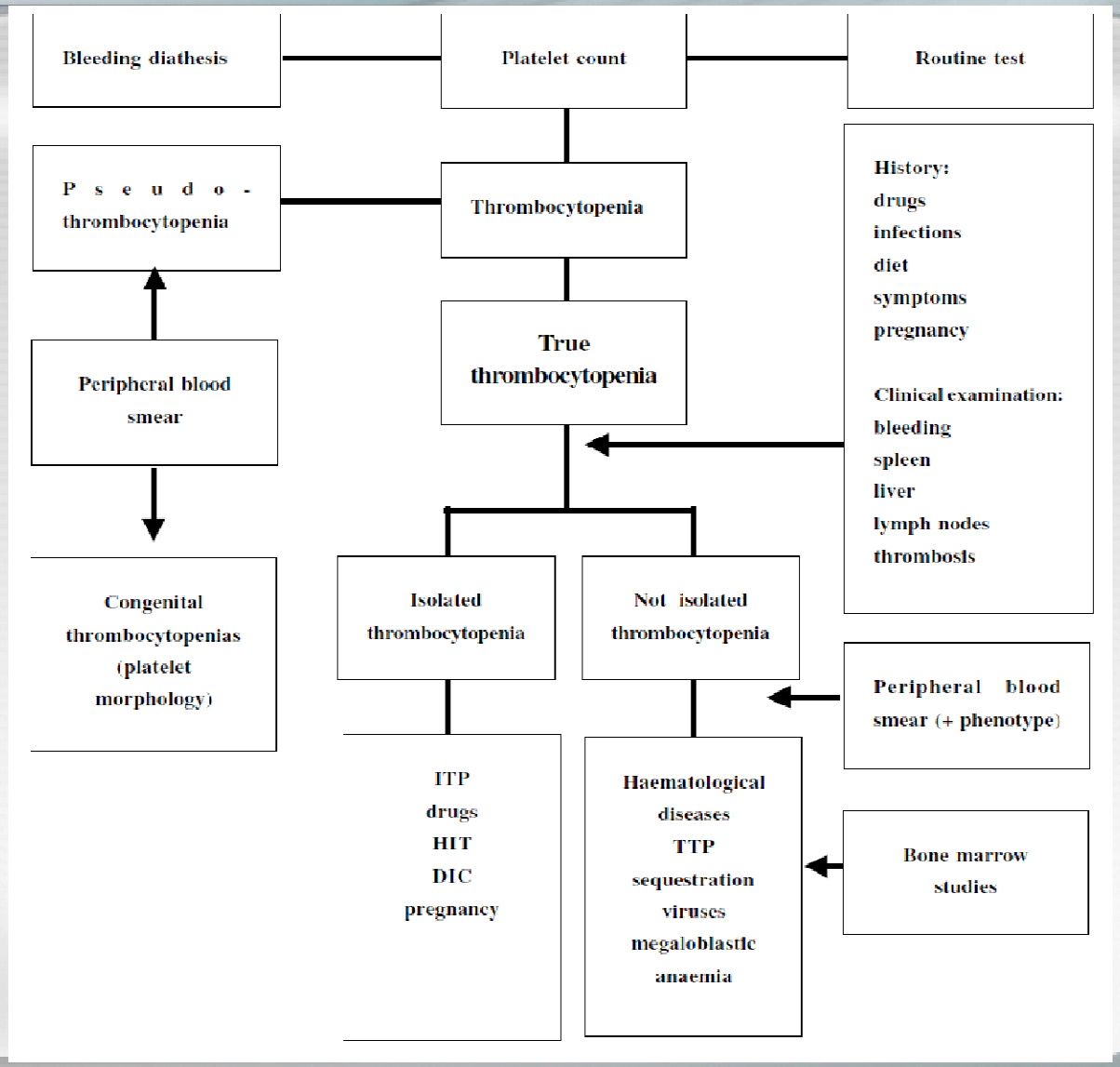
Data from patients with AITP suggests that T lymphocytes demonstrate phenotypic and functional abnormalities. Autologous mixed lymphocyte reaction–induced suppressor function is impaired. There is evidence of light chain restriction of autoantibodies which suggests clonal B–cell expansion in patients with AITP. Splenic sequestration accounts for the shortened platelet survival in most patients. Two major pathogenic roles of the spleen have been demonstrated: antiplatelet antibody production and macrophage-mediated platelet destruction. AITP leads to the disturbances of primary vascular–thrombocytic hemostasis.

## *PETECHIAE AND ECCHYMOSES IN PATIENT WITH AUTOIMMUNE THROMBOCYTOPENIA*



# ALGORITHM FOR THE DIAGNOSIS OF THROMBOCYTOPENIAS

(Blood Transfusion, 2009; 7: 75-85)



# TREATMENT OF AITP

(ASH evidence-based practice guideline.Blood, 2011;117(16):4190-4207)

**Section 1: ITP in children**

**Case 1: newly diagnosed ITP in children**

**Diagnosis of ITP**

**1.1.A.** We recommend:

- Bone marrow examination is unnecessary in children and adolescents with the typical features of ITP (grade 1B).
- Bone marrow examination is not necessary in children who fail IVIg therapy (grade 1B).

**1.1.B.** We suggest:

- Bone marrow examination is also not necessary in similar patients prior to initiation of treatment with corticosteroids or before splenectomy (grade 2C).
- Testing for antinuclear antibodies is not necessary in the evaluation of children and adolescents with suspected ITP (grade 2C)

**Initial management of ITP**

**1.2.A.** We recommend:

- Children with no bleeding or mild bleeding (defined as skin manifestations only, such as bruising and petechiae) be managed with observation alone regardless of platelet count (grade 1B).

**Initial pharmacologic management of pediatric ITP**

**1.3.A.** We recommend:

- For pediatric patients requiring treatment, a single dose of IVIg (0.8-1 g/kg) or a short course of corticosteroids be used as first-line treatment (grade 1B).
- IVIg can be used if a more rapid increase in the platelet count is desired (grade 1B).
- Anti-D therapy is not advised in children with a hemoglobin concentration that is decreased due to bleeding, or with evidence of autoimmune hemolysis (grade 1C).

**1.3.B.** We suggest:

- A single dose of anti-D can be used as first-line treatment in Rh-positive, nonsplenectomized children requiring treatment (grade 2B).

**Case 2: children who are treatment nonresponders**

**Appropriate second-line treatments for pediatric ITP**

**2.1.A.** We suggest:

- Rituximab be considered for children or adolescents with ITP who have significant ongoing bleeding despite treatment with IVIg, anti-D, or conventional doses of corticosteroids (grade 2C).
- Rituximab may also be considered as an alternative to splenectomy in children and adolescents with chronic ITP or in patients who do not respond favorably to splenectomy (grade 2C).
- High-dose dexamethasone may be considered for children or adolescents with ITP who have significant ongoing bleeding despite treatment with IVIg, anti-D, or conventional doses of corticosteroids (grade 2C).
- High-dose dexamethasone may also be considered as an alternative to splenectomy in children and adolescents with chronic ITP or in patients who do not respond favorably to splenectomy (grade 2C).

# TREATMENT OF AITP

(ASH evidence-based practice guideline.Blood, 2011;117(16):4190-4207)

**Splenectomy for persistent or chronic ITP or ITP unresponsive to initial measures**

**2.2.A.** We recommend:

- Splenectomy for children and adolescents with chronic or persistent ITP who have significant or persistent bleeding, and lack of responsiveness or intolerance of other therapies such as corticosteroids, IVIg, and anti-D, and/or who have a need for improved quality of life (grade 1B).

**2.2.B.** We suggest:

- Splenectomy or other interventions with potentially serious complications be delayed for at least 12 months, unless accompanied by severe disease defined by the International Working Group as unresponsive to other measures or other quality of life considerations (grade 2C).

***H pylori* testing in children with persistent or chronic ITP**

**2.3.A.** We recommend:

- Against routine testing for *H pylori* in children with chronic ITP (grade 1B).

**Case 3: management of MMR-associated ITP**

**3.1.A.** We recommend:

- Children with a history of ITP who are unimmunized receive their scheduled first MMR vaccine (grade 1B).
- In children with either nonvaccine or vaccine-related ITP who have already received their first dose of MMR vaccine, vaccine titers can be checked. If the child displays full immunity (90%-95% of children), then no further MMR vaccine should be given. If the child does not have adequate immunity, then the child should be re-immunized with MMR vaccine at the recommended age (grade 1B).

**Section 2: ITP in the adult**

**Case 4: newly diagnosed ITP in the adult**

**Initial diagnosis of ITP**

**4.1.A.** We recommend:

- Testing patients for HCV and HIV (grade 1B).

**4.1.B.** We suggest:

- Further investigations if there are abnormalities (other than thrombocytopenia and perhaps findings of iron deficiency) in the blood count or smear (grade 2C).
- A bone marrow examination is not necessary irrespective of age in patients presenting with typical ITP (grade 2C).

**Treatment of newly diagnosed adult ITP**

**4.2.A.** We suggest:

- Treatment be administered for newly diagnosed patients with a platelet count $< 30 \times 10^9$/L (grade 2C).

**First-line treatment of adult ITP**

**4.3.A.** We suggest:

- Longer courses of corticosteroids are preferred over shorter courses of corticosteroids or IVIg as first-line treatment (grade 2B).
- IVIg be used with corticosteroids when a more rapid increase in platelet count is required (grade 2B).
- Either IVIg or anti-D (in appropriate patients) be used as a first-line treatment if corticosteroids are contraindicated (grade 2C).
- If IVIg is used, the dose should initially be 1 g/kg as a one-time dose. This dosage may be repeated if necessary (grade 2B).

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ITP indicates immune thrombocytopenia; IVIg, intravenous immunoglobulin; anti-D, anti-D immunoglobulin; MMR, measles-mumps-rubella; HCV, hepatitis C virus; HIV, human immunodeficiency virus; and *H pylori*, *Helicobacter pylori*.

# TREATMENT OF AITP

(ASH evidence-based practice guideline.Blood, 2011;117(16):4190-4207)

**Treatment of patients who are unresponsive to or relapse after initial corticosteroid therapy**

**4.4.A.** We recommend:

- Splenectomy for patients who have failed corticosteroid therapy (grade 1B).
- Thrombopoietin receptor agonists for patients at risk of bleeding who relapse after splenectomy or who have a contraindication to splenectomy and who have failed at least one other therapy (grade 1B).

**4.4.B.** We suggest:

- Thrombopoietin receptor agonists may be considered for patients at risk of bleeding who have failed one line of therapy such as corticosteroids or IVIg and who have not had splenectomy (grade 2C).
- Rituximab may be considered for patients at risk of bleeding who have failed one line of therapy such as corticosteroids, IVIg, or splenectomy (grade 2C).

**Laparoscopic versus open splenectomy and vaccination prior to splenectomy**

**4.5.A.** We recommend:

- That for medically suitable patients, both laparoscopic and open splenectomy offer similar efficacy (grade 1C).

**Case 5: treatment of adult ITP after splenectomy**

**Treatment of ITP after splenectomy**

**5.1.A.** We recommend:

- Against further treatment in asymptomatic patients after splenectomy who have platelet counts $> 30 \times 10^9$/L (grade 1C).

**Case 6: treatment of ITP in pregnancy**

**Management of ITP during pregnancy**

**6.1.A.** We recommend:

- Pregnant patients requiring treatment receive either corticosteroids or IVIg (grade 1C).

**Treatment of ITP during labor and delivery**

**6.2.A.** We suggest:

- For pregnant women with ITP, the mode of delivery should be based on obstetric indications (grade 2C).

**Case 7: treatment of specific forms of secondary ITP**

**Management of secondary ITP, HCV-associated**

**7.1.A.** We suggest:

- In patients with secondary ITP due to HCV infection, antiviral therapy should be considered in the absence of contraindications (grade 2C). However, the platelet count should be closely monitored due to a risk of worsening thrombocytopenia attributable to interferon.
- If treatment for ITP is required, the initial treatment should be IVIg (grade 2C).

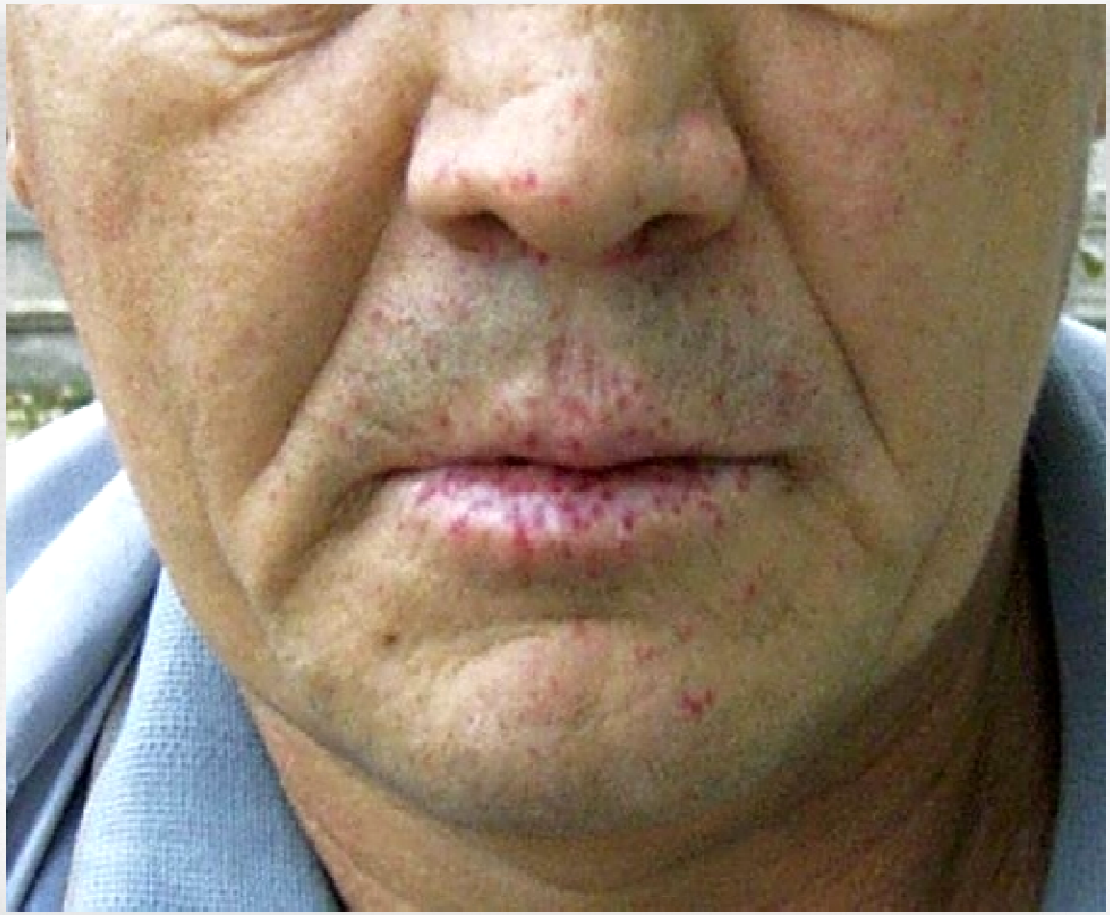
# DEFINITIONS OF RESPONSE TO TREATMENT BY AITP

(ASH evidence-based practice guideline.Blood, 2011;117(16):4190-4207)

| | |
|---|---|
| Complete response (CR) | A platelet count ≥ 100 × $10^9$/L measured on 2 occasions > 7 days apart and the absence of bleeding. |
| Response (R) | A platelet count ≥ 30 × $10^9$/L and a greater than 2-fold increase in platelet count from baseline measured on 2 occasions > 7 days apart and the absence of bleeding. |
| No response (NR) | A platelet count < 30 × $10^9$/L or a less than 2-fold increase in platelet count from baseline or the presence of bleeding. Platelet count must be measured on 2 occasions more than a day apart. |
| Loss of complete response | A platelet count < 100 × $10^9$/L measured on 2 occasions more than a day apart and/or the presence of bleeding. |
| Loss of response | A platelet count < 30 × $10^9$/L or a less than 2-fold increase in platelet count from baseline or the presence of bleeding. Platelet count must be measured on 2 occasions more than a day apart. |

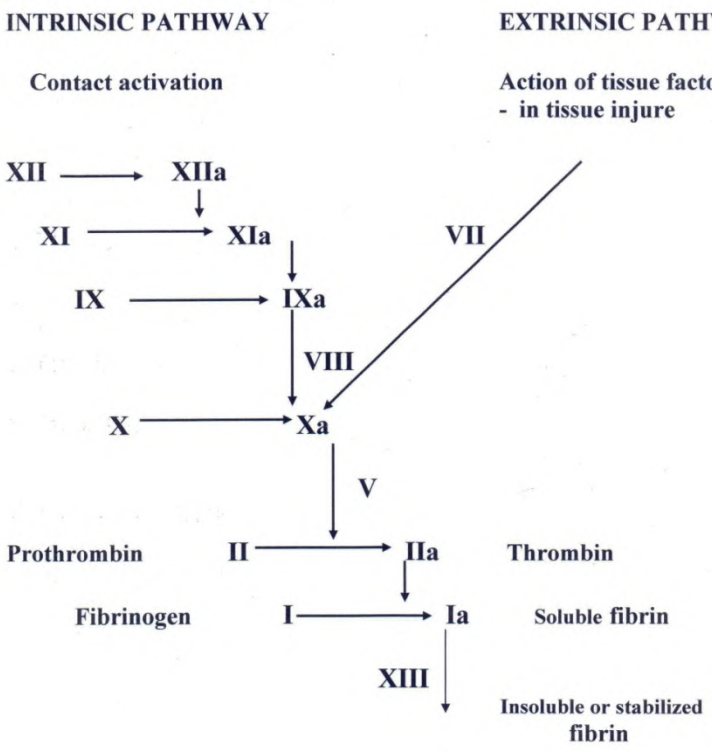
*Based on the recommendations of the International Working Group.[7]

## *HEREDITARY HEMORRHAGIC TELEANGIECTASIA*



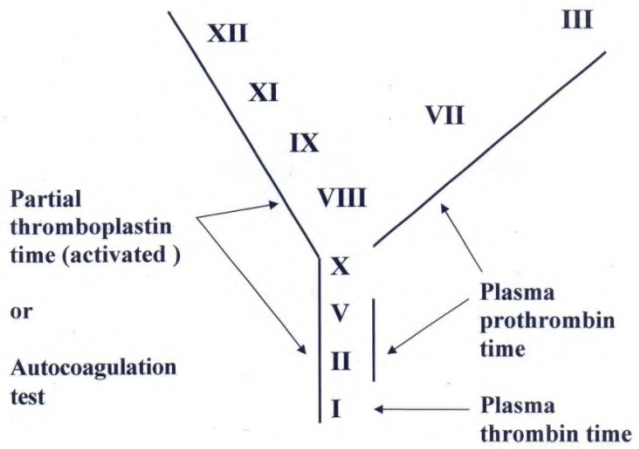
## *NOMENCLATURE AND SYNONYMS OF COAGULATION FACTORS:*

| Roman numeral | Preferred descriptive name | Synonyms |
|---|---|---|
| I | Fibrinogen | |
| II | Prothrombin | |
| III | Tissue factor | Thromboplastin |
| IV | Calcium ions | |
| V | Proaccelerin | Labile factor, accelerator globulin |
| VI | Accelerin | Abrogated |
| VII | Proconvertin | Stable factor, serum prothrombin conversion accelerator |
| VIII | Antihemophilic factor A | Antihemofilic globulin |
| IX | Plasma thromboplastin component | Christmas factor, antihemofilic factor B |
| X | Stuart factor | Prower factor |
| XI | Plasma thromboplastin antecedent | Antihemofilic factor C |
| XII | Hageman factor | Contact factor |
| XIII | Fibrin stabilizing factor | Fibrinase, Laki-Lorand factor |
| --- | Prekallikrein | Fletcher factor |
| --- | HMW Kininogen | High molecular weight kininogen, Fitzgerald factor |




**BLOOD COAGULATION CASCADE**

**(MacFarlane R.G., 1966, 1976)**




**LABORATORY METHODS FOR THE STUDY OF BLOOD COAGULATION:**

**partial thromboplastin time (activated ) – 37 – 50seconds;**

**plasma prothrombin time – 16 – 20 seconds;**

**plasma thrombin time – 28 – 32 seconds;**

**fibrinogen assay – 2 – 4.5 g/l;**

**coagulation time – 8 – 12 minutes;**

**fibrin degradation product assay – 0 – 5 µg/ml;**

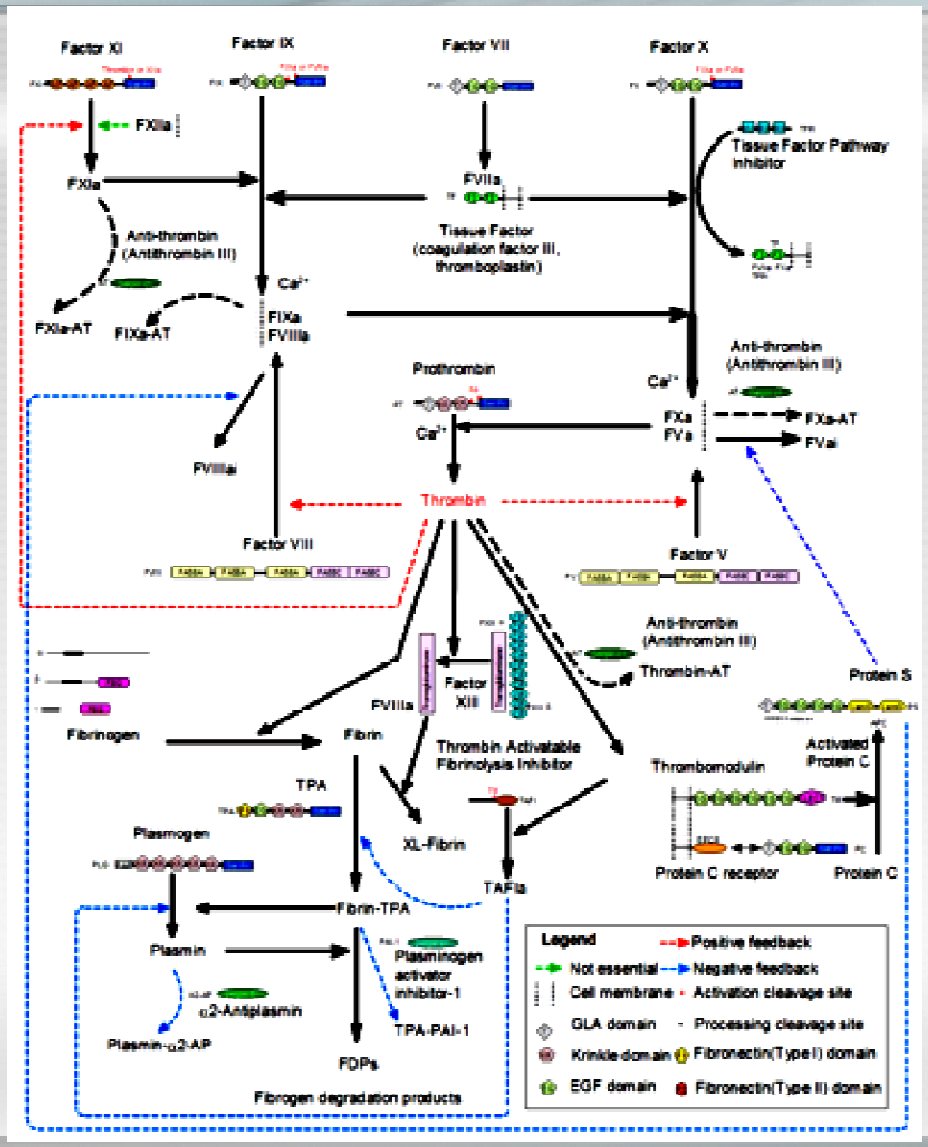
**antithrombin III assay – 80 – 125%;**

**autocoagulation test – 9 – 11 seconds;**

**fibrinolytic activity – 30 – 40 minutes;**

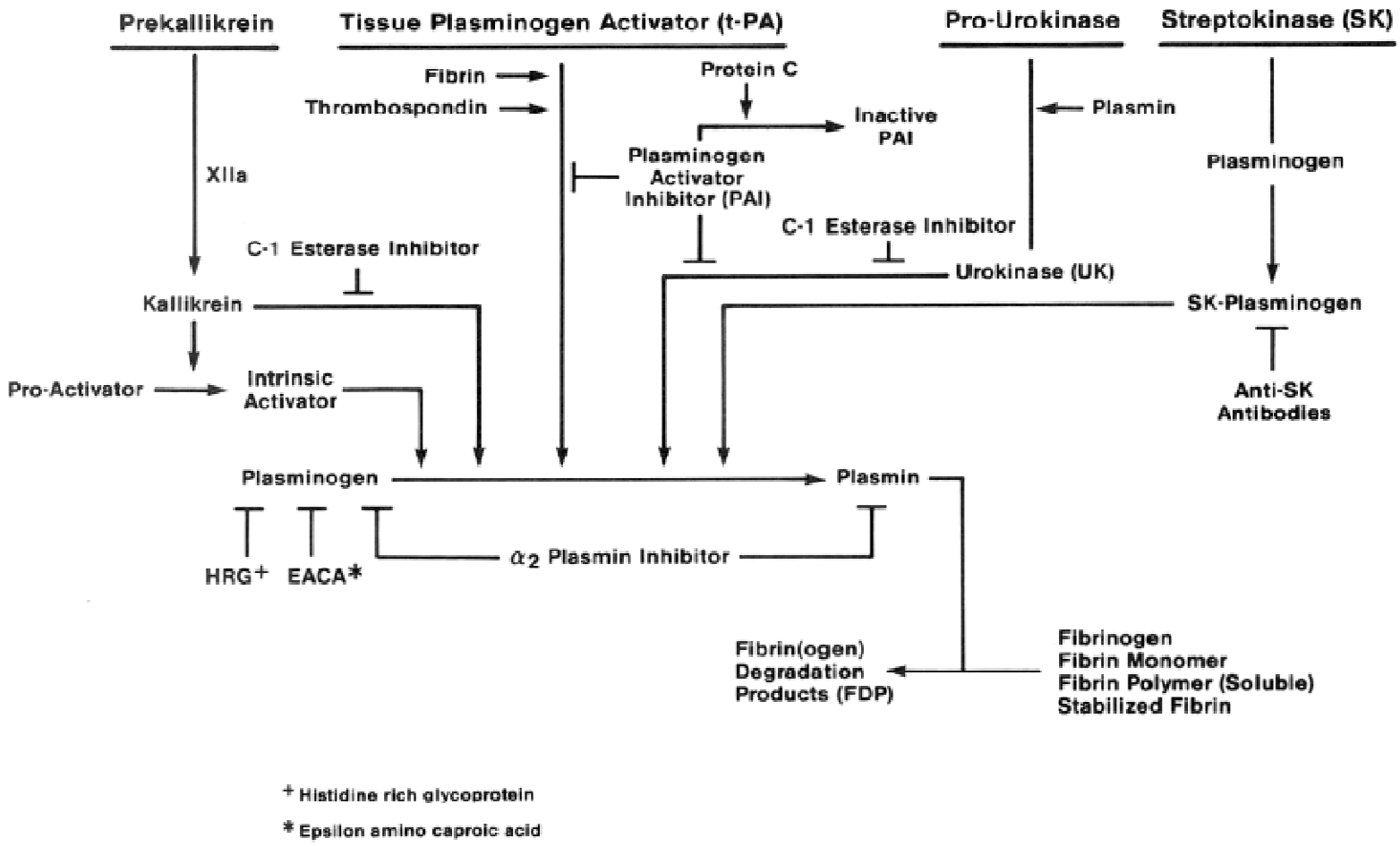
**prothrombin assay – 90 – 105%.**

# COAGULATION AND FIBRINOLYSIS



# *THE BIOCHEMICAL PATHWAYS IN THE FIBRINOLYTIC SYSTEM*

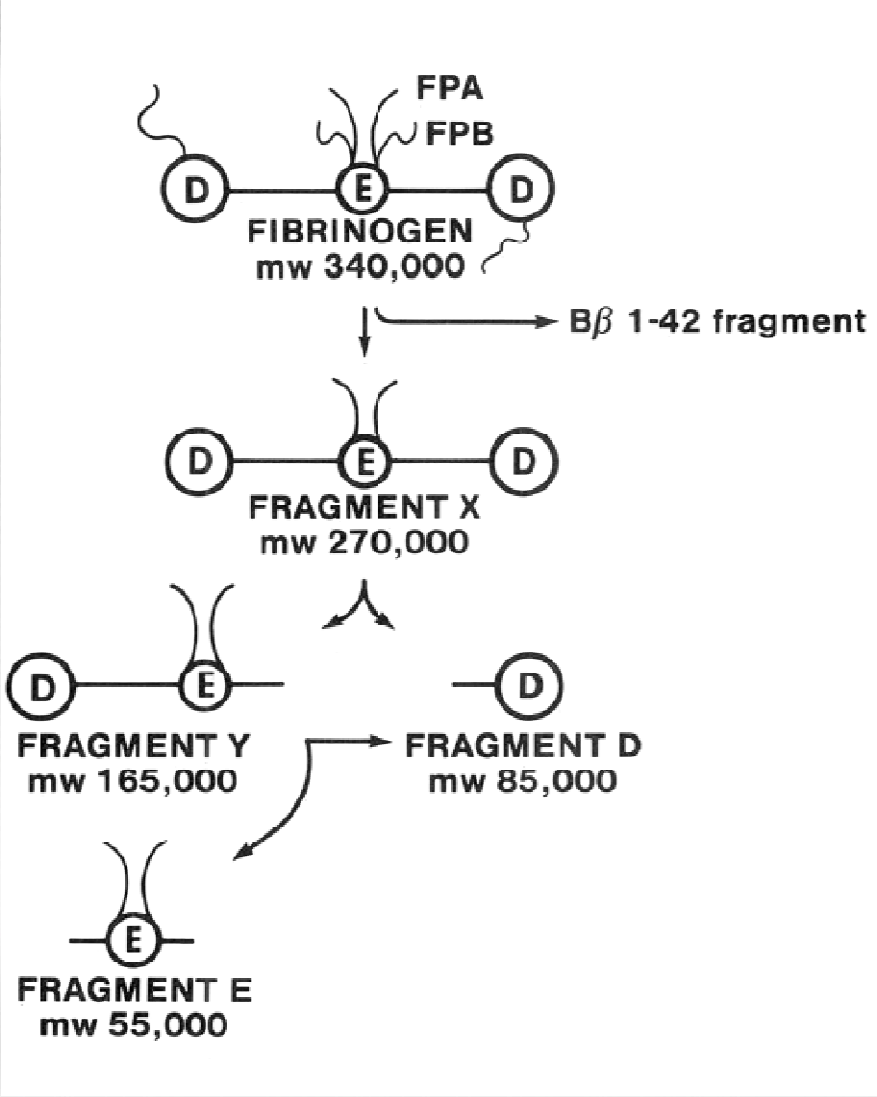
CLEVELAND CLINIC JOURNAL OF MEDICINE, 1988; 55:531-541




A variety of activators may convert plasminogen to plasmin, which then degrades fibrin.
*HRQ = histidine-rich glycoprotein; EACA = epsilon* aminocaproic acid.

# PLASMIN PROTEOLYSIS OF FIBRINOGEN

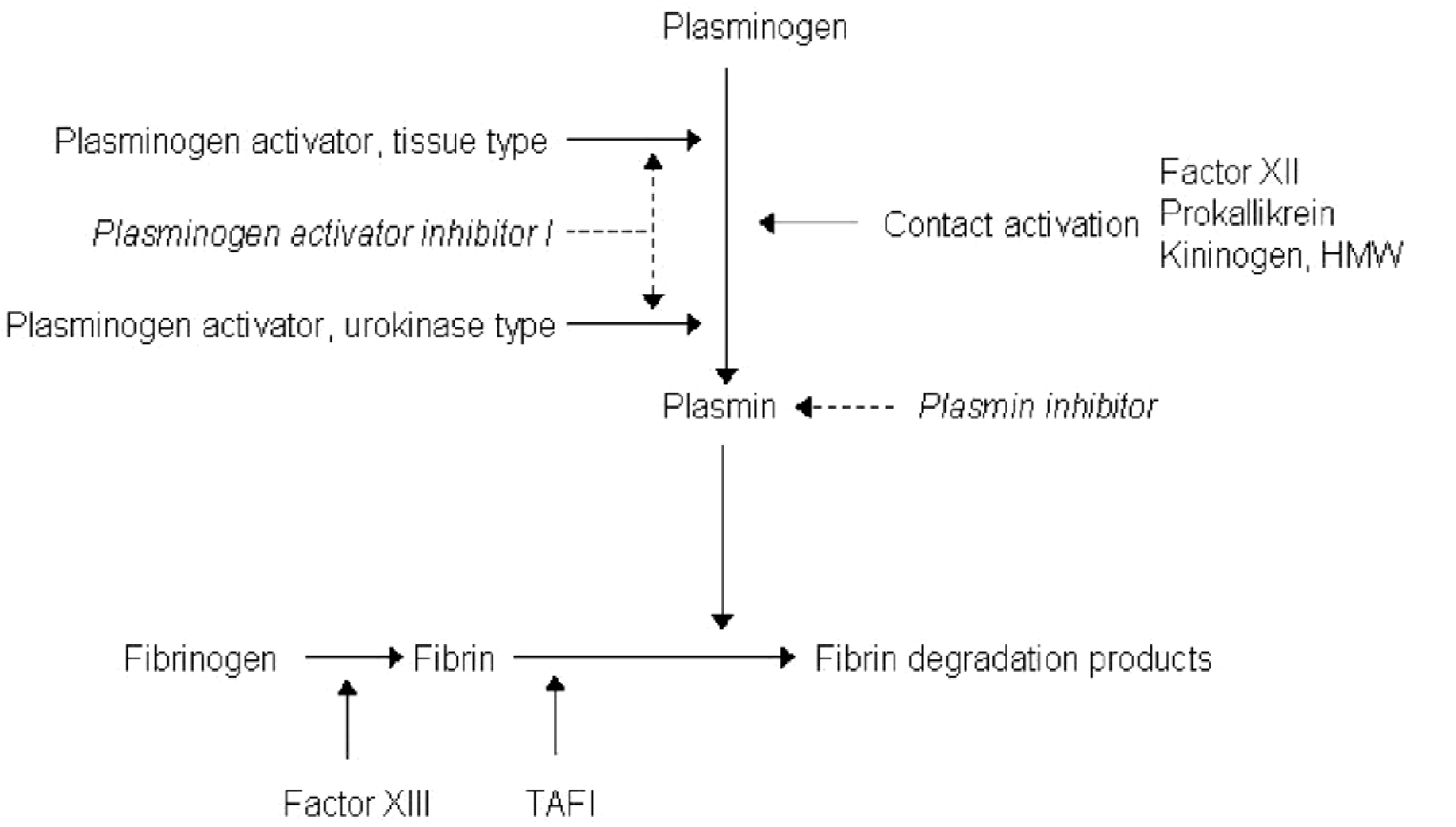
CLEVELAND CLINIC JOURNAL OF MEDICINE, 1988; 55:531-541




Plasmin proteolysis of fibrinogen produces a variety of smaller-molecular-weight species, termed fragment X, fragment Y, fragment E, fragment D, and the beta chain peptide B-beta 1-42.

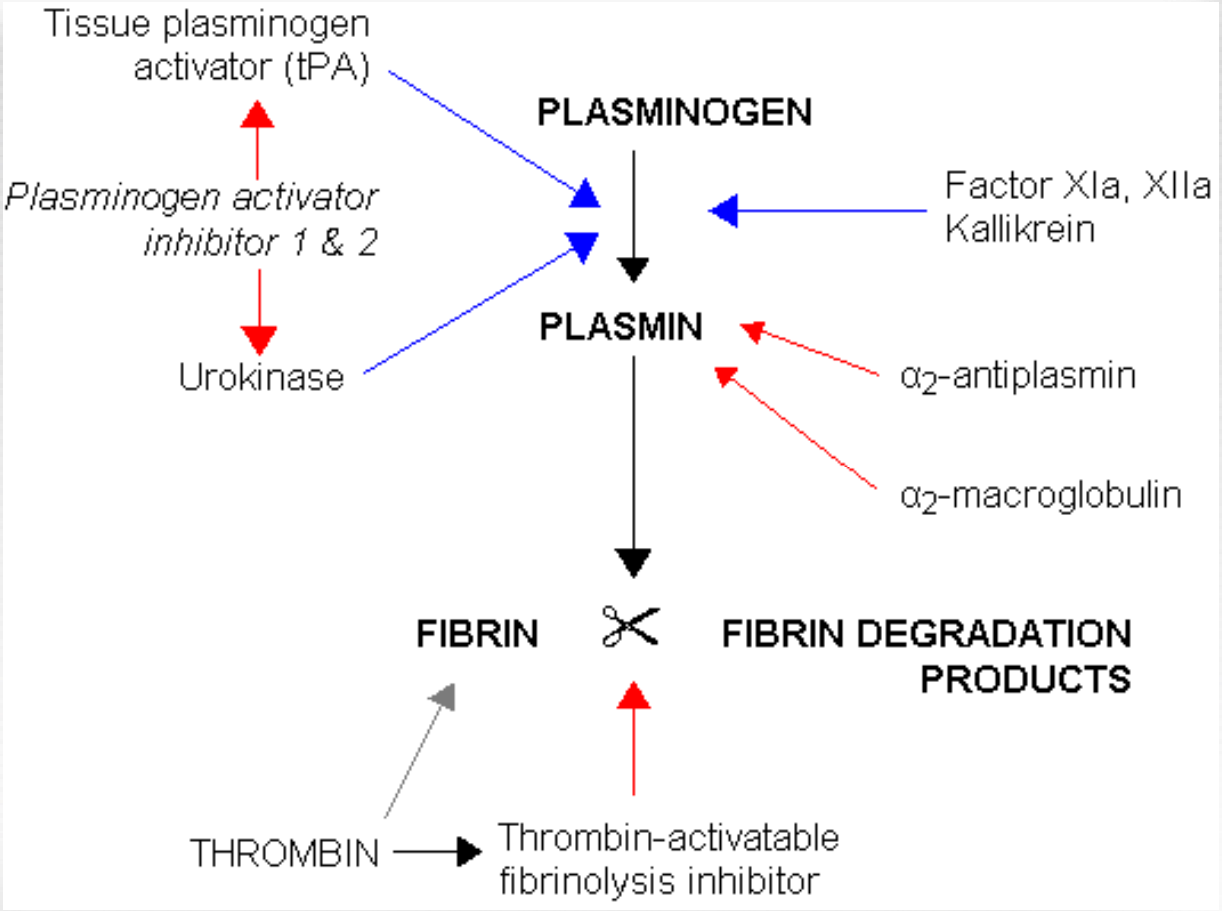
# FIBRINOLYTIC SYSTEM

eJIFCC The Electronic Journal Of The International Federation Of Clinical Chemistry And Laboratory Medicine. Number 2 2000



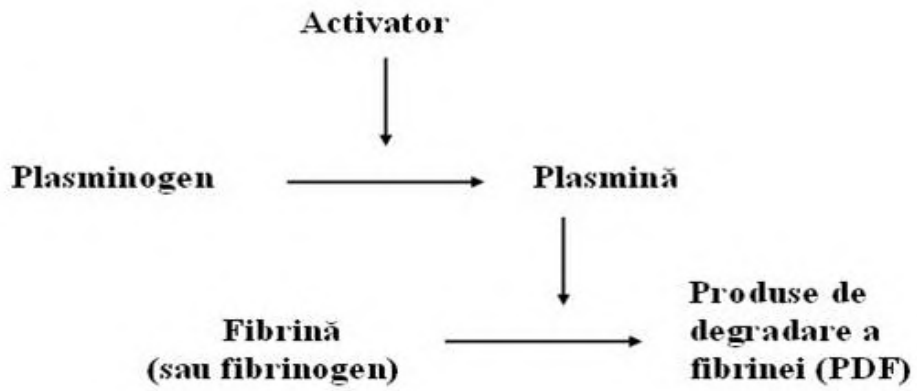

TAFI: Thrombin activatable fibrinolysis inhibitor

# *FIBRINOLYTIC SYSTEM*



# FIBRINOLYTIC SYSTEM

## SISTEMUL FIBRINOLITIC




## PRODUSELE FIBRINOLIZEI

**Fibrinogen sau fibrină** —plasmină→ **fragmentul X + peptide**

**Fragmentul X** —plasmină→ **fragmentul Y + D**

**Fragmentul Y** —plasmină→ **fragmentul D + E**

**X – inhibă formarea cheagului**
**Y – cel mai puternic anticoagulant**
**D – inhibă polimerizarea monomerilor de fibrină**
**E – inhibă complet coagularea fibrinogenului sub influența trombinei și agregarea trombocitelor**

*POSSIBLE REACTIONS WITH PARTICIPATION OF FIBRIN MONOMERS*

# Reacţiile posibile în care participă monomerii de fibrină

| | |
|---|---|
| Monomer de fibrină + Monomer de fibrină | Fibrină insolubilă (product normal) |
| Monomer de fibrină + fragmentul X sau Y | Complexe necoagulante (produse de discompunere a fibrinei) |
| Monomer de fibrină + fragmentul D sau E | Fibrină cu defect hemostatic |
| Monomer de fibrină + Fibrinogen | Complexe solubile de monomeri de fibrină, fibrinogen blocat |

# GENETICS, EPIDEMIOLOGY AND THERAPY OF INHERITED COAGULATION FACTORS DEFICIENCIES

(Williams M.E., Kahn M.J., American Society of Hematology Self-Assessment Program. Blackwell Publishing: 2005)

| Coagulation protein deficiency | Inheritance pattern | Prevalence | Minimum desired level to control active bleeding or prevent surgical bleeding | Replacement sources |
|---|---|---|---|---|
| Fibrinogen | | | | |
| Afibrinogenemia | AR | Rare | 1 g/L | Cryo/FFP |
| Hypofibrinogenemia | AD or AR | Extremely rare | | |
| Dysfibrinogenemia | AD or AR | Rare | | |
| Factor II (prothrombin) | AD or AR | Extremely rare | 30% | FFP/F IX complex concentrates |
| Factor V | AR | 1 per million births | 25% | FFP |
| Factor VII | AR | 1 per 500,000 births | 25% | FFP/F IX complex concentrates; recombinant factor VIIa |
| Factor VIII | | | | |
| Hemophilia A | X-linked recessive | 1 per 5000 male births | 100% for life-threatening bleeding; 50% for major bleeding; 30% for minor bleeding | F VIII concentrates, desmopressin for mild to moderate disease |
| VWF | | | | |
| Type 1 | Usually AD | 1 per 100 births | 50% VWF activity | Desmopressin for mild to moderate disease |
| Type 2 | Variable | Variable | Variable | VWD (except 2B); intermediate-purity F VIII concentrates containing VWF; occasionally in type 2A vWD desmopressin is utilized |
| Type 3 | AR | 1 per million births | 50% VWF activity | Intermediate-purity F VIII concentrates containing VWF |
| Factor IX | | | | |
| Hemophilia B | X-linked recessive | 1 per 30,000 male births | 100% for life-threatening bleeding; 50–80% for major bleeding; 30–40% for minor bleeding | Factor IX concentrates |
| Factor X | AR | 1 per 500,000 births | 10–25% | FFP or F IX complex concentrates |
| Factor XI | AR | Rare | 20–40% | FFP |
| Factor XII | AR | Rare | | No replacement |
| Prekallikrein | AR | Extremely rare | | No replacement |
| High-molecular-weight kininogen | AR | Extremely rare | | No replacement |
| Factor XIII | AR | Extremely rare | 3–5% | Cryo/FFP; plasma-derived FXIII (available through clinical trial) |

AD = autosomal dominant; AR = autosomal recessive; cryo = cryoprecipitate; FFP = fresh frozen plasma; F VIII = factor VIII; F IX = factor IX; VWF = von Willebrand factor.

## *CAUSES OF ↑ APTT RATIO (>1.2):*

- Hemophilia (inherited FVIII or FIX deficiency)
- von Willebrand disease (↓ FVIII due to reduced vWF carrier function)
- Factor XI deficiency
- Acquired deficiency of FII, FV, FVIII, FIX (e.g. consumption in DIC)
- Acquired hemophilia (autoantibody to FVIII or FIX)
- Contact factor deficiencies
- Phospholipid-dependent ('lupus') inhibitors
- Unfractionated heparin (UFH)

## *CAUSES OF ↑ TT (>3S >CONTROL):*

- Afibrinogenemia
- Hypofibrinogenemia
- Dysfibrinogenemia
- Inhibitors of fibrin polymerization: paraproteins, fibrin–fibrinogen degradation products (FDPs)
- Heparin (unfractionated)

## *RESULTS OF HEMOSTATIC SCREENING TESTS FOR SELECTED BLEEDING DISORDERS*

(BLEEDING AND CLOTTING DISORDERS, LAUREN L. PATTON, DDS)

| | Screening Laboratory Test | | | |
|---|---|---|---|---|
| **Bleeding Disorder** | **Platelet Count** | **PT/INR** | **aPTT** | **BT** |
| Thrombocytopenia<br>Leukemia | ↓ | N | N | ↑ |
| F VIII, IX, XI deficiency<br>Heparin anticoagulation | N | N | ↑ | N |
| F II, V, X deficiency<br>Vitamin K deficiency<br>Intestinal malabsorption | N | ↑ | ↑ | N |
| F VII deficiency<br>Coumarin anticoagulation<br>Liver disease | N | ↑ | N | N |
| von Willebrand's disease | N, ↓ | N | N, ↑ | ↑ |
| DIC<br>Severe liver disease | ↓ | ↑ | ↑ | ↑ |
| F XIII deficiency | N | N | N | N |
| Vascular wall defect | N | N | N | ↑ |

aPTT = activated partial thromboplastin time; BT = bleeding time; DIC = disseminated intravascular coagulation; INR = international normalized ratio; N = normal; PT = prothrombin time; ↑ = increased; ↓ = decreased.

## *HEMOPHILIA*

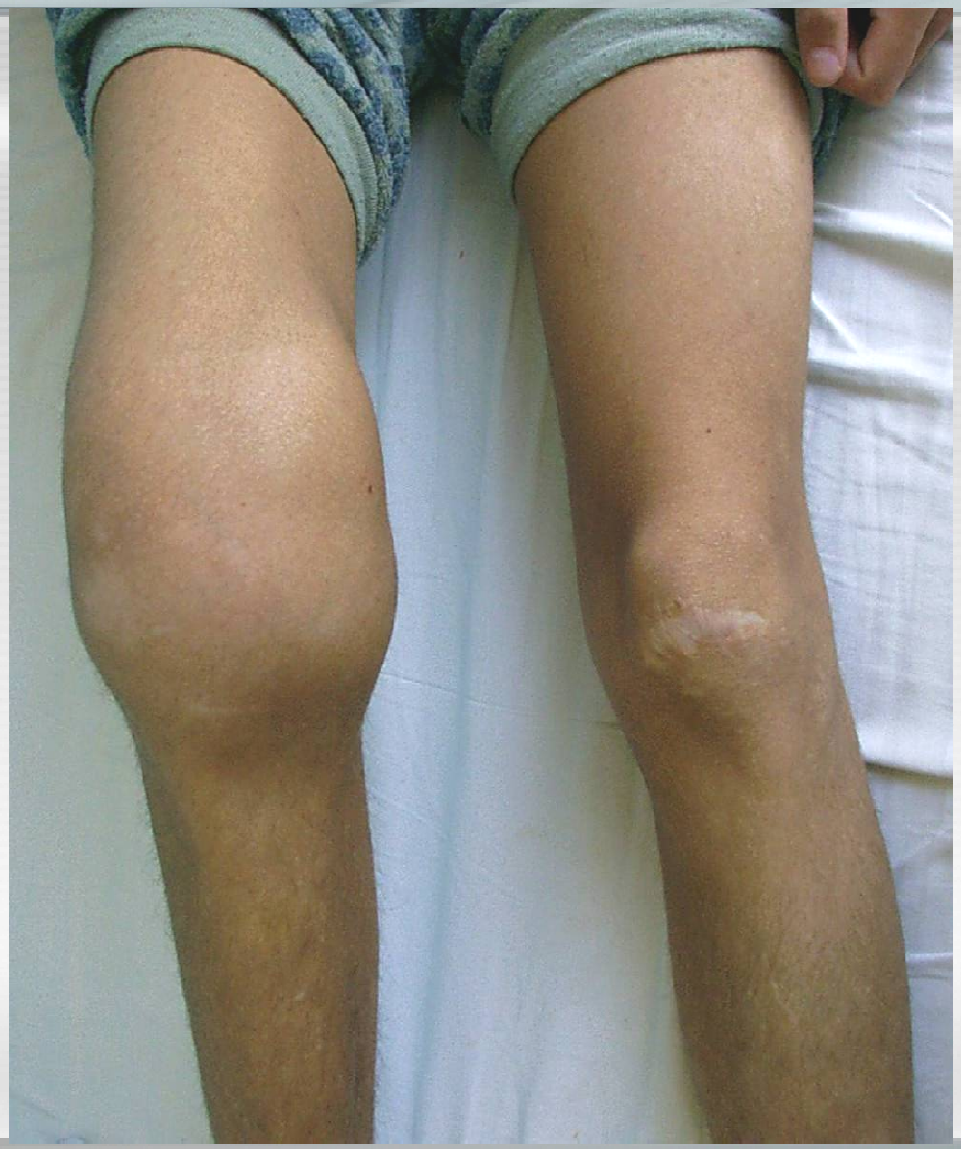
***HEMOPHILIA*** is a most frequent (96 - 98% of cases) and severe hemorrhagic diathesis that affects the male children of certain families. The term hemophilia A (80 - 85%) is synonymous with factor VIII deficiency, hemophilia B (15 - 20%) is known as factor IX deficiency or Christmas disease, and hemophilia C – as factor XI deficiency (1 - 3%).

***PATHOPHYSIOLOGY.*** The hemostatic abnormality in hemophilias is a deficiency or abnormality of a plasma protein. Hemophilia is a X-linked recessive trait. The affected male will not transmit the disorder to his sons, because his Y chromosome is normal. All of his daughters will be carriers of the trait. The female carrier will transmit the disorder to half of her sons and the carrier state to half of her daughters.

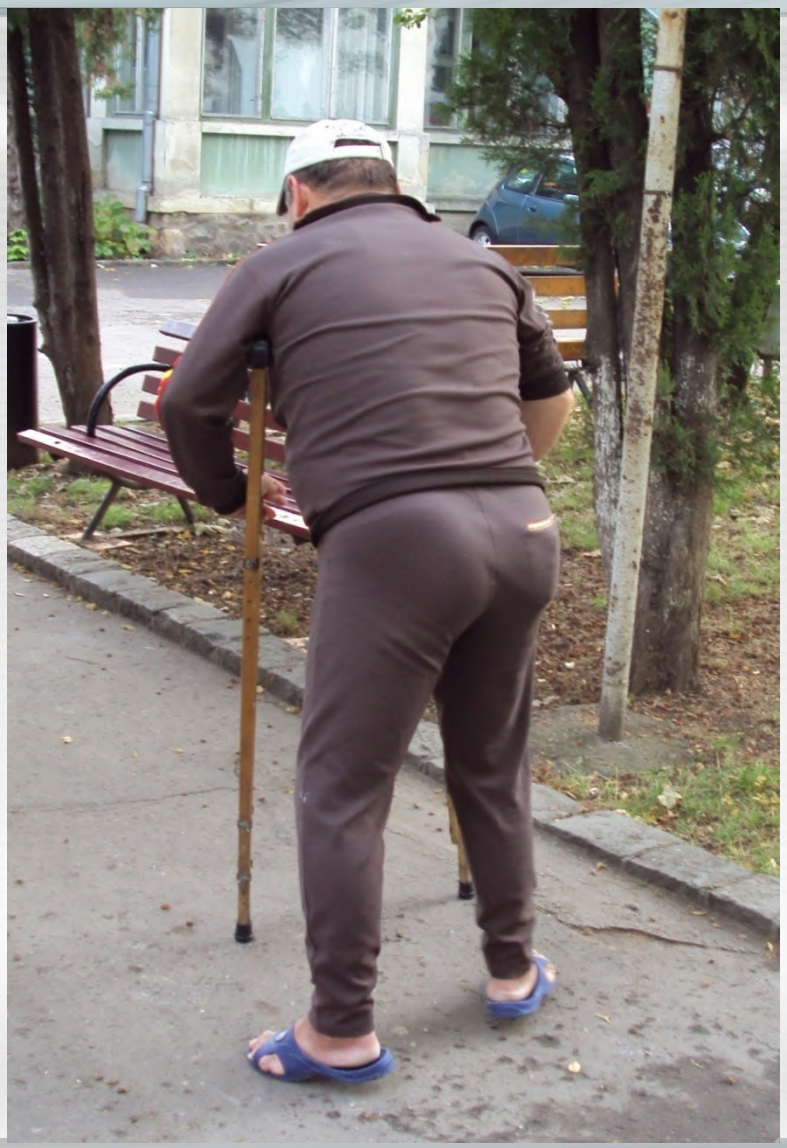
Factor VIII is an essential cofactor for factor IXa-catalyzed conversion of factor X to factor Xa. In hemophilic patients, without adequate factor VIII activity, there is delayed clot formation that results from delayed thrombin generation.

***CLINICAL FEATURES.*** Hemophilia A is clinically identical to hemophilias B and C. Hematomas and hemarthroses are highly characteristic of the disease. Hematomas are hemorrhages into subcutaneous connective tissue or into muscle. The severity of hemophilia is related to the blood level of factor VIII or IX. The disease has been classified as mild (6 - 60% of normal factor VIII or IX level), moderate (2 - 5% of normal factor level), severe (1 - 2% of normal factor level), and very severe (≤ 1% of normal factor level).

## *HEMARTHROSES IN HEMOPHILIA*



# *HEMARTHROSES IN HEMOPHILIA*



***TREATMENT.*** The principal treatment for hemophilia is replacement therapy, that is, the intravenous administration of the required factor in the form of blood products or recombinant coagulation proteins. The objective of replacement therapy is to obtain a concentration of the required factor at the bleeding site such that coagulation may become hemostatically effective.

Fresh-frozen plasma is usually preferred for therapeutic purposes. Both the rate of administration and the total dose of plasma are limited by the possibility of acute of chronic overload. The daily dosage constitutes 20 - 30 ml/kg. Doses are administered every 6 - 8 hours, because the factor VIII initial (diffusion) and subsequent (biologic) half-lives are approximately 6 and 12 hours, respectively. The factor VIII level of 5% of normal usually is sufficient for treatment of hemarthroses and hematomas.

Cryoprecipitate can be used to attain normal levels of factor VIII. The dosage is 30 - 40 units /kg/day. Cryoprecipitate is sufficiently potent to achieve and sustain even minimal hemostatic levels of factor VIII without significant expansion of the plasma volume.

Several commercial lyophilized factor VIII concentrates (Koate-HP, Humate-P, AHFM, Hemophil M, Monoclate-P) and recombinant factor VIII (Helixate, Bioclate) are now available for clinical use. The risk of virus transmission has been greatly diminished by serologic testing of the plasma for viruses and by sterilization of the concentrate by solvent-detergent treatment, monoclonal antibody purification or heat sterilization. To raise the factor VIII level to 100%, that is, 1 unit/ml, the dose of factor VIII required would be 50 units/kg body weight, assuming that the patient's baseline factor VIII level is less than 1% of normal. For some minor bleeding manifestations a single dose of 10 to 15 units/kg of factor VIII usually suffices.

A concentrated preparation of the vitamin K-depended factors (prothrombin and factors VII, IX, and X, and proteins C and S), named PPSB contains small but significant amounts of activated coagulation factors that may be thrombogenic when administered in large doses.

Recombinant factor IX Benefix is also available for clinical usage in hemophilia B. Many hemophiliacs (15 - 20%) develop antibodies (inhibitors) to factor VIII. This complication is less common in patients with hemophilia B (2 - 5%) and other inherited coagulation disorders. Such antibodies may seriously complicate the treatment of these patients. Recombinant factor VIIa Novo Seven is being studied primarily for treatment of bleeding episodes in patients with hemophilia with inhibitors. Its daily dose range from 6 to 120 μg/kg.

X-ray treatment at a total dosage of 2.5 – 5 Gy and 5 – 10 Gy has been successful in acute and chronic hemarthroses, respectively.

Synovectomy is effective in reducing the frequency of hemartrosis that cannot be controlled by replacement therapy.

For treatment of hematuria, patients may require replacement therapy (cryoprecipitate, recombinant factor VIII ) and prednisone at a dosage of 20 - 30 mg/day.

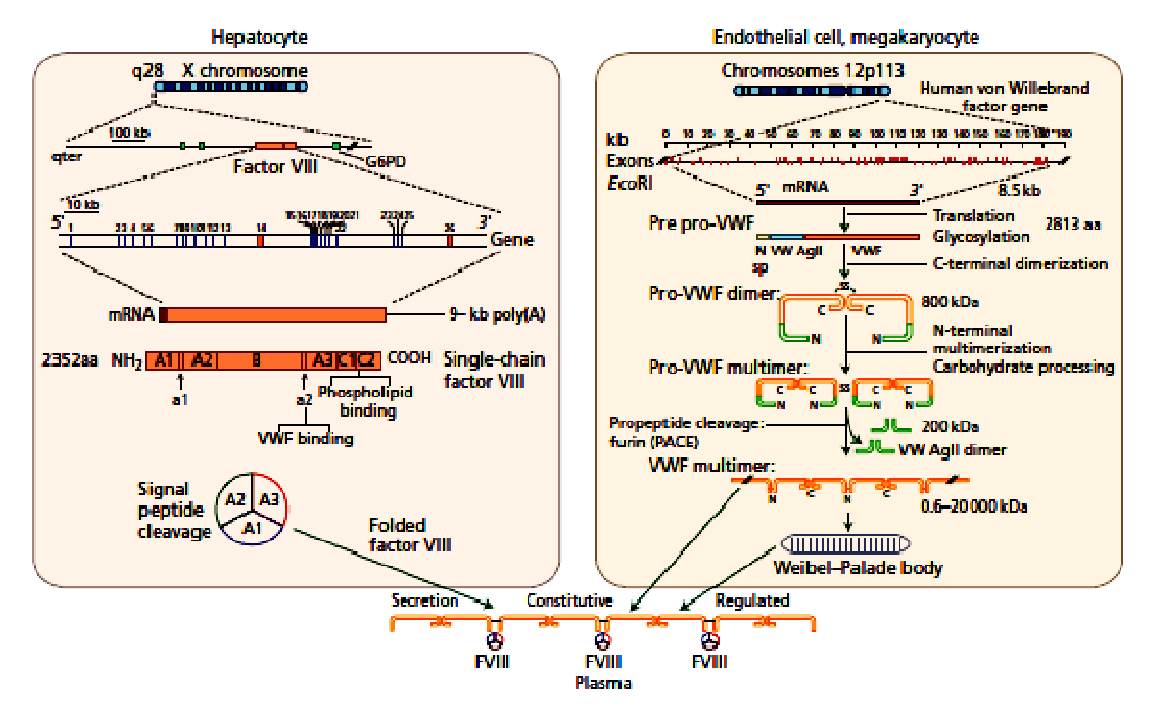
## *LOGICAL USE OF THE CLOTTING SCREEN AND PRETEST PROBABILITY*

A 66-year-old male bled for 48 h after surgery. Excess bleeding was noted from surgical drains and as a wound hematoma. Pretest probability of a bleeding disorder (from history and examination)
∴ = high

Platelet count = 245 × 109/l
APTTr ↑ (1.9) corrects with 50 : 50 normal plasma.
INR and TT normal.
∴ There is a deficiency (it corrects) of one or more factors tested only by the APTT: a contact factor, or clotting factors VIII, IX or XI.
Pretest probability suggests FVIII, FIX or FXI deficiency: all can cause excess bleeding after surgery. Contact factor deficiencies do not cause excess bleeding.
Specific activity assays of these factors are performed, starting with FVIII-C (the commoner of the two potentially severe deficiencies).
Result: FVIII:C = 190 IU/dl (normal 50–150 IU/dl)
FIX:C = 13 IU/dl (normal 50–150 IU/dl)
∴ The patient has mild hemophilia B (Christmas disease). This may not become manifest as bleeding until a major surgical challenge occurs, perhaps for the first time in later life. In the context of surgery no hemophilia is 'mild'.
∴ therapy is needed to raise the FIX level

# ASAMBLAREA COMPLEXULUI FACTOR VIII - VON WILLEBRAND FACTOR

Source: Postgraduate Haematology, 7th Edition. Wiley Blackwell, 2016.




Assembly of FVIII–VWF complex. FVIII synthesized by hepatocytes as a single chain is partially proteolysed and (possibly) complexed with VWF prior to release. VWF is synthesized as a single-chain precursor, which dimerizes with disulfide bond formation, then multimerizes with further disulfide exchange, with concomitant loss of a large propeptide segment. The propeptide is essential for multimer formation and probably functions as an acidic disulfide isomerase.

# CLASSIFICATION OF VON WILLEBRAND DISEASE

(Williams M.E., Kahn M.J., American Society of Hematology Self-Assessment Program. Blackwell Publishing: 2005)

| | |
|---|---|
| Type 1 | Partial quantitative deficiency of VWF. Autosomal dominant disorder, but only a minority of persons with a nonfunctional VWF allele have bleeding symptoms |
| Type 2 | Qualitative abnormality of VWF |
| Type 2A | Abnormal assembly or reduced half-life of high-molecular-weight VWF multimers. Autosomal dominant |
| Type 2B | Abnormal (increased) binding of VWF to platelets, causing depletion of high-molecular-weight VWF multimers and thrombocytopenia. Autosomal dominant |
| Type 2M | Abnormal (decreased) binding of VWF to platelets, but with normal VWF multimer distribution. VWF:RCo decreased by 50% compared to VWF:Ag Autosomal dominant |
| Type 2N | Abnormal (decreased) binding of VWF to factor VIII, causing low plasma factor VIII levels. Autosomal recessive |
| Type 3 | Virtually complete deficiency of VWF. Autosomal recessive (but most carriers do not manifest the abnormalities of type 1 disease) |
| Pseudo-von Willebrand disease | Abnormal platelet GP Ib-IX-V with increased affinity for large VWF multimers. Phenotype (platelet-type VWF) indistinguishable from type 2B disease |

VWF = von Willebrand factor.

# VON WILLEBRAND FACTOR (VWF) ASSAY

(Williams M.E., Kahn M.J., American Society of Hematology Self-Assessment Program. Blackwell Publishing: 2005)

| Name | Function | Assay |
|---|---|---|
| VWF activity | Activity of VWF that causes binding of VWF to platelet GP Ib in the presence of ristocetin with consequent aggregation | *Ristocetin cofactor activity*: quantitates platelet agglutination after addition of ristocetin and VWF |
| | Ability of VWF to bind to collagen | *Collagen binding activity*: quantitates binding of VWF to collagen-coated plates |
| VWF antigen | VWF protein as measured by protein assays; does not measure functional ability | Immunologic assays such as ELISA, RIA, Laurell electroimmunoassay |
| VWF multimers | Size distribution of VWF multimers as assessed by agarose gel electrophoresis | *VWF multimer assay*: electrophoresis of plasma in low-concentration agarose gel and visualization by monospecific antibody to VWF |
| RIPA | Measures the ability of patient VWF to bind to platelet receptor GP Ib in the presence of variable concentrations of ristocetin | *RIPA*: aggregation of patient platelet-rich plasma with decreasing concentrations of ristocetin |

ELISA = enzyme-linked immunoabsorbent assay; GP = glycoprotein; RIA = radioimmunoassay; RIPA = ristocetin-induced platelet aggregation.