Erythrocyte Sedimentation Rate

The erythrocyte sedimentation rate (ESR) measures the rate of settling (sedimentation) of erythrocytes in anticoagulated whole blood. Anticoagulated blood is allowed to stand in a glass tube for 1 hour and the length of column of plasma above the red cells is measured in millimeters; this corresponds to ESR.

STAGES OF ERYTHROCYTE SEDIMENTATION RATE

There are three stages of erythrocyte sedimentation:

- *Stage 1*: Formation of rouleaux or lag phase (10 minutes): Red cells stack together like a pack of coins. The ESR depends mainly on this stage.
- *Stage 2*: Sinking of rouleaux or decantation phase (40 minutes): Rapid and constant sedimentation. The longer the tube, the longer is this stage and higher the value of ESR.
- *Stage 3*: Packing of rouleaux (10 minutes): Slow sedimentation.

FACTORS AFFECTING ERYTHROCYTE SEDIMENTATION RATE

Red cells, composition of plasma, and technical factors affect ESR (Box 24.1).

1. Red cells: Alteration of ratio of red cells to plasma affects ESR. **Decreased red cell mass in anemia increases ESR.** Conversely, **increased red cell mass in polycythemia decreases ESR.** Macrocytes tend to sediment rapidly than microcytes. Sickle cells and spherocytes are unable to form rouleaux and therefore ESR is low in sickle cell disease and hereditary spherocytosis. In these conditions, ESR is not reliable as an indicator of illness.
2. Plasma: The most important factor affecting ESR is the composition of plasma. Increase in fibrinogen, other acute phase proteins (C-reactive protein, haptoglobin, ceruloplasmin, α1-antitrypsin, etc.) and immunoglobulins increase ESR. Increased proteins in plasma reduce negative charge on the surface of red cells and reduce the zeta potential (the electrical repulsion between red cells); this brings red cells closer together and facilitates rouleaux formation. Removal of fibrinogen by defibrination and increase of albumin retard ESR.
3. **Technical factors:** ESR increases with room temperature. Tilting of the tube, and length and bore of the tube affect ESR.

SIGNIFICANCE OF ERYTHROCYTE SEDIMENTATION RATE

ESR is elevated in a wide range of organic diseases. ESR is not a specific and diagnostic test for any disease. However, it is helpful in differentiating functional from organic disease. Raised ESR signifies presence of some organic disease, which needs evaluation. Most of the inflammatory and neoplastic diseases are associated with an increase in ESR (Table 24.1).

Table 24.1: Causes of increased erythrocyte sedimentation rate

1. Infections	• Acute rheumatic fever • Osteomyelitis • Bacterial endocarditis • Pyogenic arthritis • Pelvic inflammatory disease • Tuberculosis • Acute hepatitis
2. Inflammatory diseases	• Rheumatoid arthritis • Systemic lupus erythematosus • Temporal arteritis • Polymyalgia rheumatica
3. Acute myocardial infarction	
4. Malignancy	
5. Paraproteinemias	• Multiple myeloma • Waldenström's • macroglobulinemia • Cryoglobulinemia
6. Technical problems	• Increased temperature • Tilted ESR tube
7. Others	• Ruptured ectopic pregnancy • Anemia • Renal disease with azotemia • Administration of dextran or oral contraceptives

- **There are two methods**

1- Wastergerns methods. 2- Wintrobes methods.

Wastergerns methods: Equipment: wasterges tube 30cm long 2.5mm internal diameter. Volume: 1 ml. Calibration: calibrated in millimeter from top to bottom zero-200mm. Dilution of blood: (1) of 3.13% trisodium citrate to (4) parts of venous blood. Mean (0.4 ml) of citrate to (1.6 ml) of venous blood.

PHYSIOLOGY OF HEMOSTASIS

Hemostasis is the normal physiologic mechanism for keeping the blood in fluid state in vascular system and for prevention of **hemorrhage by complex interaction of blood vessel walls, platelets, and plasma proteins.** Following injury, initially vessel wall and platelets interact to control hemorrhage by forming a platelet plug at the site of injury; this is called as **primary hemostasis**.

This is followed by activation of coagulation factors by a series of enzymatic reactions to form a stable fibrin clot (platelet plug enmeshed by fibrin); this is **secondary hemostasis**. Dissolution of the clot will eventually occur by fibrinolysis when healing is complete. Hemostatic equilibrium requires normal blood vessels, normal platelets, normal coagulation factors, normal fibrinolysis, and normal coagulation inhibitor system. Role of the three main components of hemostasis is outlined below.

Blood Vessel Wall

Transient constriction of blood vessels occurs at the site of injury which helps to control blood loss. Endothelial cells synthesize von Willebrand factor (vWF), tissue factor, and platelet activating factor, which promote **hemostasis**. Also, following injury, subendothelial **collagen is exposed which provides site for attachment of platelets (adhesion).** Endothelial cells synthesize prostacycline (inhibits platelet aggregation), protein S (a cofactor for protein C, which is an inhibitor of coagulation), and tissue plasminogen activator (activates fibrinolysis).

Platelets

Platelets are produced by cytoplasmic fragmentation of megakaryocytes in bone marrow. Life-span of platelets is about 7-10 days. Normal platelet count in peripheral blood is 1.5-4.0 lac/µl. About 2/3rd of platelets in the body are circulating in peripheral blood, while 1/3rd are pooled in spleen.

Main functions of platelets in hemostasis are adhesion, release reaction, and aggregation (Fig. 29.1).

Platelets attach to exposed subendothelium following injury; this adhesion is mediated by **vWF, which binds to GpIb receptor on platelets.** This initiates activation of platelets, which change shape from a **disc to a sphere** and release their contents to the exterior (release reaction). These are mainly adenosine diphosphate (ADP), serotonin, and thromboxane A_2 (TxA_2).

Platelet aggregation refers to sticking of platelets to each other; platelet agonists such as collagen, ADP, thrombin, and TxA_2 mediate aggregation. Binding of ADP to platelets causes exposure of GpIIb/IIIa receptors which bind fibrinogen. Fibrinogen binding to multiple platelets causes formation of large platelet aggregates. Activated platelets also provide phospholipid surface for certain coagulation reactions (platelet factor 3 or platelet procoagulant activity).

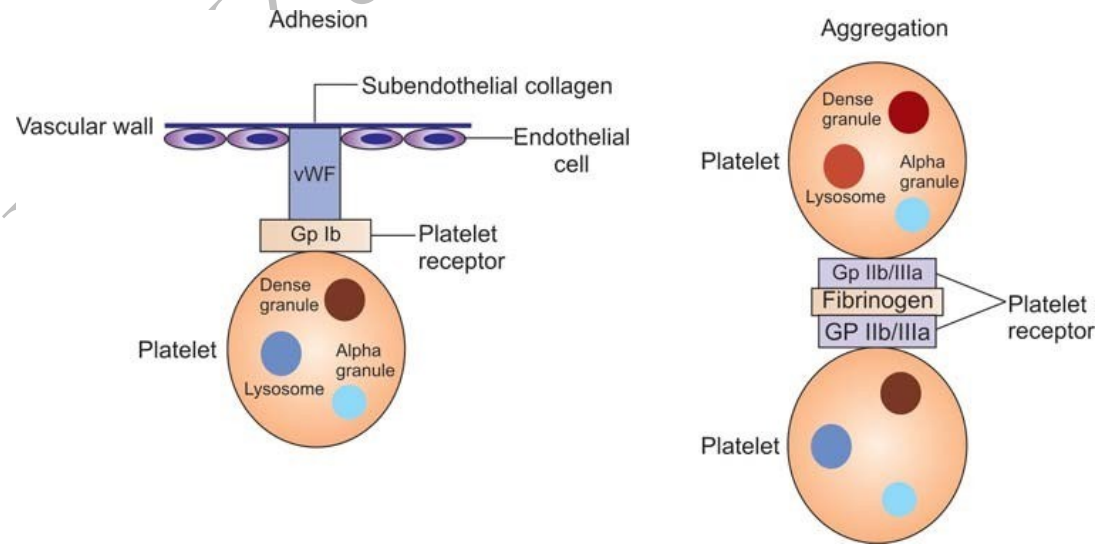


Fig. 29.1: Platelet adhesion and aggregation

Plasma Proteins

Plasma proteins, which regulate hemostasis, are coagulation factors, coagulation inhibitors, and proteins of fibrinolytic system.

Coagulation System

Normally, coagulation factors (Table 29.1) are circulating in an inactive form. Except for **thromboplastin and calcium, all the coagulation factors are proteins**. Coagulation factors have been divided into thee groups depending on similarities in structural and functional properties: (1) Fibrinogen group: I, V, VIII, XIII; (2) Vitamin-K dependent: II, VII, IX, X; and (3) Contact group: XI, XII, high molecular weight kininogen, prekallikrein. When activated, coagulation factors interact with each other in a sequential manner to ultimately form a fibrin clot and arrest bleeding.

Table 29.1: Coagulation factors

Factor	*Synonym*	*Site of synthesis*	*Significant features*
I	Fibrinogen	Liver	Normal: 200-400 mg/dl; absent in serum
II	Prothrombin	Liver	Vitamin K-dependent; multiple actions; absent in adsorbed plasma
III	Tissue factor; thromboplastin	Various tissues	Activates coagulation through extrinsic pathway
IV	Calcium	Obtained from diet and bones	Acts as a cofactor in various coagulation reactions
V	Labile factor	Liver, platelets	Cofactor in conversion of prothrombin to thrombin; absent in aged plasma
VI	There is no factor VI		
VII	Stable factor	Liver	Vitamin K-dependent; absent in adsorbed plasma; sensitive to oral anticoagulant therapy
VIII	Antihemophilic globulin; antihemophilic factor	Liver	Two subunits: VIII:C and VIII:vWF; cofactor in the conversion of F X to F Xa; absent in aged plasma
IX	Christmas factor	Liver	Vitamin-K dependent; absent in adsorbed plasma
X	Stuart-Prower factor	Liver	Vitamin-K dependent; absent in adsorbed plasma
XI	Plasma thromboplastin antecedent	Liver	Contact factor
XII	Hageman factor; Contact factor	Liver	Contact factor; activates coagulation *in vitro*; deficiency does not cause bleeding
XIII	Fibrin stabilizing factor; Laki-Lorand factor	Liver, platelets	Stabilizes fibrin clot by cross-linking fibrin monomers
Prekallik-rein	Fletcher factor	Liver	Contact factor; has many functions
High molecular weight kininogen	Fitzgerald factor	Liver	Contact factor; has many functions

Aged plasma: Stored plasma that is deficienct in factors V and VIII. Adsorbed plasma: Plasma adsorbed with ammonium hydroxide that is deficient in factors II, VII, IX, and X (vitamin K-dependent factors)

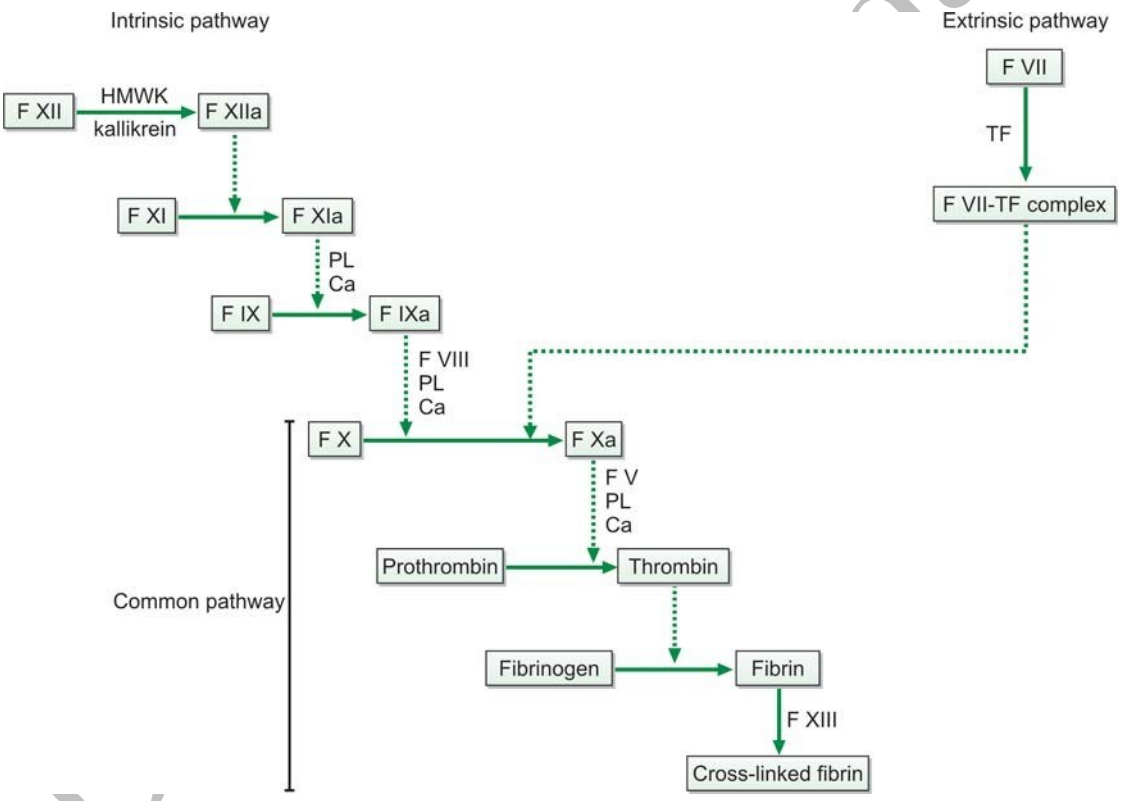
Blood coagulation occurring *in vitro* is divided into three pathways: extrinsic, intrinsic, and common (Fig. 29.2). This division is helpful in understanding the principles of common screening tests of coagulation.

Extrinsic pathway: The extrinsic pathway is initiated when F VII combines with tissue factor (released after tissue injury) in the presence of calcium ions. F VII-tissue factor complex in turn activates F X to F Xa.

Intrinsic pathway: Intrinsic system is initiated when F XII is activated *in vitro* (e.g. by glass). This activation requires presence of high molecular weight kininogen (HMWK) and prekallikrein. Activated F XII activates F XI, which in turn activates F IX to F IXa. F IXa combines with F VIII, phospholipid, and calcium to activate F X to F Xa.

Common pathway: Activation of F X to F Xa, by either extrinsic or intrinsic mechanism, marks the beginning of common pathway. F Xa binds FV, phospholipid, and calcium and converts prothrombin to thrombin. Thrombin splits off fibrinopeptides A and B from fibrinogen to form fibrin monomers, which spontaneously polymerize. F XIII stabilizes fibrin polymers by introducing covalent bonds between adjacent poly- peptide chains.

Fig. 29.2: Normal coagulation mechanism. HMWK = high molecular weight kininogen; PL = phospholipid; Ca = calcium; TF: tissue factor; solid arrow: conversion; Dotted arrow: action; subscript a: activated form of the coagulation factor



Inhibitors of Coagulation

Several physiologic inhibitors of coagulation are present in circulation to ensure that the fibrin clot remains localized to the site of injury and activation of coagulation is short-lived. Important inhibitors of coagulation are tissue factor pathway inhibitor (released from blood vessels; neutralizes VII-tissue factor complex), anti- thrombin III (most important physiological inhibitor; synthesized in liver; heparin-like substances on endothelial cells promote activity; inhibits mainly thrombin and F Xa), and protein C (activated by thrombin in the presence of thrombomodulin on the surface of

endothelial cells; causes proteolysis of activated forms of F V and F VIII; protein S acts as a cofactor in this reaction) (Fig. 29.3).

BLEEDING DISORDERS

Bleeding disorders are the result of a generalized defect in hemostasis due to abnormalities of blood vessels, platelets, or coagulation factors. Common bleeding disorders are listed in Table 29.2.

Table 29.2: Bleeding disorders

Disorders of blood vessels	
Acquired	*Hereditary*
Allergic purpura	Hereditary hemorrhagic telangiectasia
Infections: *Gram-negative septicemia, viral infections*	
Scurvy	
Senile purpura	
Disorders of platelets	
Thrombocytopenia	*Defective platelet function*
Increased destruction of platelets	**Acquired**
Idiopathic thrombocytopenic purpura	Drugs: *aspirin, other nonsteroidal anti-inflammatory drugs, antibiotics, xylocaine*
Infections: *malaria, dengue, septicemia, subacute* disorders *bacterial endocarditis, rubella, infectious mononucleosis*	Myeloproliferative
Drugs: *quinidine, quinine, heparin, procainamide*	Uremia
Heparin induced thrombocytopenia	Paraproteinemia
Disseminated intravascular coagulation	**Hereditary**
Massive blood transfusion	Storage pool deficiency
Thrombotic thrombocytopenic purpura	Bernard-Soulier syndrome
Alcohol	Glanzmann's thrombasthenia
Toxemia of pregnancy	
Increased pooling of platelets in spleen	
Hypersplenism	
Decreased production of platelets in bone marrow	
Aplastic anemia	
Bone marrow infiltration: *leukemias, lymphomas, myeloma*	
Megaloblastic anemia	
Drugs	
Infections	
Radiation	
Hereditary disorders	
Disorders of coagulation	
Hereditary	*Acquired*
Hemophilia A (F VIII deficiency)	Disseminated intravascular coagulation
von Willebrand's disease	Liver disease
Hemophilia B (F IX deficiency)	Vitamin K deficiency
Disorders of fibrinogen	Massive blood transfusion
	Heparin or oral anticoagulant therapy
	Renal disease
	Paraproteinemia
	Inhibitors of coagulation

Inherited Disorders of Coagulation

Deficiencies of all the coagulation factors have been reported. Out of these, the three relatively common disorders are hemophilia A (F VIII deficiency), hemophilia B (F IX deficiency), and von Willebrand disease (Table 29.3).

Hemophilia A

(Classical Hemophilia, F VIII Deficiency)

It is caused by hereditary deficiency or dysfunction of F VIII due mainly to point mutations or deletions of F VIII gene. It is an X-linked recessive disorder primarily affecting males; females are carriers but do not manifest the disease (Fig. 29.6). Hemophilia A is classified into three types based on the level of F VIII level in plasma: mild, moderate, and severe (Table 29.4).

In severe hemophilia, hemarthroses lead to hemorrhage can occur following minor trauma; operative and post-traumatic bleeding can be life-threatening; infections like hepatitis and acquired immunodeficiency syndrome can be transmitted through blood products. **Screening tests for hemostasis show normal bleeding time, platelet count, and prothrombin time. Activated partial thromboplastin time is prolonged. Diagnosis is made by one-stage F VIII assay**.

Hemophilia B (Christmas Disease, F IX Deficiency)

This is clinically indistinguishable from hemophilia A. Diagnosis requires F IX assay.

von Willebrand Disease (VWD)

vWD is a markedly heterogeneous congenital bleeding disorder characterized by deficiency or functional defect of von Willebrand factor (vWF). Mode of inheritance is autosomal dominant or recessive, with overall prevalence in the general population being 1%. There are three main

Table 29.3: Inheritance and incidence of inherited bleeding disorders

Disorder	*Inheritance*	*Incidence*
von Willebrand disease	Autosomal dominant or recessive	1:100
Factor VIII deficiency	X-linked recessive	1:10000
Factor IX deficiency	X-linked recessive	1:60000
Factor VII deficiency	Autosomal recessive	1:500,000
Fibrinogen deficiency	Autosomal recessive	1:1 million
Factor V deficiency	Autosomal recessive	1:1 million
Factor X deficiency	Autosomal recessive	1:1 million
Factor XI deficiency	Autosomal recessive	1:1 million
Factor XIII deficiency	Autosomal recessive	1:2 million
Prothrombin deficiency	Autosomal recessive	1:2 million

Note: Deficiencies of F XII, high molecular weight kininogen, and prekallikrein are not associated with bleeding

Acquired Disorders of Coagulation

Vitamin K Deficiency

Vitamin K is a fat-soluble vitamin necessary for the synthesis of coagulation factors II, VII, IX, and X, and also two natural anticoagulant proteins C and S. Vitamin K is required for gamma carboxylation of glutamic acid residues of the above coagulation factors. Vitamin K, being fat-soluble, requires bile salts for absorption. Vitamin K deficiency occurs in hemorrhagic disease of newborn, and in adults with poor dietary intake, malabsorption, obstructive jaundice, and drugs like oral anticoagulants.

Hemorrhagic disease of newborn Normally, vitamin K- dependent factors are low at birth; vitamin K deficiency exaggerates this deficit and causes bleeding. In classic cases, hemorrhage manifests around 2-4 days of life. Screening tests reveal normal platelet count and prolongation of both prothrombin time and activated partial thromboplastin time.

Liver Disease

Pathophysiology of hemostatic defect in liver disease is complex: (i) deficient synthesis of coagulation factors in hepatocellular disease, (ii) deficient synthesis of vitamin K-dependent factors in biliary obstruction, (iii) synthesis of dysfunctional fibrinogen (dysfibrinogenemia), (iv) decreased clearance of activated coagulation factors, (v) defective platelet function due to raised fibrinogen/fibrin degradation products, or (vi) disseminated intravascular coagulation.

Interpretation of Screening Tests

In a patient with a bleeding disorder, results of all the screening tests should be interpreted together (Fig. 29.16).

1- Hess, s test
2- Bleeding time
3- Platelets count
4- Examination of blood film for platelet morphology.
5- Whole blood clotting time
6- Prothrombin time
7- Thrombin clotting time
8- Partial thromboplastin time with kaolin

1- **Hess, s test:** capillary resistance test, measure capillary resistance capillary fragility under condition of increased pressure. Hess, s test is positive capillary disease (scurvy) and thrombocytopenia.

2- **Bleeding time:** time required for the spontaneous arrest of bleeding from skin puncture under standard conditions. The two methods:

1- Ivy methods

Time: 2-7min

Bleeding time: is prolonged thrombocytopathy and after aspirin treatment.

2- Duke methods

Time: 2-5min

Bleeding time: is prolonged thrombocytopathy and after aspirin treatment.

3- **Platelets count:**

The normal rate: 150.000-400.000 cell/ml. Examination of blood film for platelets morphology. Normal size: 2-4 in diameter. Round, oval or rod shape. Pale purple giant cells suggest platelet abnormality.

4- **Whole blood clotting time. (Lee& white method).**

Normal time: 4-9 min at 37c° is prolonged in the deficiency of factor involved in intrinsic coagulation mechanism most common cause deficiency in factor VII, IX and heparin therapy.

5- **Prothrombin**:

Normal rate: 10-14 second, it is the clotting of citrated plasma after the addition of tissue thromboplastin calcium mixture. Measure the extrinsic coagulation mechanism.

Causes of prolonged Prothrombin time

1- Oral anticoagulant therapy (comarine, warfarin) 2- Liver disease.

3- Nephritis.

6- **Thrombin clotting time**:

The time required for the clotting of citrated plasma after the addition of thrombin. Prolonged in heparin therapy and normal rate: 9-11min.

7- **Partial thromboplastin time with kaolin**

Three substance phospholipids kaolin and calcium are added to plasma. Normal rate: 35-45 second, prolonged due to deficiency of factors XII, IX, VIII and V this test is most useful in hemophilia.