HEMOSTASIS AND HEMORRHAGIC DISORDERS

R. Baklaja | M. Č. Pešić | J. Czarnecki
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BOOK REVIEW

The textbook *Haemostasis and Haemorrhagic Disorders* was written and published at the end of the 20th century. In the first decade of the 21st century, a second edition [*Hemostasis and Hemorrhagic Disorders*] has been written, discussing the recent professional and scientific issues. This updated textbook represents a significant contribution to medical science and practice.

Hemostasis and hemorrhagic disorders are significant medical issues that have, so far, been insufficiently investigated. Inherited and acquired hemostasis disorders are associated with numerous hematological diseases; however, there are many other diseases with hemostasis disorders in their genesis that are associated with various manifestations and prognoses. Clinical manifestations and laboratory investigations are not often easy or clear, and therapy can sometimes be ineffective.

Recent achievements in the fields of molecular biology, human genetics, and biochemistry have considerably contributed to understanding the factors participating in coagulation physiology, as well as to understanding the genetic basis of the occurrence and transmission of coagulation disorders. The locations of coagulation factor synthesis, genetic regulation, modes of activation, mechanism of bleeding interruption, the role of enzymes, and their biochemical structure have been discovered.
The discovery of fibrinolysis activators, known as plasminogen activators, and fibrinolysis inhibitors such as $\alpha_2$-antiplasmin, $\alpha_2$-macroglobulin, $\alpha_1$-antitrypsin, etc. is also significant.

In this textbook the authors describe hemorrhagic and vascular diseases, thrombocytopenias, and thrombocytopenias, as well as acquired and inherited coagulopathies, genetic regulation of coagulation factor synthesis, disorders caused by gene mutations, diagnostic possibilities, prenatal diagnostic, and the possible treatments. The diagnosis, modern classification, and treatment of vWD are also described. Acquired coagulation disorders are discussed, including the hemorrhagic disease of the newborn, bleeding in liver diseases, DIC hyperfibrinolysis and antifibrinolytic therapy in gynecological and obstetrical bleeding, and bleeding in gastroenterology, urology, neurology, and during neurological and cardiovascular surgery. Discovery of TFPI, thrombomodulin, antithrombin III, protein C, and protein S have considerably contributed to understanding, diagnosing, and treating hypercoagulable conditions, acquired and inherited thromboembolic diseases, acquired and inherited AT III deficiencies, and acquired and inherited PC deficiencies. Inherited and acquired thrombosis and thrombophilia are also described.

Particular significance has been given to modern diagnosis and treatment: thrombolytic therapy application, use of anticoagulant therapy, and use of streptokinase, an extracellular enzyme consisting of 415 amino acid polypeptide chains and isolated from beta hemolytic streptococcus. Streptokinase activity is reflected through a significant decrease in the quantity of circulating plas-
minogen and fibrinogen, causing blood viscosity decrease, red cell sedimentation and platelet aggregation. Indications for the administration of this anticoagulant are deep venous thrombosis, lung embolism, acute myocardial infarction, and arterial occlusions. Side effects associated with this rather significant thrombolytic drug and treatment therewith are also discussed.

Finally, the effects of other thrombolytic drugs are also described: APSAC (acylated plasminogen–streptokinase activator complex) and recombinant scu-PA (single-chain urokinase plasminogen activator). Chapter XII discusses the laboratory diagnostics of hemorrhagic disease.

This textbook contains XII chapters on over 290 pages and is illustrated with tables, schemes and color pictures. Up-to-date literature was quoted with over 200 references and an index at the end of the book. The authors provide concise knowledge on the topics in this textbook to assist a wide range of medical professionals, who are still insufficiently informed about the latest advances in the fields of hemostatic disorders, coagulation, thrombophilias, venous and arterial thrombosis, modern diagnostics and treatment possibilities.

This textbook is useful for transfusion specialists, hematologists, internists, cardiologists, neurologists, surgeons, gynecologists, anesthesiologists, as well as postgraduate and medical students.

Prof. Dr. Emilija Stojimirović
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Progress in the field of hemostasis and thrombosis has accelerated. In our book, *Hemostasis and Hemorrhagic Disorders*, the biochemistry and physiology of hemostasis, as well as the pathophysiology of thrombosis, are reviewed. This book considers the diagnosis and treatment of both.

The number of chapters has been increased because of diverse and complex new knowledge. For example, in the basic principles section on coagulation, part of the text on protein Z discusses the role of a new cofactor. In the fibrinolysis section, the part on thrombin-activated fibrinolysis inhibitor describes a new regulatory enzyme that inhibits thrombin-activated fibrinolysis (TAFI).

The section on deep venous thrombosis has been edited with new information on epidemiology, women’s health, upper extremity thrombosis, diagnosis, risk stratification, anticoagulant therapy, thrombolysis and venous thromboembolism prophylaxis.
A new chapter discusses the wide application field of streptokinase and other fibrinolytic agents, in particular for treatment of thrombosis and myocardial infarction.

We hope this new edition will serve the interests of medical and graduate students, scientists and physicians, hematologists, cardiac and vascular surgeons, pulmonologists, obstetricians, pediatricians and surgeons. We also hope you will find useful information that can be applied on a day to day basis.

The Authors
HEMOSTASIS AND BLOOD COAGULATION

Hemostasis (Physiological)

Hemostasis is one of the most significant maintenance systems of human bodily homeostasis; hemostasis plays two roles in the organism, namely:
1. to provide blood flow through blood vessels, i.e., to maintain the liquid state of circulating blood and
2. to prevent bleeding that results from blood vessel damage.

Hemostasis is a complex system that includes the participation of several factors:
- blood vessel endothelium
- platelets
- blood coagulation
- fibrinolytic process
- coagulation inhibitors.
The basic function of normal hemostasis is to prevent blood loss from undamaged blood vessels and to inhibit massive bleeding from damaged blood vessels. Blood loss from undamaged blood vessels is prevented by the normal blood vessel structure and normal platelet function. Bleeding after injury is stopped in three stages:
1. vascular stage
2. platelet stage
3. blood coagulation.

The significance of these mechanisms depends on the size and the type of the injury. Normal hemostasis starts with the vascular stage, is then followed by the platelet stage, which creates a platelet clot, and finally ends with the mutual action of coagulation factors, resulting in plug formation (Fig. I.1).
Vascular Stage - Endothelium

The role of the blood vessel in hemostasis is the constriction of an injured blood vessel – vasoconstriction. This lasts less than a minute and is a reflex action that is prolonged by serotonin from thromboxane A₂ (T A₂) and fibrinopeptide B, which, in turn, is created by the action of thrombin on fibrinogen. Blood vessel constriction prevents blood release and is sometimes sufficient for hemostasis maintenance (Fig. I.2)

When a blood vessel is injured, endothelium cells are damaged and circulating blood is exposed to the effect of various endothelium structures, such as collagen, fibronectin and von Willebrand’s factor (vWF); this results in platelet adhesion
(Fig I.3). The rapid adhesive capacity of platelets mainly depends on the glycoprotein receptors on the platelet surface.

The endothelium actively affects the function of all hemostasis components. The endothelium has two roles: activation and inhibition of hemostasis. (See Table I.1.)

Formation of vWF from Weibl-Palady’s corpusculums from endothelium cells is the most important role of endothelium activation. It also plays a role in the adhesive capacity of platelets and synthesis of vasoactive enzymes. Coagulation factors, which activate thrombin formation, are placed on the endothelium surface, synthesis of t-PA (tissue plasminogen activator) and u-PA (urokinase plasminogen activator).

**Figure I.3: Role of vWF in hemostasis**
The endothelium plays an inhibitory role in hemostasis. It synthesizes prostacycline PGI2 and nitrogen oxide (NO), which have an inhibitory effect on platelet aggregation. The endothelium contains ATP-asis (adenosine triphosphate) that destroys platelet adenosine diphosphate (ADP) and restricts platelet aggregation.

Figure I.4: Regulation of thrombin generation by the natural anticoagulant mechanisms of the endothelium. Baner K, 2006.
activation. Thrombomodulin, which is present on the surface of endothelium cells of other organs, except the brain, is found on the membrane (Fig I.4). Thrombomodulin is bound to thrombin forming a complex that activates PC. Heparin sulfate and other glycosoamines are also present on the membrane as well as some other glycosoamines that participate in AT III activation. The endothelium takes part in fibrinolysis and excretes t-PA and PAI-1 (plasminogen activator inhibitor).

**Table I.1: The Role of Blood Vessel Endothelium**

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<tr>
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<th>Activation</th>
<th>Inhibition</th>
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<tr>
<td>vasoregulation</td>
<td>synthesis of vasoactive enzymes (TA₂ and edothelin)</td>
<td>synthesis of vasodilatators (prostacycline and NO)</td>
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<tr>
<td>platelets</td>
<td>stimulates adhesive capacity and aggregation (collagen, fibronectin and vWF)</td>
<td>inhibits aggregation (prostacycline and NO)</td>
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<td>coagulation</td>
<td>phospholipid and coagulation factors are activated on the membrane</td>
<td>thrombomodulin (activates PC), heparin sulphate (activ. AT III) TFPI (inhibits TK)</td>
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<td>fibrinolysis</td>
<td>synthesis of t-PA and u-PA</td>
<td>synthesis of PAI-1 and PAI-2</td>
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Platelet Stage

Normal physiological response to vascular injury includes rapid platelet adhesion on the subendothelium and generation of thrombin, which induces platelet aggregation and fibrin formation. Platelet adhesion occurs when proteins (collagen and fibronectin) bind to specific glycoprotein (GP) receptors, also known as integrins, on platelet membranes. When the endothelium is injured, circulating vWF binds to collagen and undergoes

![Diagram of Hemostasis](image_url)

*Figure I.5: Overview of Hemostasis (Colman R. 2006.)*
a conformational change allowing it to bind to platelet receptor GPIb/IX. Patients lacking GPIb/IX (Bernard-Soulier Syndrome) or suffering from a deficiency of high molecular weight multimers of vWF, as in von Willebrand’s disease, show a prolonged bleeding time and hemorrhagic syndrome.

During adherence and activation, platelets change from discs into spheres with projections on long pseudopods. The platelet plug is developed further on the initial layer of adherent platelets by aggregation of platelets recruited to the site of vessel injury (Fig. I.5).

Platelets undergo aggregation and release the contents of their dense granules and α-granules when exposed to agonists such as ADP, epinephrine, thrombin or collagen.

Dense granules release calcium, serotonin and ADP, the latter of which promotes continued aggregation. The secreted α-granule content includes platelet factor 3, β-thromboglobulin, platelet-derived growth factor, thrombospondin, factor V and plasma proteins such as fibrinogen and IgG (immunoglobulin G). ADP and epinephrine, otherwise weak agonists for platelet aggregation, require prostaglandin T A 2 for secretion of granular contents. Thromboxane A 2 is synthesized from arachidonic acid that is released from platelet phospholipids during the aggregation process under the influence of phospholipid.
Figure I.6: Schematic diagram representing a current hypothesis of the contribution of platelets to blood coagulation. (Walsh PN, 2004.)
The Role of Platelets in Hemostasis

Two potent platelet aggregating agents are thrombin, which binds to GPV as well as to GPIb (vWF receptor), and collagen, which binds to GPIa/IIa. Thrombin and collagen can induce aggregation of platelets and secretion of platelet granular contents even if prostaglandin synthesis is blocked. A bond forms between fibrinogen and adjacent platelets through the interaction with platelet receptor complex GPIIb/IIIa. Fibrinogen binding only occurs after the platelet-activation-induced conformational change of the complex. GPIIb/IIIa is a transmembrane complex associated with actin on the inner surface of the platelet. Actin is a major component of platelet cytoskeleton. An actin-GPIIb/IIIa association is essential for clot retraction. Patients who show either the rare condition of afibrinogenemia or lack GPIIb/IIIa complex (Glanzmann thrombasthenia) have poor clot retraction and hemorrhagic syndrome (Fig. I.6).

Blood Coagulation

Blood coagulation is a series of enzyme processes in which inactive coagulation factors gradually become active. Each activated factor activates the next factor in the series. Blood remains liquid even though all coagulation factors are present in circulation. Plasma coagulation factors (F), indicated with roman numerals I to XIII, participate in the process of blood coagulation.
There is a permanent balance between coagulation activation and inhibition. If this balance is disturbed, thromboembolic complications or bleeding may occur. The hemostatic process (fibrin formation in blood vessel) is under continuous control of plasma inhibitors: antithrombin III, heparin, PC, PS, \( \alpha_2 \)-macroglobulin \((\alpha_2 \text{MG})\), C1 esterase inhibitor, \( \alpha_1 \)-antitripsin and \( \alpha_2 \)-antiplasmin \((\alpha_2 \text{AP})\).

There are three known coagulation pathways: intrinsic, extrinsic and common. (See fig. I.7 & I.8) The intrinsic pathway begins with the gradual activation of F XI, F IX, F VIII, platelet phospholipid (TrF3), and Ca\(^{++}\); F X is activated. The extrinsic pathway begins with tissue thromboplastin being released from the injured tissue, activated under the influence of F VII. The common coagulation pathway starts with F X activation. Through the activation of the intrinsic and extrinsic pathways, small quantities of fibrin are formed by transforming soluble fibrinogen into insoluble fibrin. Fibrin is the basic structural part of coagulum. Continuous coagulum formation occurs only if there is no blood flow. Blood circulation leads to dilution of coagulation factors thus preventing coagulum formation. Coagulum retraction occurs under the influence of platelets. Coagulum retreats to the injured part of the blood vessel, and blood flow is released.

Tissue factor/F VIIa complex is formed in the extrinsic pathway, and it activates F X in the presence of calcium. The intrinsic pathway is initiated by F XII activation, ending with F IXa/F
VIIIa complex formation, which also activates F X. Activation of F X initiates the common coagulation pathway in which prothrombin changes into thrombin, finally leading to fibrin formation.

Most coagulation factors are formed in the liver. The liver plays a central role in hemostasis.

When the fibrinolytic system is activated, an active enzyme called plasmin decomposes fibrin into small segments known as fibrin degradation products (FDP). FDP are normally removed by the reticulo-endothelial system (RES). High concentrations of FDP inhibit the interaction of fibrinogen monomers and fibrin formation and also prevent platelet aggregation.

Relations among activated coagulation factors, however, are far more complicated and go beyond the limits of the classical coagulation scheme. Initiation of blood coagulation through the extrinsic pathway occurs soon after blood vessel injury. Tissue factor, the cofactor required for F VII, is found in the subendothelium (is not found in endothelial cells). Injury of a blood vessel causes circulating F VII to bind to exposed tissue factor and changes small amounts of F X into F Xa. In return, F Xa activates additional F VII molecules. Tissue factor/F VIIa complex then activates new F X molecules, either directly or through F IX activation. After blood vessel injury, subendothelium collagen fibers and released endothelium proteolytic enzymes activate F XII initiating the intrinsic coagulation pathway.
The Clotting Cascade

**Figure I.7: Blood Coagulation Cascade System**
Figure I.8: Blood coagulation cascade system. Inhibitors of coagulation and fibrinolysis.
Figure I.9: The procoagulant vitamin K-dependent complexes. TFPI interacts with tissue factor - F VIIa - F Xa. (Swordo J. 2006)
F XIIa expresses a low level of enzyme activity changing small amounts of prekallikrein into kallikrein that, in turn, converts additional F XII molecules into an active form. F XIIa then activates F XI into F XIa. Prekallikrein and F XI circulate in the complex with high molecular weight kininogen (HMWK), which, as well as F XII, binds to surfaces with negative charges.

Until recently, F XII was described as the only F XI activator. However, recent studies have shown that thrombin is the most important F XI activator in vivo and not F XIIa. Besides, F XIa has autoactivation capabilities. F XIa then activates F IX; although, it has been recently demonstrated that the main F IX activator is tissue factor-F VIIa complex rather than F XIa. When F IX is activated, it forms a complex with its cofactor F VIII, and then this complex activates F X. F Xa and F Va, as a cofactor, form a new enzyme complex. These complexes play a significant role in coagulation regulation. They act as catalysts and highly increase enzyme reactions. In order to form a complex, platelets are activated and platelet phospholipid, normally situated inside the membrane, is expressed.

Localization of complexes on the platelet membrane increases complex concentration at the site of the injury and probably allows easy movement of substrates and intermediary products among adjacent complexes.
Complexes (Fig I.9) consist of vitamin K-dependent serine proteases (F IXa or F Xa) and protein cofactors (F VIIIa or F Va). They are formed in the presence of calcium and require an acid phospholipid, such as phosphatidyl serine, for their activity. Cofactors F V and F VIII are synthesized as high molecular precursors that have important identical sequences. They are primarily formed in the liver and circulate freely (F V), in plasma, or as F VIII in non-covalent complexes with vWF, which stabilizes molecules. F V is also synthesized in megakaryocytes, and it is found in platelet α–granules. Limited proteolysis of thrombin or F Xa activates these cofactors, highly increasing their binding to phospholipids. F VIIIa is then combined with F IXa, forming a complex that activates F X while F Va is combined with F Xa and forms a complex that activates prothrombin. The presence of cofactors in the complexes is necessary for maximum enzyme activity. F IXa can slowly activate F X, but in the presence of F VIII, a reaction is over 100,000 times faster. F Va combined with F Xa, calcium and phospholipid increases thrombin formation over 300,000 times. F Va is a specific receptor for F Xa when bound to the platelet. F Xa/F Va complex is bound to prothrombin, which is also bound to the platelet membrane.

The coagulation theory does not answer the question of why bleeding occurs if a coagulation factor is absent in one coagulation pathway, i.e., why the other coagulation pathway is not sufficient enough to stop the bleeding. Likewise it still is not clear why the lack of F XII, which activates the intrinsic coagulation pathway, does not contribute to the occurrence of bleed-
HEMOSTASIS AND BLOOD COAGULATION

Initial coagulation phase

Amplification phase

Propagation phase

Figure I.10: A cell based model of haemostasis (Hoffman, M., Monroe D. M., 2001)
ing. In 1992 Rapaport made a significant contribution to the understanding of coagulation when he pointed out the fact that tissue factor (TF) complex and F VIIa activate F X and F IX, causing simultaneous activation of both extrinsic and intrinsic coagulation pathways. Investigations based on the role of platelets, monocytes and endothelium in coagulation have contributed to the creation of the up-to-date coagulation cellular model.

According to the cellular model, coagulation takes place in three stages: initiation, amplification, and propagation. Initiation occurs through the binding of TF with F VIIa and activation of F X and F IX. Activated F X activates F V on the cell surface carrying TF, and this complex transforms a small quantity of prothrombin into thrombin. In the amplification stage, the created thrombin induces platelet activation, F V, F VIII, F XI and F XIII.

During the propagation stage, F IXa and F VIIIa, located on the platelet surface, create a complex that strongly activates F X. Activated F X and F Va, located on the platelet surface, create prothrombinase complex, which transforms a significant quantity of prothrombin into thrombin. Thrombin created in this way transforms fibrinogen into soluble fibrin, which under the influence of F XIIIa becomes insoluble coagulum. F XIa activates F IX and contributes to the additional formation of the thrombin surplus required for TAFI (thrombin activated fibrinolysis inhibitor) activation that ceases early fibrinolysis. This does not mean that the lack of F XI does not jeopardize co-
agulation, but, instead, compromises the inhibition of the early fibrinolysis, which contributes to a moderate bleeding risk.

All coagulation factors are synthesized in the liver. Certain coagulation factors require the presence of vitamin K for their synthesis. Vitamin K-dependent procoagulant factors II, VII, IX and X are synthesized in the liver, circulate as zymogens, are activated on the phospholipid surface and are limited by proteolysis. These factors belong to the serine proteases. Serine is found on the active site of the molecule’s carboxyterminal. On the molecule’s amino-terminal, each factor has 9 to 12 $\gamma$-carboxyglutamine residues, which are known as G1a domains, and are significant for binding calcium. Vitamin K is necessary for the carboxylation of these proteins. Inhibition of carboxylation leads to formation of factors that are incapable of binding to phospholipid and, thus, cannot manifest their procoagulant activity.

Transition of prothrombin into thrombin is caused by the cleavage of two peptide bonds. This leads to the formation of prothrombin and thrombin enzyme fragments 1.2 that are markers for hemostatic activity.

Thrombin directly activates the formation of a stable plug, changing fibrinogen into fibrin and activating F XIII, the catalyst of cross bonds within fibrin coagulum. Thrombin increases coagulation by activating F V, F VIII and F XI. This enzyme also stimulates platelet aggregation. Thrombin, however, inhib-
its continuous coagulation by binding to thrombomodulin and activating protein C, which inactivates F Va and F VIIIa (Fig. I.11).

Thrombin is one of the primary enzymes that connects the coagulation system with the inflammatory response and tissue repair mechanisms. The effect of thrombin on endothelial cells is exerted through stimulation of the secretion of prostacyclin, an inhibitor of platelet activation. Thrombin increases synthesis and release of plasminogen activator inhibitors from the endothelium as well as growth factor. It stimulates smooth muscle contractions and is a potent chemotaxis for monocytes and macrophages. The most important role of thrombin is in the transition of fibrinogen into fibrin. Fibrinogen is a glycoprotein consisting of three pairs of polypeptide chains (A-α, B-β and γ) bound by bisulphide bonds. Fibrin monomers are formed when thrombin cleaves fibrinopeptides A and B on the N-terminal part of A-α and B-β chains in the E domain. The polymerization of fibrin monomers then takes place. The formation of unstable fibrin coagulum is stabilized by F XIIIa, which is a cross catalyst of glutamine. Lysine residues bind and form bonds among the adjacent fibrin molecules. α₂ antiplasmin is included in the binding process, and the plug is relatively resistant to plasmin degradation.
The thrombin explosion following tissue damage

Figure I.11: Adapted from Beglin, T. (1996). BMJ, 312, 683-7
FIBRINOLYSIS

The fibrinolytic system removes the blood clot after the blood vessel defect is closed. In addition to this basic function, the fibrinolytic system participates in tissue reparation, ovulation, embryo implantation, malignant transformation, inflammation, and macrophage functions. Plasminogen, plasmin, plasminogen activators, inhibitor activators and antiplasmins are active fibrinolysis factors (Fig II.1).

Plasmin is the main fibrinolytic enzyme. In circulation it is found in its inactive form as plasminogen. Plasminogen is a glycoprotein and is synthesized in the liver. There are several types of plasminogen in plasma. Two of the most important are a “native“ form that contains glutamine in the amino-terminal (glu-plasminogen) and a “modified“ form that contains lysine in the amino-terminal (lys-plasminogen). Plasminogen is transformed into plasmin by an activator (Fig II.2). The most important substance for plasmin activation is fibrin. As a non-specific proteolytic enzyme, however, fibrin degrades all peptides that
contain arginyl-lysine residues. Activated plasmin degrades fibrinogen, F V, F VIII and vWF in the coagulation system, but it also activates HMWK and complementary elements.

Plasmin cleaves both fibrinogen and fibrin into a family of fragments known as fibrinogen-fibrin degradation products or fragments X, Y, D and E. They can be found in the circulation in various degrees of degradation. Fragments D and E are terminal fragments resistant to further proteolytic plasmin activity. Intermediate fragments X and Y can be further degraded to

Figure II.1: Schematic model of the structure of glu-plasminogen. Henkin T. 1994.
Fragments D and E. Fibrin and fibrinogen degradations are quite similar although, in several instances, they vary. Fragment E, resulting from fibrinogen degradation, contains fibrinopeptides A and B whereas fragment E, resulting from fibrin degradation, does not. Some fragments have biological activity. Fragments D and E, for instance, inhibit coagulation in the thrombin time test. Fragment X preserves the ability of intact fibrinogen molecules to support ADP-induced platelet aggregation and erythrocyte sedimentation. Furthermore, fragment X can be coagulated by thrombin. Terminal fragments D and E stimulate the syn-

Figure II.2: Activation of fibrinolysis and inhibitors.
thesis and the release of fibrinogen from the liver. Fragments D and E influence electric heart activity; they increase capillary permeability and vasoactive effects of bradykinin and angiotensin II.

Activators of Fibrinolysis

Numerous substances have the ability to change plasminogen into plasmin. The substances are known as plasminogen activators and are classified in endogenous and exogenous forms. Endogenous plasminogen activators are physiological constituents of hemostatic mechanisms and are divided into intrinsic and extrinsic activators. Exogenous plasminogen activators are non-physiological molecules, such as streptokinase. Intrinsic activators are plasma proteins that circulate as inactive precursors. Many of these substances act as mediators in coagulation and inflammatory processes. The intrinsic pathway of plasminogen activation requires F XIIa, which participates in the contact phase of the intrinsic coagulation pathway and induces fibrinolysis. Physiological F XII is activated by contact with a negatively charged surface. Several other coagulation factors can also activate F XII, including HMWK, prekallikrein and plasmin. Upon activation, F XII is cleaved into three peptides; the last of which contains an active part. F XIIa activates plasminogen, directly or indirectly, through prekallikrein and F XI activation (Fig II.3).
Extrinsic plasminogen can be activated by numerous tissues, including neoplasms. They are far more potent than intrinsic activators and form a heterogeneous group of molecules that can be divided into two main subgroups: t-PA and u-PA. Both directly activate plasminogen by cleaving an Arg-560-Val-561 bond.

* t-PA is produced in almost all tissues but mostly in the vascular endothelium. Increased t-PA levels can be detected in the smooth muscles of the uterus and in certain neoplasms. Tissue plasminogen activator is also found in body fluids such as tears, saliva, breast milk and sperm. Its enzyme activity is increased
in the presence of fibrin. In the absence of fibrin, t-PA weakly activates glu-plasminogen. Its concentration in plasma is 5 to 7 ng/ml, and $T_{1/2}$ (half-life) is 2.5 to 5 minutes. Over 95% of the circulating t-PA is present in complexes with plasminogen activator inhibitor type 1 (PAI-1). Vascular endothelial cells release t-PA from a depot in response to numerous physiological stimuli, such as venous stasis.

u-PA, or UK, forms the second subgroup of extrinsic plasminogen activators. Urokinase is produced in the kidney parenchyma. It is excreted in small amounts with the urine. $T_{1/2}$ is 10 to 15 minutes. Numerous other types of cells such as fibroblasts, epithelial cells, pneumocytes and decidual placenta cells produce urokinase, too. Receptors for u-PA can be found in many tissues, since u-PA has an important role in tissue repair, inflammation, tumor invasion, fertilization and embryogenesis.

Streptokinase is the most important substance among extrinsic plasminogen activators. It was first isolated from β-hemolytic streptococcus group C. It contains one single polypeptide chain and, in contrast to the other plasminogen activators, has almost no intrinsic enzyme activity. In order to develop catalytic activity, streptokinase forms complexes with plasminogen. There, an active part is exposed without any peptide bonds being cleaved. Streptokinase $T_{1/2}$ is 18-25 minutes; it is excreted through RES. $T_{1/2}$ of the streptokinase-plasminogen complex is much shorter, 1.5 to 2.5 minutes, and clearance is performed
through RES, too. Streptokinase has antigenic properties and, thus, may cause formation of anti-streptokinase antibodies.

**Inhibitors of Fibrinolysis**

Inhibitors of fibrinolysis directly inhibit plasmin (antiplasmins) or plasminogen activators. Endogenous substances that inhibit plasmin are serine proteases. The most important physiological inhibitor of plasmin is $\alpha_2$-antiplasmin, a glycoprotein synthesized in the liver with a $T^{1/2}$ of 2.5 days. The level of $\alpha_2$AP is decreased in liver diseases and in DIC. Inherited deficiency of $\alpha_2$AP, which is associated with bleeding, is very dangerous. $\alpha_2$AP inhibits fibrinolysis by forming 1:1 stoichiometric complexes with plasmin in a two-stage process.

In the first stage, $\alpha_2$-antiplasmin generates a reversible complex with plasmin through noncovalent bonds. This interaction can be prevented by the binding of certain molecules such as epsilon aminocaproic acid (EACA) to plasmin. During the second stage, serine from the active site of plasmin is irreversibly bound to the $\alpha_2$AP active site. $\alpha_2$AP interferes with the fibrin binding sites on plasminogen. In a secondary reaction, $\alpha_2$AP binds to fibrin through F XIII and calcium. It strongly inhibits fibrinolysis within the coagulum. Similar quantities of $\alpha_2$AP and plasmin are bound to fibrin on the coagulum surface. $\alpha_2$AP also inactivates several components of the coagulation cascade, including F XII, F XI, F X and thrombin. In the absence of fibrin, $\alpha_2$AP strongly
binds to the entire amount of circulating plasmin and rapidly inactivates it. Thus, fibrinolysis is increased at the coagulum formation site and inhibited in circulation.

$\alpha_2$-macroglobulin is the next important physiological plasmin inhibitor. It is a serine protease synthesized by endothelial cells, monocytes and macrophages in the liver. Increased levels are found during pregnancy, in nephritic syndrome, in lung and liver diseases, and during oral contraceptive drug use. It is an acute-phase protein. Decreased levels are found during thrombolytic therapy and in DIC. It inhibits plasmin, but it also degrades fibrin, fibrinogen and F VIII. It inhibits other components of the fibrinolytic system: t-PA, streptokinase-plasminogen complex and kallikrein.

Other inhibitors of fibrinolysis are:

$\alpha_1$AP - Increased levels can be found during inflammation in neoplasms and in estrogen therapy.

$C_1$ inhibitor - It inhibits the first complement component, as well as plasmin, kallikrein, and factors XIa and XIIa. Deficiency of $C_1$ inhibitor is associated with hereditary angioedema without bleeding.

AT III - The primary inhibitor of thrombin and some coagulation factors; it also slowly and irreversibly inhibits plasmin. The contribution of AT III to plasmin inhibition is small since only 1% of plasmin is bound to AT III.
PAI - The most important inhibitors being PAI-1 and PAI-2 while less significant ones are protease nexin I and PAI-3, otherwise identical to protein C inhibitor (PCI).

**PAI-1** is a glycoprotein. It is primarily formed in the vascular endothelium; smaller amounts are produced by hepatocytes, smooth muscle cells, fibroblasts and some malignant cells. PAI-1 is present in platelet α granules synthesized in megakaryocytes. It belongs to the serine proteases and is similar to α2AP, C1 inhibitor, AT III and α1-antitrypsin. PAI-1 efficiently inhibits both t-PA and u-PA. It is an acute-phase protein, and its level increases 20-fold in sepsis, DIC and during surgery. Increased levels are also found in DVT, during pregnancy and myocardial infarction. Many stimuli can lead to formation and excretion of PAI-1, such as interleukin-1 (IL-1), bacterial endotoxins, tumor necrosis factor (TNF), transformed growth factor β (TGF β) and dexamethasone. Activated PC decreases PAI-1 release. PAI-1 is secreted in the active form; its T1/2 is around 20 minutes. PAI-1/t-PA complex is metabolized by the liver. PAI-1 is the most significant plasmin inhibitor and the most important inhibitor of fibrinolysis, together with α2 antiplasmin. PAI-1 deficiency leads to bleeding, and increased PAI-1 is found in thrombosis. Patients with increased PAI 1 levels develop DVT more frequently.

**PAI-2** is homologous with PAI-1, although biologically different. It is a glycoprotein that inhibits both t-PA and u-PA. PAI-2 is synthesized by monocytes and by the epithelium trophoblast in fetus. Under normal conditions, PAI 2 plasma level is unde-
tectable, but it increases during pregnancy in the third trimester. Its release from monocytes is supported by bacterial toxins, TNF and colony stimulating factor (CSF-1). Dexamethasone prevents this release. The physiological role of PAI-2 is not well defined, yet. PAI-2’s major function is the inhibition of u-PA located in the extracellular space.
COAGULATION INHIBITORS

The functions of the hemostasis system must be strictly regulated. There is a constant balance between the factors that stimulate the activation of hemostasis and those that inhibit and control it. This means that hemostasis plays a very sensitive role in maintaining the balance between thrombosis and bleeding manifestations.

Inhibitors prevent the coagulation process; i.e., they limit the coagulation process at the site of blood vessel injury (AT III, thrombomodulin, TFPI, PC) (Table III.1).

TFPI (Tissue Factor Pathway Inhibitor)

TFPI exists in plasma in association with lipoproteins. An even larger quantity of TFPI appears to be bound to the endothelial surface. This source of TFPI can be released upon administration of heparin.
Table III.1: Coagulation Inhibitors

<table>
<thead>
<tr>
<th>INHIBITOR</th>
<th>INHIBITS</th>
</tr>
</thead>
<tbody>
<tr>
<td>TFPI</td>
<td>VII/TK</td>
</tr>
<tr>
<td>THROMBOMODULIN (thrombin)</td>
<td>V, VIII (PC)</td>
</tr>
<tr>
<td></td>
<td>platelet aggregation,</td>
</tr>
<tr>
<td></td>
<td>conversion of F I into fibrin</td>
</tr>
<tr>
<td>AT III</td>
<td>thrombin, IXa, Xa, Xla, Xllia, XIIa,</td>
</tr>
<tr>
<td></td>
<td>kallikrein, plasmin</td>
</tr>
<tr>
<td>PC (APC, PS)</td>
<td>V, VIII</td>
</tr>
<tr>
<td>HEPARIN COFACTOR II</td>
<td>thrombin</td>
</tr>
<tr>
<td>C₁ ESTERASE INHIBITOR</td>
<td>kallikrein, XI, XII, XIII</td>
</tr>
<tr>
<td>α₂MG</td>
<td>Ila, VIII, and t-PA</td>
</tr>
<tr>
<td>α₂AP</td>
<td>Ila, X, XI, XII</td>
</tr>
</tbody>
</table>

TFPI regulates complex tissue factor (TF/VIIa). First, TFPI must bind to F Xa, and then it inhibits factor Xa. Next, TFPI and Xa complex binds to TF/VIIa complex on the phospholipid membrane, blocking the procoagulant activity of TF/VIIa complex.

**Thrombomodulin**

Thrombomodulin has an anticoagulant effect by converting thrombin from coagulant to anticoagulant. Thrombin loses its ability to convert F I to fibrin. It does not activate platelets as well as F V and F VIII, but it activates protein C.
In order to obtain the catalytic ability to activate PC into PCa, thrombin has to bind to the cofactor thrombomodulin (TM), which is synthesized by the vascular endothelium. In complex with thrombomodulin, thrombin loses its ability to bind to fibrinogen and to activate coagulation factors V and VIII and platelets. Thrombin only maintains the ability to activate PC (Fig III.1).

Figure III.1: Thrombomodulin – Anticoagulant Effect
**Antithrombin III (AT III)**

The hemostatic balance in blood circulation is regulated by numerous complex systems (Fig. III.2). Only a few of these systems are currently well known. One of the regulating components, recognized in 1905 by Moravich, is AT III. AT III is the most important serine protease inhibitor. AT III deficiency is either inherited or acquired and is associated with the predisposition to thromboembolic diseases. Human AT III is a single-chain alpha-2 globular glycoprotein with a molecular weight of 58,000 daltons. It consists of 425 amino acids, 4 carbohydrate residues and 3 disulphide bonds. Recent studies show that AT III is formed in hepatocytes. It can also be found in endothelial cells. In the normal population, the average AT III concentration in plasma is 0.1 to 0.2 g/l. AT III concentration in plasma is less variable than other protein concentrations in plasma. AT III concentration is expressed in percentages; normal values range from 80 to 120%. Compared to adults, AT III concentration

*Figure III.2: Protein C, Protein S, and Thrombomodulin. Stearns-Kurosawa DJ, 2006.*
is lower in newborns, reaching adult values six months after
birth. Women have slightly decreased AT III levels during men-
struation. Levels decrease with age (70 to 110%). AT III half-life
is 2.5 to 3 days.

Figure III.3: Antithrombin III (AT III) and heparin cofactor II (HcII) target
enzymes. AT III inhibits coagulation factors in the intrinsic, extrinsic, and
common pathways. AT III and HcII inhibit thrombin, which has coagulant
and noncoagulant functions.

SMC = smooth muscle cell  EC NO = endothelial cell nitric oxide
The most important function of AT III is inactivation of thrombin and many other activated coagulation enzymes: Xa, XIa, IXa, XIIa, plasmin, trypsin and kallikrein. AT III binds to thrombin forming an inactive and stable complex. This reaction is considerably modified by heparin. The half-life of this complex is 9 hours in circulation. Heparin increases AT III activity 1000 times. Around half of the entire AT III amount can be found extravascularly. Extravascular AT III acts as a reservoir that adds AT III to blood whenever necessary. In tissue, though, AT III is directed towards residual fibrin during inflammatory processes (Fig. III.3).

**Protein C (PC) and Protein S (PS)**

PC is a vitamin K-dependent factor found in circulation in its inactive form as double-chain zymogen and belongs to the group of serine proteases. PC is bound to a negatively charged phospholipid, requiring Ca (Calcium). Activated PC has anticoagulation properties. As a complex formed with PS, through limited proteolysis, PC inactivates coagulation factors Va and VIIIa. PC is similar to chymotrypsin. Activation of PC, induced by thrombin, leads to cleavage of 12 amino acid peptides on the amino-terminal of its heavy chain. Thus, the zymogen changes into an active enzyme.

In order to obtain the catalytic ability to activate PC into PCa, thrombin has to bind to the cofactor TM, which is synthe-
sized by the vascular endothelium. In a complex with thrombomodulin, thrombin only maintains its ability to activate PC. Activated PC is combined with its cofactor PS, forming an anticoagulant enzyme complex on the phospholipid membrane.

PS is also vitamin K-dependent; although, it does not have any enzymatic activity. Approximately 60% of PS is found in its inactive form in circulation. The remaining 40%, circulating as free PS, is responsible for the cofactor activity. Besides FVa and F VIIIa inactivation, activated PC increases the release of plasminogen activator from endothelial cells. Like other vitamin K-dependent factors, PC and PS are synthesized in the liver. Half-life for the clearance of PC from circulation is 6 to 8 hours. It is inactivated by PCI.
HEMOSTASIS IN NEWBORNS AND IN PREGNANCY

Hemostasis in Newborns

The knowledge of the physiological values of coagulation factors in newborns has a practical significance in making a diagnosis, a differential diagnosis, and determining treatment of hemorrhagic syndromes. During investigation of hemostasis in newborns, alterations on blood vessels and platelets have not been found. Platelet count shows normal values in premature infants as well as in adults. Platelet adherence is also normal. Platelet aggregation on ADP, epinephrine, collagen and thrombin can be disturbed, though only transitory. Aggregation on ristocetin is normal.

In contrast to primary hemostasis, there are changes in blood coagulation during the neonatal period. Values of vitamin K-dependent factors are decreased (F II, F VII, F IX and F X). Fibrinogen, F V and F VIII show normal values; however, they can be moderately decreased in premature infants. Fibrinolytic
activity is highly accelerated. Despite this, fibrin degradation products are absent. These changes are even more obvious in premature infants in whom clinical manifestations of bleeding are considerably more frequent and occur as a consequence of:
- higher vitamin K-dependent factor deficiency,
- slower reaction to vitamin K administration (immature liver), and
- increased capillary permeability (hypoxia).

Certain coagulation factors (II, VII, X, XII) that were decreased at birth normalize after several weeks while normalization of other factors may take several months. Despite decreased coagulation factors values, there are certain signs of hypercoagulability in both mature newborns and in premature infants. This paradoxical hypercoagulability can be explained with the deficiency of natural inhibitors, AT III and PC. Prothrombin time is prolonged due to F II, F VII and F X deficiency. Activated partial thromboplastin time is prolonged due to deficiency of F XII, F XI, F IX, prekallikrein and HMWK.

Thrombin time is also prolonged. This is hard to explain since the level of fibrinogen is normal, and FDP and heparin are not present. Prolonged thrombin time (TT) could possibly be explained by the presence of “fetal fibrinogen” or disfibrinogenemia. False positive screening tests may occur because of blood drawn through a catheter, whose permeability is reflected by heparin administration. In order to prevent possible bleeding due to vitamin K deficiency, vitamin K administration to
newborns is recommended after birth rather than to the mother before delivery. No side effects were noted in the administration of natural vitamin K; in contrast, the administration of synthetic vitamin K that is soluble in water can result in hyperbilirubinemia and kernicterus. Mothers should not be treated with peroral anticoagulant, antivitamin K drugs prior to delivery.

Hemorrhagic Disease of the Newborn

Vitamin K deficiency found in children is known as hemorrhagic disease of the newborn. It is caused by defective synthesis of vitamin K-dependent factors because of a functionally immature liver as a result of certain drugs administered to the mother (oral anticoagulant drugs, anticonvulsive drugs, phenobarbiton, etc.) (Table IV.1).

Patients with vitamin K deficiency should be treated with parenteral vitamin K1 given subcutaneously or by slow intravenous infusion at a dose of 1 to 5 mg. Resolution of bleeding symptoms is generally rapid (within 4 to 6 hours) and, usually, precedes improvement in the results of coagulation screening studies. Any infant or child with a life-threatening hemorrhage due to vitamin K deficiency should, depending clotting factor levels, also receive FFP (fresh frozen plasma) to rapidly increase vitamin K.
The preventive routine administration of vitamin K at birth (0.5 to 1.0 mg I.M.) has significantly reduced the incidence of classic newborn hemorrhagic disease.

### Hemostasis in Pregnancy

The concentration of many coagulation factors is increased during pregnancy: F II, F V, F VIII, F IX, F X, F XII and, especially, fibrinogen, reaching doubled values at delivery compared with the values at the beginning of pregnancy. The level of F VIII/vWF is particularly increased in the last trimester of preg-

---

**Table IV.1: Hemorrhagic Disease of the Newborn (HDN)**

<table>
<thead>
<tr>
<th>Clinical Form</th>
<th>Cause</th>
<th>Appears</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early form of HDN</td>
<td>Mother was receiving anticonvulsive therapy.</td>
<td>24 hrs</td>
</tr>
<tr>
<td>Classic form of HDN</td>
<td>Reduced vitamin K synthesis by intestinal flora.</td>
<td>2-7 days after birth</td>
</tr>
<tr>
<td></td>
<td>Low placental transfer of vitamin K.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Low vitamin K concentration in breast milk.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mother was receiving anticonvulsive or drug treatment.</td>
<td></td>
</tr>
<tr>
<td>Delayed form of HDN</td>
<td>Biliary atresia.</td>
<td>2 weeks to 3 months after birth</td>
</tr>
<tr>
<td></td>
<td>Neonatal hepatitis.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cystic liver fibrosis.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Prolonged diarrheal illness.</td>
<td></td>
</tr>
</tbody>
</table>
nancy, both in healthy pregnant women and in those with vWD. In these patients, therefore, extra preparation for delivery is not necessary since there is no risk of bleeding. On the fourth day after delivery, when the vWF decreases, an adequate therapy should be administered. Despite an increased risk of thrombin formation, there is no increase of AT III, as physiological inhibitor, in pregnancy. This may explain resistance to heparin activity in pregnancy. The platelet count does not express changes in the course of normal pregnancy, and platelet function and survival time are normal.

Plasminogen level increases parallel with fibrinogen although fibrinolytic activity is not increased since the level of the plasminogen activator does not increase and antiplasmin activity is higher. There are data showing fibrinolytic activity provoked by strong stimuli and the removal of unfavorable fibrin coaguli. Contrary to increased coagulation factor levels, F XI and F XIII levels decrease. They are particularly low at birth. After delivery, considerable changes in coagulation status occur. Fibrinolysis is accelerated even 30 minutes after delivery. Fibrinogen level and platelet count in puerperium increase, possibly leading to a higher risk of thromboses. At the same time, AT III activity increases balancing the appearance of thromboses. Coagulation is normalized in the 6th week after delivery. When anticoagulant therapy is used, subcutaneous administration of heparin is recommended since antivitamin K passes through the placenta.
HEMORRHAGIC DISORDERS

Hemorrhagic syndromes are characterized by a disorder of one or more factors that participate in hemostasis. The majority of hemorrhagic syndromes are blood vessel disorders, platelet number and function disorders, or coagulation factor disorders:

- vasculopathies
- thrombocytopenias
- thrombocytopenias
- coagulopathies.

They become clinically manifest by bleeding.

Vasculopathies

Vasculopathies may be inherited or acquired. Inherited forms result from blood vessel structure disorders (inherited telangiectasia, Rendu-Osler-Weber’s disease) while acquired disorders can be a consequence of inflammatory or immune processes that damage blood vessel walls. In clinical practice, acquired dis-
HEMORRHAGIC DISORDERS

orders are found more frequently (secondary purpuras, infections, effects of some drugs, Henoch-Schonlein’s syndrome, allergic purpura, effect of aspirin, senile purpura, vitamin C deficiency, etc.).

Clinical manifestations are nose bleeding, gingival, or genital and gastrointestinal tract bleeding. Bleeding time is prolonged with normal platelet count. Bleedings are usually moderate, so that special treatment is not necessary. However, during surgical procedures, these disorders can provoke serious bleeding.

Thrombocytopenias

Thrombocytopenia, or reduced circulating platelet count, can be inherited or acquired; the acquired form being more frequent. Thrombocytopenia occurs as a result of:

- decreased platelet formation with normal platelet survival time (effects of irradiation, drugs, malignant tissue pressure on bone marrow, leukemias, aplastic anemias) or
- increased platelet degradation or platelet deposit in spleen with decreased platelet survival (DIC, effects of drugs, bacterial or viral infections, inherited idiopathic thrombocytopenic purpura, chronic leukemias, lupus erythematosus, Hodgkin’s disease, massive transfusions and liver cirrhosis).

Platelets are formed in the bone marrow, and about 80% of the platelets are found in circulation. Normal platelet survival is
8 to 10 days. Around 20% of the platelets are deposited in the spleen. In hypersplenic syndrome, however, the number deposited there may even amount to 90%. Increased deposits in the spleen are also followed by decreased platelet survival. This is significant in the assessment of effectiveness of transfusion since transfusion is one of the major therapeutic means for the prevention of bleeding in thrombocytopenias. There is no absolute connection between platelet count and severity of bleeding. However, when the platelet count is above $40 \times 10^9/l$, spontaneous bleeding rarely occurs. Platelet count above $100 \times 10^9/l$ does not usually show signs of bleeding, but if the platelet count is below this level, patients should receive a platelet transfusion.

**Thrombocytopenies**

In patients with prolonged bleeding time and normal platelet count, platelet function disorder has to be suspected. Platelet function disorders can be inherited or acquired. Acquired ones are more frequently found in clinical practice.

**Inherited Qualitative Platelet Disorders** may be due to abnormalities of
1. platelet membrane glycoproteins,
2. platelet granules,
3. platelet coagulant activity, or
4. signal transduction and secretion.
Abnormalities of Platelets Membrane Glycoproteins
- Glanzmann Thrombastenia, abnormal GPIIb/IIIa
- Bernard-Soulier Syndrome, abnormal GPIb, GPIX and GPV
- platelet-type of vWD, abnormal GPIb

Abnormalities of Platelet Granules
Platelet granule disorders are due to ADP and T A₂ release reaction disorders. These may occur due to absence of granules in platelets, storage pool disorder (characterized by disturbed platelet aggregation to collagen, adrenaline and thrombin), or disturbed release (absence of T A₂).

Abnormalities of Platelet Coagulant Activity
Only a few such patients have been described, assays for platelet factor 3 are abnormal.

Abnormalities of Signal Transduction and Secretion
Normally these abnormalities only produce a minor bleeding tendency, and therapy is not usually necessary. Abnormalities of signal transduction and secretion include:
- defects in arachidonic acid metabolism,
- cyclooxygenase deficiency, platelets unable to produce thromboxane; endothelium may not produce prostacyclin,
- thromboxane synthesis deficiency, and
- defects in platelet secretion and the second wave of platelet aggregation, found in response to epinephrine or ATP.

Acquired Qualitative Platelet Abnormalities occur with systemic diseases:
1. abnormal platelet function in uremia
2. antiplatelet antibodies
3. cardiopulmonary bypass
4. chronic myeloproliferative disorders
5. leukemia and myelodysplastic syndromes
6. multiple myeloma and other monoclonal gammopathies
7. drugs (aspirin, ticlopidine, antibiotics, cardiovascular drugs, and volume expanders like dextran and hydroxyethyl starch)
8. food and diets rich in fish oil.
The manifestations are usually mild. The usual laboratory abnormalities are prolongation of the bleeding time and/or platelet aggregation, but these results do not necessarily predict risk of clinical bleeding.

Thrombocytopathies are treated in the same manner as thrombocytopenias. If bleeding time is prolonged, 6 to 8 platelet concentrates are administered prior to surgery and are followed by postoperative transfusions if bleeding persists.¹

**Blood Clotting Disorders – Coagulopathies**

Coagulopathies are characterized by disturbed coagulation and are most frequently caused by a deficiency of one coagulation factor. This deficiency may be inherited or, more frequently, may result from disorders in organs participating in the formation of coagulation factors (Table V.1). Coagulopathies can be a consequence of the absolute absence or decrease of the synthesis of certain coagulation factors, of the production of abnormal coagulation factor molecules (inherited blood clotting disorders), of increased destruction (acquired blood clotting disorders) during intravascular coagulation, or due to the presence of inhibitors.

¹ Details about vasculopathies, thrombocytopenias and thrombocytopathies can be found in any hematology book, especially in Williams Haematology.
### Table V.1: Coagulation Factors Synonym Table

<table>
<thead>
<tr>
<th>Factor</th>
<th>Synonym</th>
<th>Kind of Protein</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Fibrinogen</td>
<td>Structural</td>
<td>Linkage Strands</td>
</tr>
<tr>
<td>II</td>
<td>Prothrombin</td>
<td>Vit K Serine Proteinase</td>
<td>Activates I, V, VII, XII, PC and Platelets</td>
</tr>
<tr>
<td>V</td>
<td>Proaccelerin</td>
<td>Binding</td>
<td>Helps Xa activate II</td>
</tr>
<tr>
<td>VII</td>
<td>Stabel Factor</td>
<td>Vit K Serine Proteinase</td>
<td>Activates IX and X</td>
</tr>
<tr>
<td>VIII</td>
<td>Antihemophilic</td>
<td>Binding</td>
<td>Helps IXa activate X</td>
</tr>
<tr>
<td>IX</td>
<td>Christmas Factor</td>
<td>Vit K Serine Proteinase</td>
<td>Activates X</td>
</tr>
<tr>
<td>X</td>
<td>Stuart-Prower Factor</td>
<td>Vit K Serine Proteinase</td>
<td>Activates II</td>
</tr>
<tr>
<td>XI</td>
<td>Thromboplastin antecedent</td>
<td>Serine Proteinase</td>
<td>Activates IX</td>
</tr>
<tr>
<td>XII</td>
<td>Hageman Factor</td>
<td>Serine Proteinase</td>
<td>Activates kinin system</td>
</tr>
<tr>
<td>XIII</td>
<td>Fibrin stabilizer</td>
<td>Transglutaminase</td>
<td>Crosslinks fibrin</td>
</tr>
<tr>
<td>von Willebrand III related Ag</td>
<td>Binding</td>
<td></td>
<td>Binds platelets and VIII</td>
</tr>
<tr>
<td>Extrinsic Pathway Inhibitor</td>
<td>Kunitz inhibitor (2 headed serpin)</td>
<td></td>
<td>Inhibits TF-VIIa and Xa</td>
</tr>
<tr>
<td>Antithrombin III</td>
<td>Serpin</td>
<td></td>
<td>Inhibits serine proteinases</td>
</tr>
<tr>
<td>Protein C</td>
<td>Vit K serine Proteinase</td>
<td></td>
<td>Inactivates Va VIIIa and PAI-1</td>
</tr>
<tr>
<td>Protein S</td>
<td>Vit K protein</td>
<td></td>
<td>Helps protein C</td>
</tr>
<tr>
<td>Plasminogen</td>
<td>Serine Proteinase</td>
<td></td>
<td>Lyses fibrin</td>
</tr>
<tr>
<td>α₂ antiplasmin</td>
<td>Serpin</td>
<td></td>
<td>Inhibits plasmin</td>
</tr>
<tr>
<td>Prourokinase</td>
<td>Serine Proteinase</td>
<td></td>
<td>Activates plasmin</td>
</tr>
<tr>
<td>Tissue plasminogen activator</td>
<td>Serine Proteinase</td>
<td></td>
<td>Activates plasmin</td>
</tr>
<tr>
<td>Plasminogen activator inhibitor 1</td>
<td>Serpin</td>
<td></td>
<td>Inactivates I-PA and urokinase</td>
</tr>
</tbody>
</table>
INHERITED BLOOD CLOTTING DISORDERS

Inherited blood coagulation defects are most frequently caused by a deficiency or abnormality of one plasma coagulation factor, rarely two or more. These diseases are relatively rare, but they require urgent treatment and the team work of various specialists: hematologists, transfusion medicine specialists, pediatricians, orthopedic specialists, dentists and physiotherapists.

The most frequent blood coagulation defects are:
- hemophilia A (factor VIII deficiency) - classic hemophilia
- hemophilia B (factor IX deficiency) - Christmas disease
- von Willebrand’s disease (von Willebrand factor defect)

Hereditary deficiencies of other coagulation factors are significantly less common, ranging in prevalence from 1 per 500,000 to 1 per million. All are autosomally recessive and may be due to a decreased synthesis of factor protein or the production of normal amounts of a factor with decreased functional activity (Table VI.1).
The main clinical characteristic common in all forms of hemorrhagic syndromes is bleeding that occurs spontaneously into skin, subcutaneous tissue, muscles and joints or as a consequence of trauma or surgical procedures (Table VI.2).

Differential diagnosis of a syndrome based only on clinical signs is difficult, almost impossible, since each type of bleeding
INHERITED BLOOD CLOTTING DISORDERS

Table VI.2: Clinical Manifestations

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Clinical Manifestations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemophilia A</td>
<td>Spontaneous or provoked, subcutaneous and intramuscular bleeding, nose bleeding, gastrointestinal bleeding, intracranial bleeding, hematuria, bleeding into joints (hemarthrosis), bleeding caused by surgery</td>
</tr>
<tr>
<td>Hemophilia B</td>
<td>Same as in hemophilia A</td>
</tr>
<tr>
<td>Hemophilia C</td>
<td>Nose bleeding, menorrhagia, after surgery and trauma, rarely spontaneous bleeding</td>
</tr>
<tr>
<td>von Willebrand's Disease</td>
<td>Gastrointestinal tract bleeding, epistaxis, menorrhagia and hematuria</td>
</tr>
<tr>
<td>A fibrinogenemia or Hypofibrinogenemia</td>
<td>Umbilical cord bleeding, subcutaneous bleeding, nose bleeding, tooth extraction trauma and bleeding caused by surgery</td>
</tr>
<tr>
<td>F II Defect</td>
<td>Subcutaneous or intramuscular bleeding after trauma and surgery</td>
</tr>
<tr>
<td>F V Deficiency</td>
<td>Nose bleeding, menorrhagia, intramuscular and subcutaneous bleeding, gastrointestinal tract and intracranial bleeding caused by trauma and surgery</td>
</tr>
<tr>
<td>F VII Defect</td>
<td>Spontaneous subcutaneous, intramuscular and nose bleeding, gastrointestinal tract bleeding, hematuria, hemarthrosis, caused by trauma and surgery</td>
</tr>
<tr>
<td>F X Defect</td>
<td>Rare spontaneous bleeding, frequent bleeding caused by trauma and surgery</td>
</tr>
<tr>
<td>F XII Defect</td>
<td>No hemorrhagic manifestations</td>
</tr>
<tr>
<td>F XIII Defect</td>
<td>Umbilical cord bleeding, hematomas, uterus bleeding, postponed wound bleeding, slow wound-healing and characteristic scars</td>
</tr>
</tbody>
</table>

is similar to the other types, and in some disorders, it is almost identical. Bleeding into joints is more frequent in hemophilia, leaving permanent consequences, e.g. joint malformations and invalidity.
Treatment

Each inherited blood coagulation defect occurs as a consequence of an inherited defect in the formation of certain blood coagulation factors. Replacement therapy, in the form of blood coagulation factor replacement with blood and blood derivative transfusions that contain sufficient amounts of the missing factors with preserved activity, is applied in all cases.

Table VI.3:

<table>
<thead>
<tr>
<th>Blood components in the treatment of coagulation disorders</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cryoprecipitate</td>
</tr>
<tr>
<td>F VIII concetrante</td>
</tr>
<tr>
<td>F IX concetrante</td>
</tr>
<tr>
<td>F IV complex</td>
</tr>
<tr>
<td>vWF concetrante</td>
</tr>
<tr>
<td>vWF/FV III concetrante</td>
</tr>
<tr>
<td>F VIIa (novo seven)</td>
</tr>
<tr>
<td>F V concetrante</td>
</tr>
<tr>
<td>F XIII concetrante</td>
</tr>
<tr>
<td>Fibrinogen concetrante</td>
</tr>
</tbody>
</table>

In order to administer blood and blood derivatives in an adequate manner, proper diagnosis and recognition of the mechanisms that induce the hemorrhagic syndrome are required. A profound knowledge of the hemostatic characteristics of conserved blood and blood derivatives, of the characteristics of coagulation fac-
tors whose defects cause bleeding, of in vivo survival $T_{1/2}$, and of hemostatic levels is also necessary (Table VI.3).

Knowledge of basic coagulation factor characteristics is of particular significance:
- stability to conservation in vitro in order to make a good choice of blood and blood components for transfusion
- survival in recipient’s organism - $T_{1/2}$ is significant in the assessment of therapy effect duration and necessity to repeat transfusion
- hemostatic level of coagulation factors is the level of coagulation factors in a patient that is sufficient for bleeding prevention. It varies significantly in spontaneous bleedings from 10 to 40%, and it is considerably higher during the preparation for surgery reaching 100%.

**Therapeutic Recommendations**

**Choice of concentrates**
When selecting a plasma-derived or recombinant concentrate, the two most important safety issues are infectious agents and inhibitor formation (including anaphylaxis).

**Hemophilia A**
Recombinant F VIII is the treatment of choice. Therapeutic products for hemophilia A include recombinant factor VIII concentrates and viral-inactivated, plasma-derived concentrates as well as cryoprecipitate in some developing countries. Most phy-
sicians and consumer groups advocate the use of recombinant therapeutic products, especially for children and newly-diagnosed patients. Many adults remain on plasma-derived concentrates, either based on personal choice or because of shortages of recombinant products. Patients with mild hemophilia A may also use desmopressin (DDAVP), which releases factor VIII and vWF from storage sites.

**Hemophilia A with inhibitor**
Therapeutic products for patients with factor VIII inhibitors include human factor VIII, porcine factor VIII, activated prothrombin complex concentrate (APCC) and recombinant activated factor VII (“Novo Seven”).

Treatment of these patients includes treatment of acute bleeding and treatment to induced immune tolerance. Bleeding in patients with congenital hemophilia should be treated with recombinant products.

**Hemophilia B**
Recombinant factor IX is the treatment of choice. The alternative to recombinant F IX is a high-purity, plasma-derived F IX concentrate. Therapeutic products for hemophilia B include prothrombin complex concentrates and factor IX concentrate, which are available as recombinant concentrates and as virally-inactivated, highly-purified, plasma-derived concentrates. Plasma is used in some developing countries. Factor IX concentrate is recommended instead of PCC (prothrombin complex concentrate) for
patients at risk of thrombosis. The recombinant product is widely advocated for children and newly-diagnosed patients.

**Hemophilia B with inhibitor**
Therapeutic products for patients with factor IX inhibitors include factor IX, PCC, APCC, and recombinant activated factor VIIa. Factor VIIa, “NovoSeven”, is the only appropriate product for patients whose inhibitor reacts with infused factor IX in such a way that causes severe allergic reactions.

**von Willebrand’s disease**
In the treatment of vWD, a concentrate containing vWF is the preferred treatment when DDAVP is not likely to be effective or is contraindicated (Level IIb, Grade B). Therapeutic products for vWD include all factor VIII concentrates, which also contain vWF. Such concentrates are now available as plasma-derived, therapeutic products. A concentrate of vWF with little F VIII has become available. Cryoprecipitate is used in developing countries.

**Factor XI deficiency**
In those with partial deficiency of F XI (15 to 17 U/dl), bleeding is more difficult to predict. When there is a clear history of abnormal bleeding and treatment is required to secure hemostasis, the use of F XI concentrate is justified. Where there is no helpful history of bleeding, tranexamic acid may be used alone. Virally inactivated plasma is recommended when F XI concentrate is contraindicated. Patients with factor XI deficiency may be treated with plasma. For surgery, an initial bolus dose of concentrate
is given to bring plasma level to 80%; half that dose is given every two to three days afterwards.

**Factor VII, Factor II and Factor X deficiency**
Recombinant F VIIa is the treatment of choice with factor VII deficiency and should be favored over F VII containing prothrombin complex concentrate because of the increased risk of thrombosis.

In the treatment of factors II and X, no specific concentrates are available, and prothrombin complex concentrates are the only possible treatment. Patients with factor VII or X deficiency are treated with recombinant, activated factor VIIa concentrate.

**Factor V deficiency**
Concentrates containing factor V are not available and plasma is the only possible treatment. Virally inactivated plasma is recommended. Patients with factor V deficiency are treated with pooled, solvent-detergent-treated plasma.

**Factor XIII deficiency**
Factor XIII concentrate is the treatment of choice. Patients with severe factor XIII deficiency have been managed successfully with prophylactic plasma infusions given monthly; the half-life of factor XIII is six days.

**Fibrinogen deficiency**
Fibrinogen concentrate is the only available treatment.
Hemophilia Treatment –
Prophylaxis, Continuous Infusion and DDAVP

Long Term Prophylaxis
Prevention of hemarthrosis is a major challenge in hemophilia care since hemophilic arthropathy due to repeated joint bleeds is the main cause of morbidity in hemophiliacs. During the last two or three decades, prophylactic treatment has revolutionized hemophilia care. Regular prophylaxis should be initiated at the age of 1 to 2 years, before arthropathy has begun to develop. Concentrate should be administered two to three times a week at annual dosages of 3000 units per kilogram of body weight. At the joint meeting of World Health Organization (WHO) and World Federation of Hemophilia (WFH) in Geneva in 1994, the following was recommended.

Since the main goal is to prevent joint bleeding and sequelae, prophylaxis should be considered optimal management for persons with severe hemophilia A and B (baseline factor VIII/IX levels <1% of normal). Possible prophylaxis schemes:

Hemophilia A (without hemarthrosis)  F VIII concentrate 20 U/kg bw
three times a week
F VIII:C level > 1 U/dl

Hemophilia B (without hemarthrosis)  F IX concentrate 40 U/kg bw
two times a week
F IX level > 1 U/dl
Patients with hemarthrosis should be treated with 20% more concentrate.

Side effects in prophylaxis
Nowadays, clotting factor concentrates are essentially safe with regard to blood-borne viruses. The risk of hepatitis and HIV transmission during prophylactic treatment should be considered negligible, whereas inhibitor development is still an important issue.

Continuous Infusion
Traditionally, replacement therapy as intravenous bolus injections with factor VIII/IX concentrates was administered. Bolus injections require high peaks in order to keep the trough levels above a minimum level and ensure that the risk of hemorrhage is minimized.

Continuous infusion would allow maintenance at a constant level above which there is no a risk of bleeding. Nowadays, a continuous infusion seems to be a convenient, safe and cost effective way of administering replacement therapy in hemophilia during bleeding episodes or as cover for surgery.

Heparin or low molecular weight heparin is often added to the concentrate to prevent local thrombophlebitis at the infusion site. According to Schulman and colleagues (1994), 5 IU of heparin is needed per one ml of concentrate. Recombinant concentrate does not require the use of heparin.
Desmopressin (DDAVP) Treatment of Hemophilia A and vWD

DDAVP (1-deamino-8-d-arginine vasopressin) is a synthetic analogue of the nonapeptide arginine vasopressin. DDAVP has been found to increase F VIII:C plasma concentration, von Willebrand factor, and tissue plasminogen activator. However, DDAVP has no effect on the F IX level. Whenever possible, DDAVP should be the treatment of choice with mild forms of hemophilia and vWD rather than blood derived concentrates.

Several different routes of administration are possible with DDAVP: intravenously, subcutaneously, or intranasally. Intravenous administration of DDAVP is a slow, intravenous infusion with doses of up to 0.3 µg/kg body weight, diluted in physiological saline. The maximum effect seems to be obtained at a dosage of 0.3 µg/kg body weight; the dosage often used for the treatment of mild hemophilia A. The peak plasma concentration of FVIII:C and vWF occurs after about 30 minutes, with that of FVIII:C increasing about 2 to 6 times and that of vWF about 2 to 4 times above the respective baseline levels.

Subcutaneous administration of DDAVP might be the ideal choice for home treatment with the increase of FVIII:C plasma concentration after 15 µg of DDAVP. Nasal administration of 150 µg spray is equal to a dose of 0.3µg administered intravenously. The dose can be repeated in 8 to 12 hours intervals. However, it is known that DDAVP doses repeated within short intervals can cause tachyphylaxis.
Since DDAVP stimulates the release of t-PA, concomitant administration of fibrinolytic inhibitor may be advisable. DDAVP is not contraindicated in uncomplicated pregnancies. No teratogenic effect has been observed. Mild hemophilia A and vWD should be treated with DDAVP (and tranexamic acid) instead of coagulation factor concentrates when ever possible.

**Side effects of DDAVP**
Some patients may experience mild, transient side effects such as headaches, tachycardia, facial flushing, abdominal pain, tremors and sweating during or shortly after intravenous administration. Water retention can occur, but it is not a clinical problem. There is a possible risk of thrombo-embolic complications after administration of DDAVP (Mannucci and Lusher, 1989).

**Vaccination Against Hepatitis A and B**
Hepatitis A and B vaccinations are highly effective in preventing infection after exposure. All patients who currently receive, or may require, blood products should be vaccinated. Hepatitis A vaccine is not licensed for those younger than 1 year of age. The vaccines should be given to patients subcutaneously, not intramuscularly, to reduce the risk of hematoma.
Hemophilia A

Hemophilia is an inherited coagulation disorder that occurs as a result of an F VIII (hemophilia A) or F IX (hemophilia B) coagulation deficiency. Basically, this disorder is the outcome of an F VIII or F IX gene mutation because of the absence of synthesis, decreased synthesis or synthesis of the altered coagulation factor molecule.

Hemophilia is inherited from a recessive gene located on X chromosome. Females who have the pathological gene are carriers, but they do not develop the disease since the healthy gene will take over the function of the pathological one. However, males are homozygous (having only one X chromosome), so the disease will develop if the pathological gene is inherited. Men suffering from hemophilia will transmit the pathological gene to all their daughters, who will be carriers, while all their sons will be healthy (since the Y chromosome is inherited from the father). If a female is a carrier, 50% of her sons will be hemophiliacs while 50% of her daughters will be carriers. In females, however, hemophilia can occur in extremely rare cases if the father is a hemophiliac, and the mother is a carrier. Most families with hemophilia A carry gene defects from independent origins.

Hemophilia is one of the most frequent coagulation disorders. The frequency of hemophilia A is 50 to 100 hemophiliacs per one million inhabitants, i.e., one hemophiliac infant per 10,000 male newborns.
Molecular Basis of Hemophilia

F VIII gene was cloned in 1984 (Gitschier 1984), but fully efficient mutation detection procedures first became available in 1991. At the time, it was the largest described gene. The F VIII gene is located on the second arm of chromosome X in the vicinity of the telomeric part in band Xq28. It is one of the largest genes in human genome with a length of 186 kb, occupying 0.1% of the chromosome length. It contains 26 exons and 25 introns (Fig VI.1).

Figure VI.1: Factor VIII Gene
The site of F VIII synthesis is still being investigated. mRNA for F VIII is isolated from hepatocytes, lymph nodes, kidneys, and the spleen. F VIII gene product is a polypeptide consisting of 2341 amino acids, located in cells in the form of precursor proteins. It consists of the signal peptide (19 residues of amino acid), three types of domains (A, B, C), and two acid peptides (\(a_1\) and \(a_2\)). A-domains are homologous with the regions of F V molecules and ceruloplasmin; this shows that these proteins originate from a mutual precursor protein. The F VIII binding site for negatively charged phospholipids is on C-domains while the \(a_2\) peptide contains the binding site for F VIII with vWF. In circulation, F VIII is found in its inactive form; thrombin activates F VIII. Activation includes proteolytic splitting of an F VIII molecule at three sites, resulting in the separation of F VIII from the protein carrier vWF, elimination of B-domain and formation of heterotrimers \((A_1a_1A_2Ba_2A_3C_1C_2)\), i.e. F VIIIa molecule. F VIII inactivation takes place thanks to the effect of activated protein C, which splits the heavy chain at position 336.

Hemophilia A is a disorder that is caused by F VIII gene mutation (hemophilia B by F IX gene mutation) located on structural or regulatory gene sequences. Mutations are heterogeneous. Point mutations are the most frequent ones (around 75%) although some other kinds of mutations can also cause hemophilia, e.g., inversions (20%), deletions (3 to 5%), insertions, and translocations (Fig VI.2).
An F VIII molecule is found in a complex with vWF in circulation. It has a significant role in the coagulation process. The F VIII molecule consists of two different proteins that have different biochemical and immunological characteristics. They are under separate genetic control and have specific physiological functions. Activated F VIII (F VIIIa) serves as a cofactor to F IX, forming a complex with F IXa on the platelet surface and dramatically accelerating the rate of factor X activation.

Figure VI.2: 3D presentation of an LRP-binding site within the A2-domain of F VIII (Saenko EL, Pipe SW. Haemophilia 2006;12:42-51.)
Clinical Manifestations

Clinical manifestations of hemophilia are bleedings that occur from the umbilical cord at birth and later spontaneously into muscles and repeatedly into joints resulting in malformations and invalidity. Other forms of spontaneous bleeding may also occur as well as bleeding caused by injury or surgical procedures. Severe hemophiliacs have frequent, spontaneous bleeding (joint bleeds) and intramuscular hematomas.

Hemarthrosis is occasionally seen in other severe congenital bleeding disorders, but it is usually a characteristic of hemophilia A and B. The most frequent sites for bleeding are the knees, followed by the elbows, ankles, hips and wrists. The acute form is characterized by initial, mild pain without any physical findings; this is followed by more intense pain, swelling of the joint and decreased range of motion. The joint develops an inflammatory reaction, resulting in a chronic synovitis, which predisposes repeated bleeding episodes in the joint (target joint). Eventually, the synovitis causes chronic, end-stage arthropathy characterized by destruction of the articular cartilage, chronic pain, and an unstable, dysfunctional joint.

Intramuscular hematomas occur in the large flexor muscles, especially the calf muscles, iliopsoas muscles and forearms. These hematomas may cause significant blood loss, compartment syndromes, nerve compressions and muscle contractures.
Development of pseudo tumors, which slowly expand, may complicate intramuscular hematomas.

CNS hemorrhage is the most common cause of bleeding mortality. Retroperitoneal bleeding and retropharyngeal bleeding may be life-threatening.

Hemorrhage into muscles surrounded by a tight fascia (e.g., the calf and forearm) can cause pressure to build up, compartment syndrome. This pressure leads to arterial occlusion with ischemia and is followed by necrosis or nerve compression by paresis, which can be permanent. A hematoma of the ileopsoas muscle as it passes through the narrow inguinal canal can exert pressure on the femoral nerve, invariably causing permanent neurological sequelae. Unless treated promptly and effectively, such hemorrhages will result in severe handicaps that could carry vocational, social and psychological consequences and, in children, poor adjustment to school.

Massive hematuria is common and may cause renal colic, but it is seldom life-threatening. Antifibrinolytics are contraindicated in the presence of hematuria.

Postsurgical bleeding can often be delayed by a few hours to several days. It is associated with poor wound healing.
Table VI.4: Replacement Therapy with Blood and Blood Derivatives

<table>
<thead>
<tr>
<th>Hemorrhagic Syndrome</th>
<th>Treatment</th>
<th>Biological T&lt;sub&gt;1/2&lt;/sub&gt;</th>
<th>Infusion Frequency</th>
<th>Hemostatic Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemophilia A</td>
<td>Fresh frozen plasma, Cryoprecipitate, F VIII concentrate, rF VIII concentrate</td>
<td>7-12 hrs</td>
<td>2x daily 6 to 8 hrs</td>
<td>30% 70-100%</td>
</tr>
<tr>
<td>von Willebrand</td>
<td>Plasma, Cryoprecipitate, vWF concentrate</td>
<td>18-30 hrs</td>
<td>1x daily</td>
<td>50-100%</td>
</tr>
<tr>
<td>Hemophilia B</td>
<td>Fresh frozen plasma, F IX concentrate, F IX complex</td>
<td>15-30 hrs</td>
<td>1x daily 8 to 12 hrs</td>
<td>30% 100%</td>
</tr>
<tr>
<td>Factor I</td>
<td>F I concentrate</td>
<td>3-5 days</td>
<td>3-4 days</td>
<td>0.7-1.0 g/l</td>
</tr>
<tr>
<td>Factor II</td>
<td>F IX complex</td>
<td>2-4 days</td>
<td>2-4 days</td>
<td>30-40%</td>
</tr>
<tr>
<td>Factor VII</td>
<td>F IX complex, rF VIIa concentrate</td>
<td>4-6 hrs</td>
<td>4-8 hrs</td>
<td>5-15%</td>
</tr>
<tr>
<td>Factor X</td>
<td>F IX complex</td>
<td>30-70 hrs</td>
<td>4 days</td>
<td>10-20%</td>
</tr>
<tr>
<td>Factor XI</td>
<td>Plasma, F XI concentrate</td>
<td>30-70 hrs</td>
<td>4 days</td>
<td>20-30%</td>
</tr>
<tr>
<td>Factor V</td>
<td>Plasma</td>
<td>15-18 hrs</td>
<td>6-8 hrs</td>
<td>10-30%</td>
</tr>
<tr>
<td>Factor XIII</td>
<td>Plasma, F XIII concentrate</td>
<td>4 days</td>
<td>7 days</td>
<td>10%</td>
</tr>
</tbody>
</table>

Diagnosis of Hemophilia A

Hemophilia A can easily be diagnosed based on a positive individual and family history, clinical findings, and laboratory test
results. PTT is prolonged in all cases of hemophilia A, except in some moderate cases when F VIII is above 50% (0.50 U/ml). PT and TT are normal. Assays for F VIII level determination are very significant in the assessment of the severity of clinical symptoms, which are otherwise directly dependent on the F VIII level, by determining the hemostatic levels. This assessment is also important in preparing for surgical procedures and for determining follow-up therapy. Normal hemostasis is generally seen with levels in excess of 30%. The normal range of F VIII is between 50 and 150% with a mean of 100 percent.

With a differential diagnosis, hemophilia A must be distinguished from vWD (especially the Normandy variant), which is an acquired inhibitor of F VIII and combined congenital deficiency of F VIII and F V.

Treatment of Hemophilia A

General guidelines for the treatment of hemophilia are: treat bleeding episodes promptly, avoid aspirin and other antiplatelet agents, avoid intramuscular injection, and plan surgical procedures carefully (Table VI.4).

Immediate administration of intensive blood transfusion is essential in the treatment of hemophilic bleeding episodes. The aim of a blood transfusion is not to reach the level of healthy persons but, rather, to achieve an adequate hemostatic level (30%; 0.30 U/ml) sufficient enough to prevent spontaneous bleeding.
Transfusions are repeated after 8-12 hours due to a short $T_{1/2}$. F VIII is administered during therapy for spontaneous bleeding in doses of 20 to 30 U/kg/b.w. (body weight) (Table VI.5). During the preparation for a surgical procedure, a dose of 40 to 60 U/kg/b.w. of F VIII should be administered in order to reach a hemostatic level of about 70% and should be followed by a maintenance dose two times less than the initial dose and repeated twice daily during wound-healing. Doses can be higher, and the hemostatic level can reach 100%. For F VIII replacement therapy, cryoprecipitate and F VIII concentrate should be administered.

Table VI.5:

<table>
<thead>
<tr>
<th>Blood Component</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Cryoprecipitate</td>
<td></td>
</tr>
<tr>
<td>F VIII concentrate</td>
<td>80 to 100 IU (4 to 8 IU/ml) of F VIII, 250 mg of fibrinogen, around 70 IU of F XIII, and also fibronectin. Hypersensitivity to proteins and red cell hemolysis due to the presence of high titers of isoagglutinins anti-A and anti-B can occur; thus, administration of cryoprecipitate of the corresponding blood group is necessary. Cryoprecipitate, until recently the best preparation for the treatment of hemophilia, and fresh frozen plasma have gradually been replaced by safe concentrated preparations; still, a complete elimination of transfusion risks is probably unob-</td>
</tr>
</tbody>
</table>
tainable. Cryoprecipitate is not virally attenuated. Whole blood is used only in acute bleeding episodes.

Currently available concentrates include plasma-derived, intermediate-purity concentrates; plasma-derived, monoclonally-purified concentrates; and recombinant DNA-derived concentrates.

F VIII concentrate is prepared from large pools of plasma donated by numerous donors. Use of these preparations from 1970 to 1980 changed the quality of life for hemophiliacs, prolonging their lives significantly. In 1962, their average lifetime was 37 years while in 1985 their life expectancy was the same as the healthy population. Until 1990, the preparations presented a high risk because of virus transmission, hepatitis B and C, and HIV, in particular.

F VIII concentrates widely differ with regard to the contaminating protein content. Until the mid 1980s, the specific activity of concentrates (expressed in international units of F VIII per mg of total protein) varied from 0.5 to 5.0. In addition to F VIII, these intermediate-purity concentrates contained other plasma proteins such as immunoglobulins, fibrinogen, immuno-complexes and others. Proteins that lead to immunity disorders have since been removed (decrease of T4-lymphocytes in patients that were given high doses of these concentrates). Now most manufacturers produce purer concentrates, with specific activities ranging from 30 to 50 to 2,000 to 4,000 IU/mg. There are
basically two methods for F VIII concentrate purification. In one
an immunoaffinity chromatography with monoclonal antibod-
ies directed against F VIII of vWF is employed. In the other an
ion-exchange step, affinity or gel filtration chromatography af-
ther production of intermediate-purity concentrates, is added. As
a result, concentrates contain almost exclusively F VIII and vWF,
which co-purifies with F VIII and stabilizes this protein.

The purification process also removes a substantial amount
of the blood-borne infectious viruses that might otherwise have
contaminated the plasma pool. This removal, however, is usu-
ally not sufficient to eliminate the risk of infection. With the
thrust provided by the AIDS epidemic, commercial manufac-
turers have developed an array of virucidal methods that still
preserve the biological activity of such labile proteins as coagu-
lation factors.

F VIII concentrates are exposed to virus inactivation meth-
ods, so that virus-transmissible diseases have considerably been
reduced. Various methods for virus inactivation have been used
in F VIII preparation.

Methods for virus inactivation:
1. Dry heating, 80°C, 72 hours; dry heating, 60°C, 144 hours
2. Heating in solution (pasteurization) 60°C, 10 hours
3. Vapor heating (60°C, 10 hours, 1160 mbar)
4. Solvent/detergent (TNBP and TWEEN 80, Triton X-100, or
cholate)
5. Solvent/detergent plus dry heating  
6. Beta-propiolacton and UV-irradiation  
7. Monoclonal antibodies  
8. Recombinant technology - rDNA (genetic engineering)

Heat inactivates hepatitis B virus and HIV, but it does not inactivate hepatitis C. Solvent detergent virus inactivation method prevents hepatitis C transmission because it affects the virus C lipid coat and inactivates it. The new generation of F VIII concentrates, prepared using purification by monoclonal antibodies and genetic engineering, is rather impressive with regard to the exclusion of possible risks of virus transmission via concentrate. The proper choice of concentrate is necessary. Most authors think that HIV-positive patients should be treated with highly purified preparations that will not lead to immunity disorders in patients with already compromised immunity. In “virgin” patients, i.e. patients who have never been treated with replacement therapy, only preparations with the lowest risk of infectious disease transmission should be used (monoclonal or rDNA F VIII concentrate, or doubly inactivated F VIII concentrate).

Due to the possible risks of blood transfusion such as infectious diseases, in particular virus transmission, (hepatitis B, hepatitis C and HIV), an adequate choice of blood derivatives is needed. It is uncertain which is the best concentrate.

Biological characteristics of rF VIII are the same as the those of natural F VIII, but the purity of rF VIII preparation is much
higher compared with the purity of F VIII preparation obtained from human plasma,. The rF VIII end product is stabilized using human albumin and stored in a lyophilized form.

rF VIII and rF IX concentrates have the same biological characteristics as human concentrates. However, the purity of these preparations is considerably higher; and it can be prepared in large quantities. The risk of viral disease transmission has been greatly reduced since the first generation of recombinant preparations where human albumin was used as a stabilizer. The risks have not been completely eliminated, though. In second generation recombinant preparation, there were no protein stabilizers, but bovine serum was used in the cell culture medium. Now, in the third generation recombinant preparation, animal proteins are no longer used in the culture medium.

rF VIII half-life in patient’s circulation is around 16 hours ($T_{1/2}$ of F VIII obtained from human plasma in the same group of patients is around 14 hours). Laboratory investigation shows a similar degree of PTT shortening after the application of both preparations. The study of the therapeutic effects of rF VIII confirms the efficiency of this product during perioperative treatment of patients suffering from hemophilia A without side effects.

Whenever possible, replacement therapy should be substituted with deamino-D-arginine vasopressin (DDAVP). DDAVP (a synthetic antidiuretic hormone) has been used for the treat-
ment of moderate forms of hemophilia since 1977. DDAVP increases F VIII levels in circulation by 3 to 4 times. It can be administered intravenously, subcutaneously and intranasally. It can be used along with antifibrinolytic therapy.

**Side Effects of Hemophilia Treatment**

Due to the great progress in the prevention of infectious blood-borne viruses, careful donor selection, viral inactivation processes, and new fractionation procedures, the risk of complications has been drastically reduced (Table VI.7); however, it has not be completely eliminated. The possibility of viral disease transmission by blood derivatives still exists.

**Table VI.6:**

<table>
<thead>
<tr>
<th>Side Effects of Hemophilia Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood born viruses (HAV, HBV, HCV, AIDS, parvovirus)</td>
</tr>
<tr>
<td>Immuno modulation</td>
</tr>
<tr>
<td>Thromboembolic complication</td>
</tr>
<tr>
<td>Inhibitors</td>
</tr>
</tbody>
</table>

**Transfusion of Transmitted Infections**

Hepatitis A (HAV) can be transmitted by inadequately virus-inactivated concentrates. Vaccination of all newly diagnosed patients is recommended; otherwise, a double inactivation procedure should be performed.
Hepatitis B (HBV) – Most hemophilia patients have serological markers pointing to HBV infection. In most cases, the infection has a subclinical form with gained immunity, and a certain number of patients is a constant virus carrier. The best preventive measure available is vaccination performed right after diagnosis of hemophilia and treatment using rF VIII and rF IX.

Hepatitis C (HCV) – 60 to 80% of the adult hemophilia patients are infected with HCV. In 70 to 80% of the infected patients another consequence can be expected: liver cirrhosis or, in 30% of infected patients, hepatocellular cancer. Treatment with ribavirin and alpha interferon can be effective in around 40% of patients. Therapy requires improvement of liver function and prevention of liver cirrhosis and hepatocellular cancer development.

AIDS – The available preventive measures in HIV transmission have been successful, and HIV transmission by blood-derived products has not been reported in the past 15 years.

Parvovirus B 19 is a small DNA virus without a virus envelope, so far none of the available virus inactivation methods have been effective. Acute infections lead to febrile reactions, and chronic infections lead to aplastic anemia. Patients with compromised immune systems run a higher risk of contracting this disease.
Safety of Concentrates Nowadays

HIV has not been transmitted by a US-manufactured concentrate since early 1987. Transmission of HBV and HCV from concentrates has disappeared. Hepatitis A, a rare contaminant of blood, can be transmitted by concentrates treated with solvent-detergent. The B-19 parvovirus cannot be inactivated with any of the current viral-inactivation methods.

In recent years, advances in the treatment of hemophilia have been achieved: introduction of prophylaxis, continuous infusion and pharmacological treatment with DDAVP.

Elimination of viruses from blood derivatives can be performed using the polymerase chain reaction (PCR) technique for HAV, HBV, HCV, HIV, parvovirus B-19, and FFP and also through double inactivation of viruses in blood derived concentrates.

Immune Modulation

Repeated administration of large quantities of foreign proteins causes an immunomodulatory effect (immune suppression) in the recipient. The patient becomes more susceptible to immune deficiencies, bacterial infections and malignant diseases. This is why highly purified F VIII and F IX concentrates or recombinant preparations are recommended.
Thromboembolic Complications

All bypassing agents (PCCs, APCCs and rF VIIa) currently used for the treatment of patients with high-titer inhibitory antibodies carry the risk of thrombotic complications, including thromboembolism, DIC, and myocardial infarction.

Thrombotic problems most commonly occur in patients with underlying atherosclerotic disease and in those immobile for long periods. Premature infants and patients with pre-existing liver disease are particularly susceptible to DIC when treated with PCCs. Purified F IX concentrates have been shown to have a lesser effect on sensitive markers of coagulation than PCCs.

Use of F XI concentrates, particularly in high doses, has been associated with thrombosis. Elderly patients and those with a previous history of thrombosis or ishemic heart disease are particularly at risk.

The use of a vWF-containing concentrate for the treatment of vWD may result in a high plasma F VIII level, which is known to be a risk factor for thromboembolisms. Venous thromboembolisms have been reported following the use of concentrates containing vWF.
F VIII Inhibitors in Hemophilia Patients

Therapy complications in hemophilia patients could arise, e.g., allergic reactions (uncommon with highly purified concentrates), primary pulmonary hypertension, HIV infection and chronic hepatitis in patients who were exposed in the past to concentrates that had not been virally attuned (Fig VI.3).

![Figure VI.3: Summary of factors that may influence the risk of inhibitor formation in patients with hemophilia.](image)

One of the major complications in the treatment of hemophiliac patients is the development of inhibitors- antibodies directed against F VIII. These anticoagulants are IgG antibodies, usually IgG subclass 4, which develop in hemophilia A patients after exposure to transfused F VIII. The majority of the anti-
bodies react with a small segment of the amino-terminal portion of the A2 domain or with the carboxy-terminal of the C2 domain of F VIII. They occur in at least 15 percent of transfused patients who have a familial predisposition. The development of inhibitors is most common during the first 5 to 10 years of life and appears to be related to the number of days of exposure to transfused F VIII. Inhibitor development usually occurs within the first 20 days of exposure.

The inhibitor level may be quantified by the Bethesda assay in which patient plasma, both undiluted and diluted with imidazole buffer, is mixed with normal plasma, incubated for 2 hours, and then the F VIII level is compared to the F VIII level of normal plasma diluted with buffer alone. One Bethesda unit/ml (BU) is the amount of inhibitor that decreases the residual F VIII activity from 100% to 50%.

“Low responders” are patients who have 0.6 to 5 BU inhibitors and do not have anamnestic responses after treatment with F VIII. “High responders” are patients who exhibit anamnestic responses. They may have very high titer inhibitors (1000 BU).

It is controversial whether the likelihood of inhibitor formation is related to the purity of transfused F VIII. Incidence of persistent inhibitors was reported in clinical trials with recombinant F VIII, monoclonally purified F VIII and intermediate purity F VIII concentrates (Fig VI.4).
Exogenous infused F VIII binds to antigen presenting cells (APC) (Step 1). After endocytosis, oligopeptide are formed by proteolytic cleavage (Step 2). These peptides bind to the major histocompatibility complex (MHC) class II molecules, having the correct recognition sequence. The MHC - F VIII complexes are then transferred to the cell membrane and presented to the T-cell receptors (TCR) on the CD4+ T\textsubscript{H} lymphocytes (Step 3). Co-stimulatory signals have to be provided by the binding of B7.1 (CD80) / B7.2 (CD86) to CD28 (Step 4a) in order to fully activate the T\textsubscript{H} lymphocytes and stimulate the release of cytokines (Step 5). The subsequent binding of these cytokines to the corresponding receptors (CK-R) up-regulates immune response genes and co-stimulatory molecules on the cell surfaces of both B and T-cells.
(Step 6). The enhanced action of cytokines and co-stimulatory molecules, including the interaction between CD40 and CD40L, induces B cell proliferation, differentiation and F VIII antibody production (Step 7). The activation of the T_\text{H}_1\text{ cells is down-regulated by the competitive binding of cytotoxic T-lymphocyte anti-gen 4 (CTLA4) to the B molecules on the APC (Step 4b).}

Treatment for hemophilia A patients who have developed inhibitors depends on the titer. Low-titer inhibitors can be saturated with the administration of large amounts of F VIII. This is not usually feasible when the inhibitor level is more then 10 Bethesda units (BU) per ml of plasma. In that case, one method is to administer either F IX complex or a specially activated F IX complex, termed anti-inhibitor coagulant complex, to “bypass” F VIII. These concentrates are effective in 50% to 60% of the cases, but thromboembolic complications have occurred in some patients treated in this way.

If a hemophilic patient has developed inhibitors, treatment should include the following:
- high doses of human F VIII concentrate
- F VIII of animal origin, porcine F VIII (Advanced reactions include thrombocytopenia, hypersensitivity, and the development of inhibitor antibodies against porcine F VIII.)
- prothrombin complex concentrates (PCC), factor eight bypassing activity (FEIBA), Autoplex
- rF VIIa, which is able to activate F X to F Xa independent of F VIII and F IX activity, and it is efficient in the treatment
of hemophilia A, hemophilia B, acquired hemophilia, F XI
deficiency and vWD

- plasma replacement, plasmapheresis and affinity chroma-
  graphy, which remove IgG using protein A Sepharose and
can transiently lower the inhibitor level to allow transfusion
  therapy with F VIII
- immunosuppressive therapy
- immunotolerance inductor – continuous intravenous ad-
  ministration of F VIII/F IX from one week to one month
  in varying doses and frequencies. Highly purified F VIII
  monoclonal concentrate or rF VIII concentrate are used.
  Administration should be initiated in the first year the
  inhibitors appear. If therapy proves to be ineffective, it
  should be interrupted after two years. The failure rate is
  only 5%.

Patients with very low inhibitor titers can be treated for hemor-
rhage in the same way as non-inhibitor patients, by substitution
of the deficient clotting factor, although much higher doses are
required. Sufficient clotting factor must be given to neutralize
circulating inhibitors and be followed by more to obtain the
desired hemostatic effect. Attempts to reduce the amount of
clotting factor concentrate required using a continuous infusion
have sometimes proved successful (Gordon et al. 1994).

The immune system of inhibitor hemophilia A patients will
sometimes accept porcine F VIII, but this has been abandoned
because of side effects that include thrombocytopenia and aller-
gic reactions. These problems persist despite improved methods of purification.

The most widely used therapeutic alternative for inhibitor patients is probably PCC, usually as APCC. PCC is effective in about 50% of the treatment episodes. Moreover, serious side effects, including myocardial infarction and DIC, have been reported in patients receiving frequent high doses of PCC or APPC.

Intensive plasmapheresis, preferably combined with immune adsorption, may lower inhibitor titers enough to allow successful replacement of the missing factor. However, if the initial inhibitor titer is high, this technique is too time-consuming to be used in life-threatening hemorrhages. Moreover, there is always an anamnestic response with this treatment, and after 5 to 7 days, it may be impossible to keep inhibitor levels low enough.

Clinical trials suggest that rF VII is highly effective at restoring hemostasis in inhibitor patients (Birch et al. 1992).

The ultimate goal in the treatment of inhibitor patients is to eliminate inhibitors. Hemophilia patients with inhibitors are more difficult to treat than those without. The bleeding tendency of patients with acquired hemophilia would disappear altogether if their inhibitors could be completely removed.

Patients with congenital hemophilia do not usually respond to the above treatments. Instead, in these patients,
immune tolerance induction (ITI) with frequent injections of the deficient clotting factors (i.e., antigen) is the most widely accepted technique (Table VI.8). Higher and daily doses of clotting factor make the treatment more successful (Mariani et al. 1994). Sometimes the regimen is initiated with a short course of cyclophosphamide and intravenous gammaglobulin although it is not certain whether this increases the treatment success rate.

Table VI.7: ITI success depends on dosage of F VIII and immunosuppressive therapy

<table>
<thead>
<tr>
<th>Protocol</th>
<th>F VIII Dose</th>
<th>Per Month</th>
<th>Success Rate in %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bonn (-)</td>
<td>200-300 U/kg/daily</td>
<td>15.0</td>
<td>73</td>
</tr>
<tr>
<td>Malmo (+)</td>
<td>200 U/kg/daily</td>
<td>1.3</td>
<td>80</td>
</tr>
<tr>
<td>Dutch (-)</td>
<td>25 U/kg three times a week</td>
<td>11.5</td>
<td>83</td>
</tr>
<tr>
<td>Kasper (+/-)</td>
<td>50 U/kg/daily</td>
<td>3.0</td>
<td>73</td>
</tr>
</tbody>
</table>

(+ immunosuppression) Nigel S. Key: Brit J of Haem 2004

Inhibitors in Non-hemophilic Patients

Spontaneous F VIII inhibitors may appear in patients who do not have hemophilia. This occurs idiopathically in older adults, pregnant and postpartum women, and patients with immunological disorders such as systemic lupus erythematosus and rheumatoid arthritis.
Acquired hemophilia is a condition in which previously hemostatically normal persons develop inhibitors, autoantibodies rather than alloantibodies. This is a rare disease, though, with one new case per million people occurring per year. Such individuals suddenly develop a bleeding tendency, although the usual hemarthroses are rare. Vast subcutaneous, intramuscular and retroperitoneal hemorrhages are typical. Acquired hemophilia is fatal in 10% of the cases each year (Green and Lechner, 1981).

Underlying causes of acquired hemophilia can often be identified, since around 20% of these individuals have an autoimmune or systemic connective tissue disorder, 7% a malignant disease, while 6% are on some kind of drug therapy. Another 7% of patients who develop acquired hemophilia are postpartum. In these women, alloantibodies could be involved. In exceptional cases, though, chronic dermatological, respiratory and gastrointestinal diseases may be the underlying cause of acquired hemophilia. In about 50% of these cases, however, an underlying cause cannot be identified (Green and Lechner, 1981).

Inhibitors usually disappear spontaneously from the plasma of women who acquired hemophilia postpartum. Patients with acquired hemophilia respond to corticosteroids, cyclophosphamide, or a combination of the two (Green et al. 1993). High doses of intravenous gamma globulin have also been effective in a limited number of cases (Sultan et al. 1984). Transfusion
therapy to achieve hemostasis is identical to the treatment of hemophiliacs with inhibitors.

Gene Therapy for Hemophilia

Hemophilia has become a major target for gene therapy for several reasons.
- It is a single-gene disorder.
- Although replacement treatments are effective, they are expensive, and the potential risk of disease transmission by plasma-derived products remains.
- Even with prophylactic regimens, replacement therapy must be administered intravenously at frequent intervals.
- Tight regulation of expression is probably unnecessary, and tissue-specific expression is probably important.

With the present technology, gene therapy is unlikely to be able to provide a total “cure” for hemophilia. Current technology is not sufficiently advanced to affect a life-long gene transfer that ensures continuous production of coagulation factors at normal levels. A broadly accepted and feasible goal for many scientists working in this field is to achieve a plasma level of 5 to 10 U/dl of the relevant coagulation factor using a simply administered gene treatment every few months. This level could protect against spontaneous bleeding and provide a substantial lifestyle improvement. In essence, this method would achieve prophylaxis through endogenous factor production without regular infusions of concentrate (Fig. VI.5).
In the field of hemophilia, ex vivo and in vivo methods of transfection using retroviruses, adenoviruses and direct gene transfer have been reported. Many studies have been performed using factor IX cDNA. This is because the large size of the factor VIII gene places constraints upon transfer by available vector systems.

Studies of factor VIII were carried out using smaller, partially deleted genes that do not contain the large internal B
domain of the protein. This reduces the size of the factor VIII cDNA by approximately 30%. In this truncated form, factor VIII can be inserted into retroviral vectors. B-domain-deleted factor VIII remains fully functional in vivo because the B domain is not required for coagulant activity. Clinical data using B-domain-deleted recombinant factor VIII concentrate in previously treated hemophilic patients have shown that the deleted protein is clinically effective. Besides, factor VIII is less stable than factor IX. For these reasons, hemophilia B has proven to be a more popular model for gene therapy studies than hemophilia A.

These days around 80% of the world population is without effective hemophilia therapy. Severe hemophilia remains a crippling and fatal disorder in many areas. Even with the increasing availability of factor concentrates, present approaches using standard replacement therapy are impracticable in many areas of the third world, not least because of the population size and costs. Gene therapy, if available as an in vivo modification treatment simply administered, offers a real practicable alternative therapy. Whether all concerns about safety should be neglected in these areas of the world in order to provide some form of treatment for an otherwise fatal disorder is a debatable ethical issue.

**Prenatal Diagnosis of Hemophilia**

The best way to provide genetic counselling is through national confidential databases of mutations and pedigrees (Fig. VI.6). The preliminary step to prenatal diagnosis is carrier assessment.
Women with a family history of hemophilia can be potential or obligate carriers.

Figure VI.6: Inheritance in hemophilia. Peyvandi F et al Haemophilia 2006; p3.
By definition, an obligate carrier is a woman who fulfills one or more of the following criteria:
- Her father has hemophilia.
- She has given birth to more than one hemophiliac son (identical twins excluded) or one hemophiliac son and a daughter who has a hemophiliac son.
- She has given birth to a hemophiliac son and there are hemophiliac men in the maternal line of the pedigree.

An obligate carrier is somebody whose genetic probability of carriership is 1.0 and who, thus, requires no further carriership analysis. If more than one hemophiliac exists in the pedigree, the case is familial. If the hemophiliac is the only known case in the family, the case is considered sporadic. A sporadic case of hemophilia can be a new mutation in the mother or a new mutation in the hemophiliac herself.

Between the 9th and 11th week of gestation, a sample of chorion villus can be taken to analyze DNA in order to determine if the woman is a carrier and to diagnosis hemophilia as well. The next step after carrier assessment is determination of fetal sex in the 16th gestational week using ultrasonography or analyzing the cells in the amniotic fluid. During gestational weeks 18 to 22, fetal blood is sampled from male fetuses for analysis of F VIII. Normal values are: Daffos 35+/-13 IU/dl and Mibashan 45+/-12 IU/dl. Fetal blood samples are obtained by cordocentesis and supervised with ultrasound.
Prenatal diagnosis of hemophilia A and B is possible by performing a chorionic villus biopsy, which traces the mutation or informative genetic markers during the first trimester. If possible, direct gene analysis of the mutation is preferred. The starting point with hemophilia A is to ascertain if the disease is due to inversion of the X-chromosome, which is the case in almost half of the severe cases. In hemophilia B, most families carry a unique mutation that needs to be characterized.

In the near future, much of the prenatal diagnosis will be based on indirect genetic markers and repetition of polymorphisms, F VIII and IX genes. Currently, chorionic villus sampling is the most widely used method, but amniotic fluid, fetal blood and pre-implantation genetic diagnostics can also be used in selected cases. Prenatal diagnosis must be preceded by adequate genetic counseling, with risk assessment of the potential carrier and with subsequent support during the diagnostic process.

New methods are based on genetic investigation, such as determination of the length of the restrictive fragment of the gene polymorphism and PCR.

**Surgical Procedures in Hemophilia Patients**

Surgical procedures in hemophiliacs should only be performed in hospitals with specialized laboratories for routine coagulation factor investigation and where continuous consultation with hematologists and transfusion medicine specialists responsible
for the treatment of hemophiliacs is possible. Surgeon and anesthesiologist must be aware of the specific requirements of such a surgical procedure, and sufficient quantities of matching blood derivatives must be readily available.

F VIII level and presence of inhibitors should be determined prior to a surgical procedure. About one hour prior to the procedure, patients should be transfused with F VIII, dose of 40 to 60 U/kg b.w., in order to reach a hemostatic level of 70 to 100%, depending on the extent of the procedure. Following the procedure, patients are transfused with 20 to 30 U/kg twice a day for 7 to 10 days. F VIII level is monitored before, after, and for the next 7 days following the operation.

**Hemophilia B**

Hemophilia B is a consequence of F IX activity disorder and is inherited the same way as hemophilia A. Hemophilia B is mostly due to small changes in the F IX gene, affecting either its transcription, mRNA maturation, mRNA translation or the fine structure of F IX. Only 2 to 3% of the patients show cross deletions or rearrangements. The great variety of missense mutations reported to cause hemophilia B indicates that this multidomain protein is highly constrained.

It is well known that F IX molecular mass is $55 \times 10^3$ daltons. It is a single-stranded protein exposed to vitamin K-dependent
gamma carboxylation (necessary for the expression of F IX biological activity). The F IX gene contains eight exons mutually divided by corresponding introns that form a considerable part of the gene. The kDNK nucleotide sequence for F IX shows that F IX is synthesized as “pre-protein” with the leading peptide consisting of 46 amino acids. In the course of protein maturation, this peptide is removed from F IX structure. Total gene weight is around 34 kb, and mRNK for F IX consists of 2.8 kb. Using kDNK probes, gene mapping for F IX has been performed. It was concluded that it is located on the distal part of the long side of X chromosome. Despite the fact that F IX was the first factor to be cloned in 1982, rF IX preparations are not in routine therapeutic use, yet. Sufficient supply of concentrated F IX for the treatment of hemophilia B patients is not available, and there is a relatively low yield of F IX using the rDNK method.

Table VI.8:

<table>
<thead>
<tr>
<th>Blood component and treatment in hemophilia B</th>
</tr>
</thead>
<tbody>
<tr>
<td>F IX concentrate</td>
</tr>
<tr>
<td>F IX complex</td>
</tr>
<tr>
<td>F IX recombinant</td>
</tr>
</tbody>
</table>

Clinical Manifestations

Clinical symptoms are indistinguishable from symptoms found in hemophilia A; however, bleeding episodes caused by injury are far more intense in hemophilia B patients, and the risk of surgical pro-
The frequency of hemophilia B is four times lower than hemophilia A. Diagnosis is made according to prolonged PTT and F IX activity determination. Because of the T_{1/2} of F IX (15-30 hrs), patients are transfused once a day. For surgical procedure preparation, a 100% level of F IX should be reached in time for the operation. This can be achieved by administering 50 to 60 IU of F IX/kg b.w. The maintenance dose is two times lower and is administered once a day until the wound has completely healed. During surgical procedures and postoperatively, it is necessary to watch for effects of the transfusion and possible complications.

**Treatment and Side Effects**

Two types of F IX concentrates are available today.

1. F IX complex (also known as PCC), which contains significant amounts of other vitamin K-dependent proteins, including factors II, VII, and X and PC and PS
2. Coagulation factor IX, a generic name for preparations substantially free of these other proteins (Table VI.8)

F IX complex (F VII, F II, F X, F IX), the first factor IX-rich concentrate used for the treatment of hemophilia B, was developed in France over 30 years ago. The most important development in the production of PCCs was the introduction of ion-exchange chromatography using DEAE-cellulose or DEAE-Sephadex. This was one of the first major uses of chromatography in plasma fractionation. F IX concentrate is exposed to virus inactivation methods just like F VIII concentrate.
Possible complications in the treatment of hemophilia B using concentrated F IX can be caused by prothrombin activation, resulting in thromboembolic complications or DIC. Thus, F IX complex administration should be combined with the administration of 5 to 10 IU of heparin in each milliliter of dissolved concentrate (heparin is sometimes contained in the concentrate). Accelerated fibrinolysis is a possible complication. Virus inactivated F IX concentrate is being administered more and more frequently. Highly purified F IX concentrates have also been prepared by immunoaffinity chromatography using monoclonal antibodies to F IX. Clinical studies with one of these preparations have shown it to be effective in stopping bleeding episodes. Proof of the absence of thrombogenicity includes the measurement of prothrombin fragment F 1+2 levels as markers for activation of the clotting system as well as clinical observations.

Considerable progress has been made in the past few years in the field of gene therapy (gene transaction) and embryo manipulation in experimental animal studies. Namely, procedures for the production of human plasma proteins using large transgenic animals (the required recombinant protein is found in the animals' milk) have been developed with much higher yield. One liter of their milk contains 1 to 10 g of this protein while in the supernatant liquid of the cell culture system only 0.1 g/l of recombinant protein is found. Besides, proteins formed in transgenic animals are more easily exposed to posttranslation modifications required for obtaining bioactivity of recombinant molecules.
von Willebrand’s Disease (vWD)

In 1926, Dr. Eric von Willebrand of Helsinki published, in Finnish, his astute observations on a large family from one of the Aland Islands on the gulf between Sweden and Finland with a bleeding disorder.

vWD is an autosomally inherited congenital bleeding disorder in which there is a deficiency of dysfunction of von Willebrand factor. vWF is a large multimeric glycoprotein made in the endothelial cells and in megakaryocytes; it is present in plasma and platelets. vWF has two functions:
1) It attaches to subendothelial collagen and to platelets, promoting information for a platelet plug at the site of small blood vessel injury.
2) It binds and transports F VIII.

von Willebrand’s disease is the most commonly inherited bleeding disorder and is the result of quantitative and qualitative disorders of von Willebrand factor. In contrast to hemophilia, it is inherited through the autosomal chromosome; thus, males and females are affected. Most patients are heterozygous, suffering from the mild or moderate form of the disease.

The vWD gene is located on chromosome 12, occupying 178 kb in human genome. It is interrupted by 51 introns (Fig. VI.7). A pseudo gene, or a gene duplicate, has recently been identified on chromosome 22 with domains that are 98% identical to the
authentic gene. This gene located on chromosome 22 has the same phenotype expression as the original gene. Genetic investigations show that a defect in another locus, outside vWF gene, can result in a phenotype similar to vWD phenotype. vWD may be caused by various genetic disorders. Gene deletion is not a frequent cause of the disorder; it much more frequently occurs as a consequence of gene mutation, particularly point mutation. The pathological gene is inherited as an autosomal dominant gene; in contrast, type III is inherited through a recessive gene and presents the most severe form of the disease in the homozygous.

![vWF gene diagram](Mayo Clinic Proc, May 1991, Vol 86)
vWF is synthesized in endothelial cells and megakaryocytes. It is found in plasma, subendothelium (Weibel-Palade bodies), and platelets (α granules). Stored endothelial vWF is released after stimulation by insulin, epinephrine, vasopressin, or the modified form of vasopressin, DDAVP. vWD is the main adhesive protein and is needed for the adhesion of platelets on subendothelium. Furthermore, it serves as a carrier and as a protective protein for F VIII. The vWF molecule presents several functional domains on its surface for binding to F VIII, collagen, and GP on the platelet membrane. The specific GP on platelet membrane for vWF binding is GPIb, which takes part in primary platelet adhesion. vWF is also bound to GPIIb IIIa, which is a specific receptor for fibrinogen, fibrinectin and thrombomodulin, through which vWF participates in platelet aggregation. In circulation, vWF is noncovalently bound to F VIII. It serves as a transport protein for F VIII, protecting it from degradation and fast elimination. F VIII bound to vWF is resistant to the effect of PC (Table VI.9).

Table VI.9: Recommended Nomenclature of Factor VIII/vWF

<table>
<thead>
<tr>
<th>Factor VIII</th>
<th>von Willebrand factor</th>
</tr>
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<tbody>
<tr>
<td>Protein</td>
<td>VIII</td>
</tr>
<tr>
<td>Antigen</td>
<td>VIII:Ag</td>
</tr>
<tr>
<td>Function</td>
<td>VIII:C</td>
</tr>
</tbody>
</table>

Isolation of von Willebrand’s factor proved that vWF protein consists of a series of polymers of various sizes circulating in plasma as a set. Some of these polymers have a very high mo-
lecular weight, and they seem to be of considerable significance for the maintenance of hemostasis. Only high molecular weight vWF multimers take part in platelet adhesion, but all multimers, regardless of molecular weight, are bound to F VIII. vWF purified from plasma has a molecular weight ranging from 500,000 to 20 million, depending on the degree of multimerization.

Clinical Manifestations

Clinical manifestations of the disease are spontaneous bleeding from mucous membrane, epistaxis, gingival bleeding, menorrhagia, gastrointestinal bleeding, and after surgical procedures, in particular tonsillectomy and tooth extraction, posttraumatic bleeding and postpartum bleeding. Type III is characterized by far more severe forms of bleeding such as metrorrhage, gastrointestinal tract bleeding, and bleeding into joints (hemarthrosis) and muscles.

Diagnosis of vWD

Sometimes it is difficult to diagnose vWD because of the varying results obtained over time in the individual. Moreover, the level of vWF is influenced by age and blood group (patients with type AB blood have significantly higher levels than those with type O). vWF is an acute-phase reactant and is increased by pregnancy, estrogen therapy, recent exercise, hyperthyroidism, uremia and liver disease (Table VI.10).
Many diseases influence vWF. High levels are found in liver diseases, uremias, coronary arterial diseases and diabetes. Major surgeries are also associated with an increase of F VIII activity. In a patient, vWF levels are variable over time and also vary among members of the same kindred.

Significant alterations of F VIII were noted with stress, during infusion of epinephrine and vasopressin, and during
physical exercise. This activity induced by stimuli is short-termed, maximum 24 hours. There is clear evidence that in those situations F VIII is released from vascular endothelium.

Profound investigation of all functions has led to significant and valid diagnostic criteria for vWD. Once these criteria were introduced in routine laboratory practice, it appeared that problems related to vWD diagnostics were solved. For the diagnosis of vWD, an array of tests is usually required, especially in mild forms of disease.

**Diagnositc Tests for vWD**

- **Bleeding time** is best measured with the Ivy template method, which is more sensitive than the Duke bleeding time. The bleeding time is a useful complement to the diagnosis, even if its prolongation does not invariably predict a more severe bleeding tendency.

- **Activated partial thromboplastin time** is sensitive only to significantly reduced factor VIII levels.

- **Factor VIII coagulant activity** (F VIII:C) assay may be normal or only slightly reduced in the variant forms, apart from type 2 Normandy subtype; however, in classical deficiency it is low.

- **Ristocetin-induced platelet aggregation** (RIPA) is platelet aggregation in fresh, platelet-rich, patient plasma induced by high and low doses of ristocetin and is useful in screening for variants of vWF. Increased aggregation occurs at a very low ristocetin level such as type 2B and platelet type
INHERITED BLOOD CLOTTING DISORDERS

vWD. These patients usually have thrombocytopenia after administration of DDAVP.

- **Ristocetin cofactor activity (vWF:RCo)** The most sensitive test for identifying patients with vWD is vWF:RCo. It tests the ability of vWF to bind GPIb on platelet membranes. The activity of plasma vWF in promoting the aggregation of fixed or washed platelets is measured in response to the antibiotic ristocetin, which induces aggregation of platelets through GPIb. Ristocetin cofactor is responsible for prolonged bleeding time and absence of platelet adhesion. Its presence is demonstrated by platelet agglutination with ristocetin.

- **von Willebrand factor antigen, vWF:Ag (Laurell),** has a lower sensitivity but is useful during the diagnostic work-up since it helps to identify patients with dysfunctional vWF, that is, patients with normal or slightly reduced vWF:Ag and low vWF:RCo. Moreover, its lower sensitivity can be improved by repeating this assay on different occasions. The presence of vWF proteins is determined by immunological tests (rocket electrophoresis according to Laurell or radioimmunoassay - IRMA), which are rather sensitive.

- **Multimeric analysis** enables determination of the amount and size distribution of the vWF multimers through gel electrophoresis and immunostaining using anti-vWF antibodies. It also allows a broad distinction to be made between type 1 (all multimers present at reduced concentration) and type 2 (large multimers absent), even though patients with type 2N and some with type 2M have a normal multimer pattern.
Patients with type 3 have no or minimal detectable traces of vWF.

Type 2B vWD should be differentiated from platelet-type (pseudo) vWD. In this disorder RIPA occurs at low ristocetin levels, the largest vWF multimers are absent, and desmopressin-induced thrombocytopenia is present. The defect in platelet-type vWD involves GPIb on platelet membrane whereas in type 2B vWD the defect involves the vWF molecule. Normal plasma (vWF) aggregates platelets from patients with platelet-type vWD but not type 2B platelets. Furthermore, plasma vWF from patients with platelet-type vWD, unlike type 2B vWD, does not have increased binding affinity for normal platelets in the presence of ristocetin.

There are certain variations related to the disorders described. Platelet agglutination with ristocetin can be decreased in primary platelet disorders while in vWD it can remain normal, in pregnancy for instance. If one takes the varying clinical expressions of the disease and the laboratory findings in various patients with vWD or laboratory findings in one patient done at different time intervals into consideration, it is obvious why there have been numerous attempts to classify the types of this disease.

Classification of vWD

Classification of vWD has been done according to the results of immunological investigations and study of the vWF structure. Various expressions of the disease in certain types of vWD have
been explained by different combinations of vWF polymers that carry various biochemical activities. In vWD type I, the vWF structure is normal. Investigation of vWD type III is impossible because of the absence of molecules. Homozygous type III is found in a rather small number of patients. The majority of other types shows an absence of large polymers and fast anode migration followed by cross-electrophoresis. Further investigation using SDS polyacrylamide electrophoresis, otherwise used in the identification of abnormal polymer distribution in vWD, enabled classification of vWD types.

According to one classification, von Willebrand’s disease can be classified in five types (Ruggeri and Zimmerman) (Tables VI.11 & 12).

### Table VI.11: Classification of vWD by Sadler

<table>
<thead>
<tr>
<th>New (Sadler)</th>
<th>Old (Ruggeri &amp; Zimmerman)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>I platelet normal, I platelet low, IA, I-1, I-2, I-3</td>
</tr>
<tr>
<td>2A</td>
<td>IIA, IB, I platelet discordant, IIC, IID, IIE, IIF, IIG, IIH, III, IIA-1, IIA-2, IIA-3</td>
</tr>
<tr>
<td>2B</td>
<td>IIB, I New York / Malmoe</td>
</tr>
<tr>
<td>2M</td>
<td>B, Vicenza, IC, ID</td>
</tr>
<tr>
<td>2N</td>
<td>Normandy, defective binding to factor VIII</td>
</tr>
<tr>
<td>3</td>
<td>III</td>
</tr>
</tbody>
</table>

*Type I (70% of cases) probably results from a mutation in the regulatory genes responsible for the release of vWF. Decreased*
production of a normal vWF protein shows altered laboratory characteristics.

*Type IIA* (20% of cases) is a qualitative disorder caused by the loss of the high molecular weight multimers.

*Type IIB* (5% of cases) is a qualitative disorder resulting in increased affinity of vWF to GPIb, absence of high molecular weight multimers in the plasma, and increased RIPA. Thrombocytopenia occurs due to in vivo platelet aggregation; desmopressin may worsen thrombocytopenias. Clinical manifestations of the disease are spontaneous bleedings from mucous membranes, especially of the nose and gingiva; bleedings after surgical procedures, particularly in tonsillectomy and tooth extraction; and characteristic for types I and II.

*Type III* is a consequence of the absence of vWF synthesis and is the most severe form. Type III is characterized by far more severe forms of bleeding such as menorrhage, gastrointestinal tract bleeding and bleeding into joints and muscles. It is easily diagnosed since all laboratory characteristics are pathological.

*Platelet type* von Willebrand’s disease is caused by mutation in platelet GPIb resulting in increased binding of normal vWF to GPIb.

*Normandy* variant of von Willebrand’s disease occurs due to point mutations of vWF in the amino terminal portion, which is the binding site for F VIII:C.
### Table VI.12: The 1994 Classifications of vWD Subtypes by the ISTH

<table>
<thead>
<tr>
<th></th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Type I</td>
<td>Plasma contains multimers of all sizes; quantity decreased</td>
<td>Type 1</td>
<td>Partial quantitative deficiency of vWF</td>
<td>Dominant. Variable expression, phenotype influenced by multiple genes</td>
</tr>
<tr>
<td>Type II</td>
<td>Largest multimers absent from plasma</td>
<td>Type 2</td>
<td>Qualitative defect of vWF</td>
<td>(subtypes below)</td>
</tr>
<tr>
<td>Type IIA</td>
<td>Largest multimers absent from platelets and plasma, even after stimulation with DDAVP</td>
<td>Type 2A</td>
<td>Decreased platelet-dependent function with absence of largest multimers</td>
<td>Dominant. Some mutations, large multimers do not form. With others, vWF is vulnerable to rapid proteolysis</td>
</tr>
<tr>
<td>Type IIB</td>
<td>Largest multimers present in platelets; they appear in plasma after stimulation with DDAVP</td>
<td>Type 2B</td>
<td>Increased vWF affinity for platelet GPIb</td>
<td>Dominant. May be associated with thrombocytopenia, especially after DDAVP</td>
</tr>
<tr>
<td>Type III</td>
<td>No multimers (severe vWD)</td>
<td>Type 2M</td>
<td>Decreased platelet-dependent function with presence of largest multimers</td>
<td>Dominant. In the “Vicenza” variant, extra-large multimers are present</td>
</tr>
<tr>
<td>Type IIIB</td>
<td>No multimers (severe vWD)</td>
<td>Type 2N</td>
<td>Decreased vWF affinity for F VIII</td>
<td>Recessive. Often mistaken for mild-moderate hemophilia A.</td>
</tr>
<tr>
<td>Platelet-type vWD</td>
<td>Largest multimers absent from plasma</td>
<td>(pseudo vWD)</td>
<td>No defect of vWF; not to be considered a form of vWD</td>
<td>Dominant. A platelet disorder: increased affinity of platelet GPIb for vWF. Thrombocytopenia may be present</td>
</tr>
</tbody>
</table>
Several classifications of vWD have been used in the past. Recently, the vWF Subcommittee of the ISTH (International Society on Thrombosis and Hemostasis) formulated a new classification (Sadler), essentially based on a revisitation of the previous classification by Ruggeri and Zimmerman. Three main types of vWD have been identified.

Type 1 with classical autosomal dominant inheritance, accounting for about 70 to 80% of cases, is characterized by equally low amounts of factor VIII and vWF with no evidence of structural abnormality on multimeric analysis.

Type 2 is usually characterized by the lack of large multimers and abnormally low levels of vWF:RCo in comparison with vWF:Ag. In this group several subtypes, sometimes with recessive inheritance, have previously been recognized on the basis of subtle differences of the inner structure of the multimers on high-resolution gels (A-I).

The new classification identifies the type 2 phenotypes as:
A - patients with the absence of high molecular weight vWF multimers and decreased platelet-dependent function,
B - patients with hyperresponsiveness to ristocetin,
M- patients with qualitative variants with platelet-dependent function but with high molecular weight multimers present, and
N - patients with defective factor VIII-vWF binding, including Normandy subtype.
Type 3 is recessively transmitted, and patients have very low or undetectable levels in all VIII/vWF measurements. There are very few of these patients (about 1 in $10^6$ of the population), and they are affected by moderate to severe bleeding diathesis.

In the past few years, considerable progress has been made in diagnosis and differential diagnosis of vWD. However, many aspects of the disease still need to be investigated, particularly with regard to pathophysiology, classification, laboratory diagnosis and treatment.

What is vWD in the year 2006? (Casper K)

In 1984 vWD was classified in three types based on multimers.
- Type I reduced multimers but all sizes are present.
- Type II no large multimers.
- Type III no multimers at all (severe vWD)

The 1994 definition demanded a mutation on the vWF gene.
- Type 1 as a quantitative deficiency
- Type 2 as qualitative deficiency
- Type 3 as severe
- Platelet-type was to be called pseudo vWD

In 2006, in recognition of the sparse availability of genotyping and complexity of causation of phenotype type 1 vWD, the requirement that there be a mutation in the vWF gene was dropped (Table VI.13).
Accquired vWD

vWD syndrome may appear in patients with hypothyroidism, autoimmune disorders, lymphoma, macroglobulinemia or other similar conditions. vWD may appear before the underlying disorder. It may be difficult to demonstrate that the vWD is acquired and not congenital. Attempts to demonstrate an antibody to vWF are successful only in a few instances. The plasma levels of the vWF propeptide are typically higher than normal. Propeptide levels are assayed in reference laboratories.

vWD syndrome with a type 2A phenotype has been described in people with aortic stenosis; this is due, presumably, to consumption of HMW multimers in the churning blood at the valve. Excessive bleeding from gastro-intestinal angiodysplasia is commonly reported in older patients. The syndrome, including gastrointestinal bleeding, disappears after valve replacement.

Table VI.13

<table>
<thead>
<tr>
<th>Classification in 2006</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Type 1</td>
<td>is a partial quantitative deficiency of vWF; it is able to react normally with platelet receptors and to attach to F VIII. Types 1 are said to be generally capable of satisfactory DDAVP response.</td>
</tr>
<tr>
<td>Type 2</td>
<td>is qualitative deficiency. vWF does not react normally with one or more of its platelets or F VIII receptors.</td>
</tr>
<tr>
<td>Type 3</td>
<td>is a virtually complete deficiency of vWF.</td>
</tr>
</tbody>
</table>
Treatment of vWD

The main objective in the treatment of vWD is the correction of bleeding time and the increase of F VIII level.

Treatment for vWD type 1 requires administration of DDAVP and contraceptive drugs. In type 2B, DDAVP is contraindicated. vWF and FVIII concentrate should be administered in all other types of vWD. Recombinant or monoclonal F VIII is not administered in the treatment of vWD.

Patients with vWD have been treated with plasma, cryoprecipitate, and F VIII concentrates containing variable amounts of vWF. It is known that lyophilized F VIII concentrates are usually deficient in the higher molecular weight, multimeric forms of vWF. As early as 1984, Nilsson recognized that intermediate and high purity concentrates of F VIII that lacked these multimers did not correct the vWD hemostatic defect.

Replacement therapy is seldom required in the treatment of spontaneous bleedings in types 1 and 2. However, it is necessary in the preparation of patients for surgical procedures. Correction of bleeding time is particularly significant for surgical procedures, so patients must be transfused in frequent time intervals, i.e., in three-hour intervals. Replacement therapy in patients with vWD type 3 is necessary both in spontaneous bleeding episodes and in the preparation for surgical procedures.
In the majority of vWD patients, F VIII:C and vWF can be corrected by administering synthetic DDAVP. Desmopressin can be administrated as an intravenous solution, as intranasal spray or subcutaneously.

DDAVP is efficient in the treatment of patients with vWD type 1 since it stimulates vWF release from blood vessel endothelium and platelets. Patients with type 3 and type 2B do not respond to desmopressin treatment (20% of patients). These patients require application of substitution therapy with F VIII/vWF concentrate.

The required dose of F VIII concentrate in spontaneous or posttraumatic bleeding episodes is one infusion of 20 to 40 IU/kg; this is usually sufficient. With tooth extraction the dose is the same, but antifibrinolytic therapy is necessary for the following 7 days. In preparation for surgical procedures,
F VIII concentrate is used to obtain a hemostatic level higher than 50% in the first week to 10 days.

**von Willebrand’s Disease in Pregnancy**

vWF increases during pregnancy, especially in the third trimester, so that bleeding episodes seldom occur in vWD patients during pregnancy. Patients with the classical type 1 vWD with the increased vWF during pregnancy, usually do not require any treatment. If there is a bleeding episode in the history of pregnancy, however, desmopressin should be administered immediately after delivery with the next dose administered 24h later. In patients with types 2A and 3, F VIII/vWF concentrate is usually required during delivery. Monitoring is required after delivery, and if a vWF decrease occurs, substitution therapy should be introduced.

**Inhibitors of von Willebrand Factor**

Alloantibodies develop in 9.5% of patients with type 3 vWD. These antibodies inhibit vWF:RCo activity and belong to precipitating antibodies. Development of antibodies is in correlation to the presence of homozygous gene deletion. Treatment of such patients can be very difficult. Allergic reactions frequently occur after substitution therapy, which usually does not result in vWF increase and does not correct bleeding time. Highly purified concentrates that do not contain vWF or recombinant F VIIa can be used. Intravenous immunoglobulin can raise the level of vWF in some patients. In life threatening bleeding episodes, immunoadsorption to protein-A columns and substitution therapy can be applied.
ACQUIRED BLOOD CLOTTING DISORDERS

Acquired blood clotting disorders occur more frequently than inherited forms and usually consist of several coagulation factor deficiencies. They occur in:
- vitamin K deficiency,
- liver diseases,
- liver transplantation,
- disseminated intravascular coagulation,
- renal diseases,
- massive blood transfusion syndrome,
- primary pathological fibrinolysis, and
- during the course of anticoagulant therapy.

Vitamin K Deficiency

Vitamin K is the catalyst of glutamine acid carboxylation on specific sites in vitamin K-dependent factors (F II, F VII, F X, F IX) as well as in PC and PS. \( \gamma \)-carboxy-glutamine acid is
responsible for calcium binding and is important for the functional activity of vitamin K-dependent factors.

Vitamin K is taken in with food. It is dissolved in lipids; thus, its resorption depends on the presence of pancreas lipases and bile. It is absorbed from the intestinal wall and participates in F II, F VII, F IX and F X synthesis in the liver. In vitamin K deficiency states, these coagulation proteins do not bind calcium, normally a prerequisite for their normal activation and function.

Hemorrhagic complication due to vitamin K deficiency can occur in older people as a result of biliary obstruction, fat malabsorption, by total parenteral nutrition, or oral anticoagulant therapy.

Vitamin K deficiency:
- biliary obstruction
- malabsorption states (cystic fibrosis, celiac disease)
- pancreas disorders
- kidney diseases
- broad spectrum antimicrobial therapy
- hypoalbuminemia
- total parenteral nutrition
- oral anticoagulant therapy
- hemorrhagic disease of the newborn

Deficiency of vitamin K-dependent coagulation factors causes the prothrombin time to be prolonged, and in severe de-
iciency, the partial thromboplastin time will be prolonged as well. Differential diagnosis includes DIC and liver disease. In vitamin K deficiency, the platelet count, fibrinogen level and FV activity are all normal but are reduced in DIC and liver disease.

The management of vitamin K deficiency consists primarily of its repletion, usually by intravenous or subcutaneous routes in critically ill patients. Concern about the possibility of anaphylactoid reactions with the intravenous use of vitamin K exists. This risk is almost completely negated when the drug is given as an infusion over 30 to 45 minutes in a small volume of fluid rather than as a bolus. This is the preferred method of drug administration in hemodynamically unstable patients. The usual dose of vitamin K is 5 to 10 mg intravenously or subcutaneously. In an otherwise healthy person, the PT should correct within 12 to 24 hours after application.

When the patient is actively bleeding, it is not sufficient to give vitamin K alone. A more immediate restoration of coagulation is required. FFP usually contains sufficient amounts of vitamin K-dependent coagulation proteins to stem further bleeding. To restore hemostasis to an acceptable level (30% to 50%) of normal enzyme activity, 4 to 6 units of plasma or F IX complex are required. Replacement therapy with FFP or F IX complex should be used only for a life-threatening hemorrhage.
Protein Z (PZ)

In 1977, Prowse and Esnouf identified an additional vitamin K-dependent protein circulating in bovine plasma and named it protein Z. PZ serves as a cofactor for the inhibition of F Xa by another plasma protein called PZ-dependent protease inhibitor.

The PZ gene is at chromosome 13q34, the location where the genes for F VII and F X reside side by side. PZ spans 14 kb and consist of nine exons, including an alternatively spliced exon that inserts a unique peptide of 22 amino acids in the pre-pro-leader sequence of PZ. The exon-intron organization of the PZ gene is identical to that of F VII, IX, X and PC, indicating that these genes were derived from a common ancestor during evolution. Several polymorphisms have been identified in the PZ gene.

The range of PZ plasma levels in normal individuals is very broad (95% interval of 32% to 168% of the mean) and appears to be influenced predominantly by inheritable factors. Reported mean concentrations of PZ in adult plasmas have varied from 1.2 to 2.9 µg per mL. PZ circulates in plasma complexed with ZPI (protein Z-related protease inhibitor).

Similar to other coagulation factors, the liver appears to be the major source of PZ. The level of PZ is reduced in individuals with severe liver disease and is low in newborn infants. Oral contraceptive use substantially increases PZ levels. Plasma PZ
reportedly increases with chronic hemodialysis and is reduced in the nephrotic syndrome. Immunoreactive PZ has been detected in atherosclerotic plaques.

In contrast to other plasma vitamin K-dependent proteins, the coumarin class of oral anticoagulants dramatically affects the levels of PZ.

An interaction between F Xa and PZ occurs as an inhibitory effect of PZ on F Xa activity. In one stage, coagulation assay was due, at least in part, to a plasma ZPI that recognizes the F Xa-PZ complex. ZPI was isolated from human plasma in 1998.

It has been suggested that PZ deficiency is associated with a hemorrhagic disorder, perhaps related to capillary fragility. Prothrombin complex concentrates, which contain PZ, have been used to prevent perioperative hemorrhage in individuals with a bleeding history and perceived PZ deficiency.

Fedi et. al found an association between low PZ levels (<15th percentile) and acute coronary syndrome that was increased further by concomitant smoking. Low plasma concentrations of PZ have also been reported in ischemic colitis. Leiden Thrombophilia Study (LETS) showed a modestly increased risk of venous thrombosis with low PZ (<10th percentile) in men.
Hemostatic Disorder in Liver Disease

The liver plays a central role in the regulation of hemostasis.
- Synthesis of plasma coagulation factors: vitamin K-dependent (F II, F VII, F IX, F X) and other coagulation factors: F I, F V, F IX, F XII, prekallikrein, HMWK, F XIII, and vWF
- Synthesis of the natural coagulation factor inhibitors: AT III, PC, PS, and antiplasmins ($\alpha_2$MG and $\alpha_2$AP)
- Elimination of activated coagulation factors (hepatic clearance)
- Synthesis of factors participating in fibrinolysis: synthesis of plasminogen, synthesis of PAI-1 and PAI-2, and sequestration and degradation of t-PA

In liver failure, there is a simultaneous decrease of the levels of several hemostatic factors. The degree of decrease depends on the extent of the liver damage. The most frequent disorders are the following:

1. reduced and disturbed synthesis of coagulation factors and inhibitors
2. lack of hepatic clearance
3. disorders of platelets (number and function)
4. pathological fibrinolysis
5. DIC.
Reduced and Disturbed Synthesis

In cases of moderate liver damage, disturbances of vitamin K-dependent factors (F II, F VII, F IX, F X) develop as a consequence of decreased synthesis and also as a consequence of decreased carboxilation due to vitamin K deficiency. With severe liver damage due to reduced synthesis, a decrease in the levels of F V, F XI, F XII, prekallikrein, HMWK, F XIII and α₂AP occurs. AT III and plasminogen disorders may occur in parenchymal liver diseases as a consequence of the decreased synthesis but also due to increased consumption in DIC.

Fibrinogen (F I) levels can be decreased in acute liver disorders because of decreased synthesis. In inflammatory and neoplastic diseases that are caused by a decrease in hepatic clearance, F I and F VIII levels, as reactive proteins, can be increased.

Lack of Hepatic Clearance

In physiological conditions, activated coagulation factors are inactivated in the liver. In severe liver disease (cirrhosis), T₁/₂ of activated factors is prolonged. This may predispose for DIC occurrence. t-PA is normally sequestered and degraded in the liver. Thus, activation of the fibrinolytic system may be caused by lack of clearance activity.
Disorder of Platelets (Number and Function)

Hemostatic disorders in liver diseases are often caused by a decline of the platelet count and a disturbed platelet function, most frequently due to hypersplenism. In normal conditions, 30% of the platelets are deposited in the spleen. In hypersplenism up to 90% of the platelets can be deposited in the spleen causing a hemorrhagic syndrome associated with thrombocytopenia. The short survival of platelets may be a consequence of hypersplenism, of increased consumption in DIC, or of the presence of anti-platelet antibodies. Thrombocytopenia most frequently develops due to hypersplenism, congestive splenomegaly or the toxic effect of alcohol. In cases of alcohol-dependent liver cirrhosis, there is a decreased platelet production and an increased degradation. Bleeding usually does not occur if platelet count is above 40 x 10^9/l.

Qualitative platelet disorders with decreased aggregation due to a decreased content of arachidonic acid in platelets and also because of the presence of FDPs and fibrin in liver cirrhosis have been reported. Defective synthesis, disorders of hepatic clearance, and consequences of hypersplenism may be simultaneously present.

Pathological Fibrinolysis

Fibrinolysis is the coagulum degradation process formed by the effect of plasmin enzyme. Plasminogen activators, which change
Plasminogen, fibrinolysis inhibitors, PAI-1, PAI-2, and plasmin inhibitors (α₂AP, α₂MG, α₁ antitrypsin, and C-1 inactivator) are formed in the liver. If the liver clearance remains intact, endogenous and extrinsic plasminogen activators, such as t-PA and urokinase u-PA, are inactivated and eliminated by the liver.
In liver insufficiency, pathological fibrinolysis frequently occurs since fibrinolysis inhibitors are formed in insufficient numbers and the liver is not capable of neutralizing plasminogen. It is often very difficult to determine whether a hemorrhagic syndrome is the result of a primary or a secondary fibrinolysis, which may be caused by DIC. Laboratory tests can confirm or exclude one of these disorders.

DIC in Hepatic Failure

DIC is an acquired hemostatic disorder that is characterized by simultaneous bleeding and microthrombosis. Causes of thromboembolic complications in DIC in liver disease are:
- release of thromboplastin due to hepatocyte necrosis,
- vascular endothelium damage,
- effect of endotoxin,
- deposition of coagulation factors due to the lack of hepatic clearance,
- inhibition of AT III formation, and
- blockage of the RES.

DIC is aggravated by stasis since coagulants are normally inactivated by natural inhibitors and eliminated in liver and RES. Fibrinolysis activation is a secondary manifestation.
Treatment of DIC is very complex; it requires both prevention of thrombosis and arrest of bleeding caused by consumption of coagulation factors and increased fibrinolysis. Thus, treatment of DIC is based on clinical symptoms and laboratory findings. There are different therapeutic approaches for DIC using heparin in liver disease. For many authors, it is a treatment of choice; others point to the complications that arise during treatment as a direct consequence of heparin administration as
well as to the formation of heparin-platelet complex. The use of the low molecular heparin (fraxarin) is increasing these days because it does not form heparin-platelet complex and does not result in thrombocytopenia.

Clinical signs may worsen considerably if AT III is decreased when the stage in which microthrombi are formed in circulation is prolonged. Low values of PC reported in liver cirrhosis may decrease even more with the use of heparin. This is why in some liver diseases heparin administration should be carefully considered. Under normal circumstances, the liver quickly metabolizes heparin. In liver diseases, however, heparin half-life is prolonged due to a clearance disorder.

**Hemostasis Disorder in Liver Transplantation**

The number of transplanted livers has increased, particularly in the last decade, with ever better results thanks to improved surgical methods, increased knowledge of hemostatic disorders and their treatment, better storage conditions for the liver prepared for transplantation, introduction of cyclosporine as an immunosuppressive drug, assessment of the preoperative condition and function of the liver, and early diagnosis and prevention of graft rejection. The survival rate of patients after transplantation has increased, and patients’ lives have been prolonged. However, liver transplantation may still be associated with intraoperative bleeding. Increased blood loss during liver
transplantation contributes to postoperative morbidity and mortality rate. Intraoperative bleeding requires immediate diagnosis and treatment, most frequently using replacement therapy with blood and blood derivatives.

Most patients undergoing a liver transplant suffer from a severe preoperative hemostatic disorder that may contribute to very extensive intraoperative bleeding. The underlying liver disease is one of the main non-surgical factors influencing orthoptic liver transplantation (OLT). The surgical procedure itself may induce certain complications, particularly during the liver removal stage. Furthermore, neither coagulation factors nor coagulation inhibitors can be removed. The foreign surface and venous bypass activate the intrinsic coagulation system and contribute to the platelet function disorder. The coagulation process may be activated by ischemic endothelial cells of the liver prepared for transplantation. Perfusion of an anoxic liver prepared for transplantation often leads to some critical coagulation disorders.

Hemostatic disorders may occur during preoperative, operative and postoperative periods. There are various causes that can induce the disorders.
- Pre-existing hemostatic disorder
- Pathological disorders that may arise during the surgical procedure (increased fibrinolysis, DIC)
- Evaluation of transplanted liver function, changes in the transplanted liver caused by liver ischemia, most frequently thrombocytopenia
- As a prognostic factor F V level (returns to normal value after 24-48h)
- Early detection of transplanted liver rejection
- Side effects of immunosuppressive therapy - hypercoagulability
- Adequate therapy using blood components
- Dilution coagulopathy which occurs during surgical procedures due to extensive blood compensation

**DIC**

Disseminated intravascular coagulation is caused by activation of coagulation. Inhibitors of coagulation such as AT III, PC, and TFPI are depleted.

DIC is an acquired hemostatic disorder that is characterized by simultaneous bleeding and microthrombosis. DIC is the only syndrome known in which thrombosis and bleeding occur simultaneously. DIC is a secondary phenomenon which complicates numerous clinical conditions.

Common causes of DIC are:
- infection, meningococcemia, serious infections with the more common gram-negative bacteria, pseudomonas aeroginosa, and sepsis with gram-positive organisms
- malignancies, solid tumors such as prostate carcinoma and pancreatic carcinoma
- acute promyelocytic leukemia (APL)
- complications of pregnancy: abruptio placentae, amniotic fluid embolism, retained dead fetus, infection and septic abortions, HELLP (hemolysis, elevated liver enzymes, low platelet count) syndrome, preeclampsia and eclampsia
- trauma- DIC due to tissue destruction, infection, ischemia, shock, or fat embolization,
- hemolytic anemias, ABO-incompatible blood transfusions, liver disease, burns, snake envenomation, heat stroke, and treatment with prothrombin complex concentrates.

Table VII.1

<table>
<thead>
<tr>
<th>DIC is caused by coagulation activation.</th>
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</thead>
<tbody>
<tr>
<td>A. Through the internal pathway endothelium damage</td>
</tr>
<tr>
<td>acidosis</td>
</tr>
<tr>
<td>endotoxins</td>
</tr>
<tr>
<td>immune complex</td>
</tr>
<tr>
<td>B. Through the external pathway tissue damage – released tissue thromboplastin</td>
</tr>
<tr>
<td>complications in pregnancy</td>
</tr>
<tr>
<td>malignant tumors</td>
</tr>
<tr>
<td>leukemia</td>
</tr>
<tr>
<td>C. By direct activation of FX</td>
</tr>
<tr>
<td>amniotic fluid pancreas enzymes</td>
</tr>
</tbody>
</table>

Secondary fibrinolysis most frequently follows DIC. It is usually an acute disorder although it can appear in subacute and chronic forms. Microthrombi in circulation may cause coagulation process activation, blood vessel damage and increased platelet aggregation, or penetration of tissue thromboplastin into circulation.
DIC is impaired by stasis. Coagulants are normally inactivated by natural inhibitors and removed in liver and RES while stasis prevents penetration of circulating inhibitors to coagulum. The activation of the coagulation process causes thrombus formation in blood vessels. Thus, blood vessels in vital organs (kidneys, brain, etc.) may be blocked, which may lead to ischemia and organ necroses. Due to intravascular thrombosis, coagulation factors are consumed: F I, F II, F V, F VIII and platelets; bleeding occurs. Erythrocytes pass through the fibrin tissue network, and this occurrence leads to microangiopathic hemolytic anemia.

Probably the most severe consequence of DIC is shock. It is sometimes hard to determine if the shock is a consequence of the underlying disease (sepsis, trauma, and burns), the result of a great loss of blood from surgical or obstetric procedures, or a consequence of DIC. Activation of fibrinolysis is a secondary issue. Fibrinolytic enzymes effect the formation of fibrinogen and fibrin degradation products, acting as anticoagulants since they prevent transition of fibrinogen into fibrin and platelet aggregation.

A diagnosis is made based on screening tests, decreased platelet count, prolonged PT, PTT and TT tests as well as the presence of an increased concentration of soluble fibrin monomer complexes, circulating FDPs, and plasma D-dimer concentration. The fibrinogen level is decreased.
The fibrinogen level may be normal despite consumption because of its elevation as an acute-phase reactant. Characteristically, FDP levels are significantly elevated in the face of normal platelet counts and D-dimer levels in primary fibrin(ogen)olysis. Additionally, there are often rapid whole blood clot lysis and shortened euglobulin lysis times.

The treatment of DIC is rather complex because it simultaneously prevents thrombosis and arrests bleeding caused by coagulation factor consumption and increased fibrinolysis. Therefore, the treatment of DIC is based on clinical symptoms and laboratory findings. Treatment possibilities include the following:

- Elimination of the underlying cause.
- Inhibition of the coagulation process and prevention of thrombosis by administration of heparin, one of the most significant coagulation inhibitors that affects many plasma coagulation factors (XII, XI, IX, X and thrombin). Unsuccessful treatment with heparin may be a consequence of a reduced AT III concentration. In such cases, administration of FFP or AT III concentrate is indicated. Initial doses of heparin are high (5,000 IU) and decreased continuously in order to avoid sudden increases of heparin concentration in blood. PTT should be 2 to 2.5 times longer than the normal values. Specific indications for heparin treatment include purpura fulminans, dead fetus syndrome, aortic aneurysms, migratory thrombophlebitis due to malignancy, and DIC with large vessel thrombosis. Less established indications for heparin therapy include skin necrosis and acral gangrene, amniotic fluid embolism, ABO-mismatched blood transfusion, and septic abortion. The decision to use heparin should be made on an individual basis; the risks and benefits should be carefully considered.
- Replacement therapy should be considered when the signs of bleeding are predominant. FFP, as a source of all plasma coagulation factors, and AT III concentrate are administered as well as platelet concentrate. Decreased concentration of fibrinogen and F VIII is best compensated by administration of cryoprecipitate. Antifibrinolytic therapy is not recommended.
Hemostasis Disorder in Renal Disease

Hemostasis disorder in renal disease is a complex process resulting from deposition of toxic metabolic products in plasma (urea, hydroxyphenolic acid, guanidinosuccinic acid). This is because these products inhibit coagulation factors, platelets in particular. In both acute and chronic renal disease, there is a bleeding tendency caused by several different disorders.

1. Platelet function disorders:
   - decreased TrF3
   - platelet aggregation disorder
   - platelet adhesion disorder
   - PGI₂ synthesis disorder
2. Decreased platelet count
3. Plasma coagulation factor deficiency
4. DIC
5. Primary pathological fibrinolysis
6. Presence of heparin
7. Presence of FDP
8. Isolated F IX and AT III deficiency (selective loss via urine)

Thrombocytopenia is common in renal failure and arises as a result of bone marrow failure and peripheral consumption. Uremic bleeding is not correlated with platelet count, and significant hemorrhage can be found with normal counts. A major reversible platelet defect is present in renal failure. All tests of platelet function including bleeding time, clot retraction,
platelet factor III availability, retention in glass bead column and platelet aggregation tests show abnormal results; the underlying mechanism is the inhibition of the platelet release reaction. Both laboratory abnormalities and bleeding tendency can be corrected by regular dialysis. Guanidinosuccinic acid, hydroxyphenolic acid, and possibly urea itself are the substances responsible for the platelet dysfunction. They are found in high concentrations in uremic plasma, can be rapidly removed by dialysis, and if incubated with normal platelets in vitro, provoke platelet abnormalities identical to those found in uremia.

It has been reported that PGI₂ production from venous tissue was higher than normal in patients with uremia. Thus, an imbalance of PGI₂ and thromboxane synthesis will also contribute to bleeding problems. Fibrinogen and F VIII:C concentrations are high in renal disease. In patients with glomerular disease, vWF:Ag concentration is particularly high, often disproportionately in comparison to F VIII:C. Pathological partial thromboplastin time and one-stage prothrombin time are often found. This prolongation is due to a reduced concentration of F V, F VII and F X. The deficiency may arise as a result of an associated hepatic impairment or from vitamin K deficiency caused by malnutrition, antibiotic therapy or uremic enteritis. Besides, some patients with nephrotic syndrome may develop an isolated deficiency of F IX due to selective loss via urine. F XIII is reduced in patients with renal insufficiency.
In the acute phase of renal disease, during allograft rejection and in chronic renal insufficiency, laboratory markers of low grade DIC are often found: raised FDP concentration, long thrombin time, partial thromboplastin time and one-stage prothrombin time as well as a reduced platelet count. It is usually impossible to determine whether these abnormalities are due to the local changes in kidney or arise as a result of uremic and metabolic effects leading to DIC.

Fibrinolytic activity of blood is decreased in renal disease because of the excess of plasma inhibitors. The urinary concentration of urokinase also decreases simultaneously with the decline of renal function. In contrast, fibrinolytic activity of the glomeruli is found to be increased in many types of renal disease, possibly as a response to glomerular fibrin deposition. In acute and chronic cases of renal disease, there are hemostic disorders such as bleeding or thrombosis. Bleeding is most frequently localized in skin and mucous membranes, gastrointestinal tract (hematemesis and melena), pericardially, and intracranially (makes treatment very difficult and endangers patients’ lives).

Uremic bleeding rarely occurs in patients undergoing regular dialysis. If bleeding occurs, inadequate heparin neutralization and/or DIC associated with another condition, septicaemia for example, should be excluded. In patients not undergoing dialysis, hemorrhage may be difficult to arrest with replacement therapy unless the metabolic defects are corrected by
dialysis first. Platelet transfusions are only temporarily effective because the donor’s platelets acquire the uremic defect within hours. Specific deficiency of vitamin K-dependent factors and factor V may at least be partly corrected by vitamin K1 and FFP administration, respectively. If isolated factor IX deficiency is the cause of bleeding, F IX concentrates can be given. Despite elevated F VIII:C and vWF:Ag, cryoprecipitate can temporarily improve hemostatic function.

Recommended therapy includes:
1. Dialysis
2. Transplantation
3. Blood component therapy (in accordance with the disorder):
   - red cell concentrate, erythropoietin
   - FFP
   - F IX concentrate
   - platelet transfusion
   - cryoprecipitate or DDAVP (improves platelet adhesion).

If blood transfusions are considered, particular attention should be paid to possible sensitization, which might compromise transplantation.

**Massive Blood Transfusion**

Transfusion of large quantities of stored blood can result in a multifactorial hemostatic defect. Increased bleeding may occur
in patients who usually receive large quantities of whole blood or erythrocytes due to intraoperative bleeding. If mechanical injury of blood vessels can be excluded, the most frequent multiple coagulation disorder is due to the diluting effect of conserved blood (decreased platelet count and labile coagulation factors F V and F VIII). This syndrome is far more expressed in patients with reduced production of platelets or coagulation factors due to an underlying illness (thrombocytopenia, hemophilia, liver disease or in increased destruction of coagulation factors in idiopathic thrombocytopenic purpura [ITP] and DIC). The hemostatic effect depends on the quantity of transfused blood, frequency of transfusions, duration of blood conservation and underlying disease. Thrombocytopenia regularly occurs if more than 10 units of conserved blood are transfused in 24 hours. If blood is transfused rapidly, thrombocytopenia is more frequent.

Massive transfusions with crystalloid and colloid solutions, such as dextran and albumin, can also lead to a considerable decrease of coagulation factor levels. After surgeries with massive blood transfusions, PTT and platelet count screening tests should be performed at a minimum. FFP should be used if PTT is prolonged. If platelet count is decreased, platelet concentrates should be administered. Administration of platelets or FFP according to established schemes (3 units of platelets and 2 units of plasma on 10 blood units) as a preventative measure provided no satisfactory results.
HYPERFIBRINOLYSIS AND ANTIFIBRINOLYTIC THERAPY

Table VIII.1

<table>
<thead>
<tr>
<th>Abberrations of the fibrinolytic balance of thrombosis and bleeding</th>
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<tr>
<td><strong>BLEEDING</strong></td>
</tr>
<tr>
<td>Excessive activation of fibrinolysis</td>
</tr>
<tr>
<td>Defective inhibition of fibrinolysis</td>
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Hyperfibrinolysis

Excessive local or systemic fibrinolysis can disrupt hemostasis and lead to significant bleeding. Inhibition or deficient activation of fibrinolysis can lead to local or systemic thrombosis.

Primary pathological fibrinolysis is a hemorrhagic syndrome caused by increased fibrinolytic enzyme activity in plasma. The activity of the fibrinolytic enzyme plasmin causes degradation
of fibrinogen and other coagulation factors. The constant balance between activation of coagulation and fibrinolysis is disturbed. Fibrinolysis occurs as a primary process during lung and gland surgery, in malignant diseases of blood, metastasis of malignant tumors, and in severe traumas. It is particularly expressed in liver disorders due to decreased activity of antiplasmin ($\alpha_2$AP). Fibrinolysis can be increased in hypoxia caused by physical effort and in stress.

Clinical treatment of fibrinolysis can hardly be separated from DIC. Bleeding during surgery or postoperatively is very severe. Diagnosis is made according to an euglobulin fibrinolysis test, which is most frequently used. Normal euglobulin fibrinolysis is longer than 2 hours, but in pathological fibrinolysis, lysis time is shortened. In contrast to DIC, platelet count is normal, as well as other coagulation screening tests. PTT, PT and TT are almost normal. TT can be prolonged due to the presence of FDPs. Contrary to DIC, paracoagulant tests used for the determination of soluble fibrinogen monomers are not pathological. Sometimes it is hard to define whether the cause of symptoms is DIC or the presence of heparin using laboratory screening methods. Reptilase time is a significant differential diagnostic test. While TT is prolonged in both cases, reptilase time is normal in the presence of heparin and prolonged in DIC.
Antifibrinolytic Therapy

Antifibrinolytic agents have been found to be useful in the treatment of a wide variety of bleeding disorders that occur secondary to systemic hyperfibrinolysis, primary coagulopathies, localized bleeding conditions, surgery and thrombocytopenia. Antifibrinolytic agents have also been found to be useful in the treatment of bleeding complications associated with thrombolytic therapy in patients receiving urokinase or streptokinase.
Bleeding may occur in association with a localized increase in fibrinolytic activity in the presence of normal coagulability. Antifibrinolytic agents have been found useful in controlling bleeding localized to the genitourinary or gastrointestinal tracts or the central nervous system.

Antifibrinolytic therapy is indicated for:

1. **Bleeding in Gynecology and Obstetrics**
   Endometrial tissue has been associated with increased fibrinolytic activity, especially during the secretory phase of the menstrual cycle and with alterations of normal menses. Antifibrinolytic therapy has been proven to reduce bleeding in patients with essential menorrhagia, menorrhagia associated with intrauterine devices, and bleeding after cervical conization. Bleeding during pregnancy is associated with an increase in perinatal mortality. Bleeding of small vessels at the borders of the placenta may be due to defective sealing of the microcirculation. EACA and AMCA (tranexamic acid) cross the placental barrier and may secure local hemostasis and reduce premature labor.

2. **Bleeding in Urinary Tract**
   Urinary tract bleeding also has been associated with localized increases in fibrinolytic activity, mostly due to the action of u-PA. Antifibrinolytic agents effectively control bleeding after prostatectomy and renal biopsy as well as bleeding secondary to hemorrhagic cystitis. In patients with
upper urinary tract bleeding, antifibrinolytic therapy can lead to obstructing clots in the urinary collecting systems.

3. **Bleeding in Gastrointestinal Tract**
Gastrointestinal tract bleeding also responds to antifibrinolytic therapy. Gastric tissue contains plasmin activators that are released during pathological states such as surgical manipulation and active peptic ulcer disease. Lower gastrointestinal bleeding secondary to ulcerative colitis also has been shown to respond to EACA.

4. **Neurosurgery and Neurology (Intracranial Hemorrhage)**
Antifibrinolytic therapy is not routinely used in the treatment of subarachnoid hemorrhage, but antifibrinolytic therapy may be effective in preventing or delaying re-bleeding in patients with ruptured intracranial aneurysm. Antifibrinolytic agents can also produce delayed cerebral ischemic complications.

Indications for antifibrinolytic therapy in the treatment of intracranial hemorrhage are not as clear. It is well-established that plasminogen activator activity, which is normally absent from the cerebral spinal fluid, is increased following subarachnoid hemorrhage. This increased activity is thought to disrupt initial hemostasis following rupture of an aneurysm and to be responsible for some incidences of rebleeding that complicates a subarachnoid hemorrhage. Because both EACA and AMCA cross the blood-brain barrier, these
agents should theoretically be of benefit in preventing re-bleeding.

One possible complication of antifibrinolytic therapy is a higher incidence of hydrocephalus due to fibrin deposition and eventual fibrosis of the subarachonid space.

In brain surgery, hemostasis may sometimes become a problem as a result of the high concentration of plasminogen activators in meningiomas, medulloblastomas and cerebellar sarcomas. Aprotinin solutions have been applied topically to stop oozing and prevent postoperative rebleeding.

5. **Bleeding in Cardiovascular Surgery, Extracorporeal Circulation**

Although excessive blood loss has considerably decreased with more refined cardiac bypass methodology and equipment, there is still concern about reducing postoperative bleeding in cardiac surgery.

Earlier studies have shown that aprotinin can reduce fibrinolytic activity during open heart surgery with extracorporeal circulation. It is also worth noting that aprotinin has been used successfully to prevent leakage of fibrin-coated aortic allografts.

6. **Bleeding in Liver Transplantation**

Aprotinin has been found to be useful in the treatment of bleeding complications associated with liver transplan-
tion. During surgical procedures fibrinolysis is increased. Use of aprotinin reduces blood loss in liver transplantation. Initial dose is 2,000,000 KIU before surgery as a bolus and 500,000 KIU by infusion during operation.

7. Clinical Efficacy in Patients With a Generalized Bleeding Disorder

Antifibrinolytic drugs have been used as prophylaxis against spontaneous bleeding episodes after tooth extraction in patients with hemophilia. The oral mucosa and the salivary gland have been found to contain a high concentration of plasminogen activators. This may explain why hemorrhage after oral surgical procedures may be caused by local fibrinolytic activity.

Before dental extraction 15-25 mg tranexamic acid per kg body weight is given intravenously together with a factor VIII or IX concentrate. After surgery 15-25 mg tranexamic acid per kg are administered orally 3 to 4 times daily for 8 days. Usually, there is no further substitution therapy with coagulation factor concentrates required after surgery. The use of tranexamic acid has reduced the need for factor VIII or IX concentrates after dental extractions by 80%, also avoiding potential hazards inherent to blood products.
Antifibrinolytic agents

Epsilon-Aminocaproic Acid (EACA)
The most widely employed synthetic antifibrinolytic agent is EACA. The substance is freely soluble in water and, therefore, is almost completely absorbed by the gastrointestinal tract. Peak levels after oral dosage are reached within 2 hours and are comparable to those reached after intravenous administration. EACA is widely distributed, including passage into synovial fluid and across the blood-brain barrier. Direct measurements of serum levels following intravenous administration estimate the elimination half-life to be $294 \pm 183$ minutes.

The main antifibrinolytic effect of EACA occurs through prevention of plasminogen binding to fibrin. In higher concentrations, EACA inhibits the enzymatic activity of plasmin. EACA has also been found to competitively inhibit the action of urokinase and to prevent binding of t-PA to fibrin.

Tranexamic Acid (AMCA)
Tranexamic acid (trans-4-aminomethycyclohexanoic acid) is another lysine analogue that has been found to be ten times as potent as EACA on a molar basis. The mechanism of action is identical to that of EACA. AMCA is freely soluble, but oral bioavailability is only about 40% of the corresponding intravenous dose. Tissue distribution and the mechanism of elimination are the same as with EACA. The plasma elimination half-life has
been found to be about 2 hours with an apparent biological half-life of 1 to 2 hours.

AMCA should not be used in association with FEIBA or other prothrombin complex concentrates because of the potential for thrombogenity. In contrast, administration of AMCA may enhance the efficacy of recombinant F VIIa (Novo Seven). AMCA should not be applied in the treatment of hematuria because of blood loss from the upper urinary tract; this can provoke painful clot retention and even renal failure associated with bilateral ureteric obstruction.

Side effects of AMCA are rare and mainly limited to nausea, diarrhea or abdominal pain. These symptoms are usually associated with high doses, and they often subside when dose is reduced. Hypotension is occasionally observed, typically after rapid intravenous infusion.

Aprotinin
Aprotinin, polyvalent proteinase inhibitor, was first isolated from bovine lung in 1936. It was not until the 1960s that the compound was developed for clinical use. Aprotinin is not absorbed orally and has an elimination half-life of 150 minutes (Fig. VIII.2).

The molecule consists of a single polypeptide chain containing 58 amino acid residues and has a molecular weight of 6.5 kDa. Half-life of aprotinin is about 60 min. Aprotinin inhibits a wide array of proteases other than plasmin including trypsin, chymotrypsin, and kallikrein. Its antifibrinolytic effect
is exerted by reversible binding to plasmin via a lysine residue in the molecule's active site.

Aprotinin acts as an inhibitor on the following enzyme systems.
- pancreas enzymes (trypsin, chymotrypsin)
- kinin system (granular and plasmatic kininogenases)
- coagulation system (contact factors, F XIIa, F VIIa tissue factor complex)
- fibrinolysis system (plasmin, urokinase)
- activated protein C

Furthermore, aprotinin demonstrates inhibitory effects on leukocytic proteinases and tissue proteinases as well as factors of the complement system.

Aprotinin prevents the spontaneous formation of microaggregates in stored blood, protects clotting factors, and depresses the fibrinolytic system. Under transfusion conditions, platelet function is immediately restored so that the patient’s coagulation status remains unchanged. In concentrations between 200 and 500 KIU/ml, aprotinin effectively inhibits platelet aggregation.

Aprotinin activity is expressed in Kallikrein Inactivator Units (KIU). One KIU is equal to the quantity of the substance that will inactivate a given quantity of kallikrein. One milligram of aprotinin contains approximately 7,160 KIU. Elimination is through renal metabolism and excretion. The drug does not distribute across cell membranes or cross the blood-brain barrier.
Based on the various enzyme systems in which aprotinin exerts its inhibitory effects, its application is recommended in the following clinical conditions:

- acute pancreatitis
- prevention and management of shock
- hyperfibrinolytic hemorrhage

Figure VIII.2: Modeled complex of the interaction between Aprotinin and plasmin’s protease domain
- prevention of possible post-operative and post-traumatic complications (shock)
- reduction of blood loss in open heart surgery (cardiopulmonary bypass)
- reduction of blood loss in liver transplantation.

Aprotinin is also used in diabetic patients refractory to subcutaneous insulin. In these cases, aprotinin prevents the destruction of insulin by insulinases.

Side-Effects of Antifibrinolytic Therapy

The most common side effects of EACA are nausea, vomiting, and diarrhea, which occur in approximately 20% of cases. They appear to be related to direct gastrointestinal irritation rather than to toxic serum levels of the agent. Less common side effects are dizziness, pruritus, rash and headache. Inflammatory myopathy has only been reported in a few patients taking large doses for prolonged periods. By far, the most serious complication is thrombosis, usually in patients with predisposing hypercoagulable states, especially DIC.

Antifibrinolytic agents are generally contraindicated in disorders, such as DIC, that manifest a hypercoagulable state as part of their pathophysiology. In these instances, inhibition of the fibrinolytic component may unmask the hypercoagulable state and lead to clinically significant thrombosis. Just because it is now recognized that disorders of pure hyperfibrinolysis are
rare, it does not mean that antifibrinolytic agents can not be employed indiscriminately.

Side effects of aprotinin are rare and include a 1% incidence of allergic reactions, including anaphylaxis.

In general, aprotinin has been proven to be well tolerated, even at massive doses. However, due to its polypeptidic nature, it may be responsible for allergic reactions. Such reactions occur quite rarely but call for the immediate discontinuation of the treatment. Signs of intolerance may already be evident with the first administration of the drug to subjects with a previous history of allergic manifestations or when the cycle of therapy is repeated.

Decreased Fibrinolysis

Predisposition to thrombosis can result from an excessive procoagulant tendency that overwhelms a physiologic fibrinolytic response or from an inadequate fibrinolytic response to otherwise benign thrombophilic processes. Thrombolytic disease caused by defective fibrinolysis, secondary to either decreased plasminogen activation or increased inhibition, is difficult to evaluate because fibrinolytic assays are less sensitive to decreased activity than to increased activity. Cogenital defects in the fibrinolytic system have been associated with thrombosis, specifically due to abnormalities of fibrinogen and plasminogen. Aoki et al. described a patient with recurrent venous thrombotic
disease, who had a normal plasminogen antigen concentration but a 50% functional activity. This was the result of amino acid substitution near the reactive site.

**Thrombin-activated fibrinolysis inhibitor (TAFI)**

Activated TAFI (also known as carboxypeptidase U (CPU)) or plasma carboxypeptidase B can inhibit fibrinolysis by removing these lysine residues. Consequently, this results in a decreased plasmin formation and a prolongation of clot lysis time. This prolongation of clot lysis time is abolished when activated TAFI is inhibited directly by inhibitors. TAFI plasma levels are significantly increased in individuals with MI at a young age (24), acute CHD (3), or stable angina pectoris.
Thrombophilia is defined as the tendency to develop clots in veins or arteries. A hemostatic system functional reactivity increase without signs of its activation is termed as prethrombosis condition or thrombophilia. Hypercoaguoble states may be a result of an inherited abnormality or may be acquired.

**Inherited Thrombophilia**

**Table IX.1**

<table>
<thead>
<tr>
<th>Clinical features indicating the presence of an inherited hypercoagulable state.</th>
</tr>
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<tbody>
<tr>
<td>thrombosis at an early age</td>
</tr>
<tr>
<td>family history of thrombosis</td>
</tr>
<tr>
<td>unusual sites of thrombosis, such as hepatic or mesenteric veins</td>
</tr>
<tr>
<td>recurrent thrombosis, with or without precipitating factors</td>
</tr>
<tr>
<td>recurrent thrombosis despite adequate therapy</td>
</tr>
<tr>
<td>warfarin-induced skin necrosis</td>
</tr>
</tbody>
</table>
Venous thrombosis is more common in hypercoagulable states then arterial thrombosis.

Congenital Risk Factors for Development of Thrombophilia

→ AT III deficiency
→ PC deficiency
→ PS deficiency
→ Prothrombin 20210A allele
→ F V Leiden activated protein C (APC) resistance
→ Disorders of plasminogen and plasmin activation
→ Dysfibrinogenemia
→ Homocysteinemia

The following are the most frequent causes of inherited thrombophilias.

AT III Deficiency

Antithrombin III (AT III) deficiency is caused by a genetic defect that leads to a decreased function in AT III. This causes a relative increase in clotting factors XI, X and IX, creating a state of hypercoagulability.

AT III deficiency is an autosomal dominant inheritance of thrombophilia. Prevalence in general population is 1 in 350. There are two types of deficiency. Type I deficiency is due to related synthesis of the AT III protein. Type II deficiency is due to production of an AT III preotein with abnormal function. Values of AT III below 70% are considered to be deficient. 55% of bio-
chemically affected individuals develop venous thrombosis, occurring spontaneously or, more frequently, related to stress such as pregnancy, oral contraceptive use, surgery or trauma. The risk of thrombosis increases with age.

Inherited AT III Deficiency
Egeberg was the first to describe inherited AT III deficiency in a study of a Norwegian family with a history of recurrent thrombosis and with several fatal thromboembolic diseases (1965). The inheritance was autosomally dominant, and the disease appeared in a heterozygous form. Cases of homozygous inheritance were not reported; thus, the homozygous form was considered incompatible with life. Incidence of the disease in the overall population was 0.02 to 0.05% and in patients with a history of DVT the frequency was 2 to 3%. Both males and females suffered from this disease. Biological activity of AT III ranged from 50 to 60% of the normal values in all affected members of described families. Thromboembolisms appeared either spontaneously (in 1/3 of cases) or after certain predisposing situations (in 2/3 of cases), such as pregnancy, delivery, oral contraceptive drug use, surgeries, traumas and infections injuring platelets. Hypertriglyceridemia was an additional risk factor.

Thrombosis episodes most frequently occurred during (or even before) pregnancy or use of oral contraceptive drugs among female members of affected families. Thrombosis caused by the effect of estrogen was described in males, too, in cases of accidental use of di-ethyl-stilbestrol. In cases of inherited AT III
deficiency, DVT was typical. It is well known that in certain cases of AT III deficiency, the AT III level can increase during the use of coumarin therapy while in some cases, however, there is no increase.

Acquired AT III Deficiency
Acquired AT III deficiency is similar to the inherited form. Early recognition of the deficiency is significant for efficient prophylaxis of DVT and lung embolism. Acquired AT III deficiency results from decreased synthesis, increased consumption, increased loss, or a combination thereof. Patients with acquired AT III deficiency have an increased risk of thrombosis, both locally (venous thrombosis) and systemic (DIC). Acquired AT III deficiency is most frequently found in the following conditions.

1. Decreased synthesis
   – liver cirrhosis and chronic active hepatitis
2. Increased consumption
   – preeclampsia
   – use of oral contraceptives
   – DIC
   – sepsis
   – malignant neoplasmas
3. Increased loss
   – nephrotic syndrome
   – entheropatia
   – therapeutical plasmapheresis
   – massive blood loss
4. Combination of these three causes
   - postoperatively (major surgical procedures of thorax and abdomen, in orthopedic surgical procedures - the first and the second day after surgery)
   - heparin therapy (Due to AT III consumption, thrombosis can occur; thus, AT III should be monitored during heparin therapy, including replacement therapy whenever necessary.)
   - myocardial infarction

AT III Deficiency Treatment
Treatment of acute thrombosis in the AT III deficient patient is similar to thrombosis in a patient not AT III deficient: higher doses of heparin, long-term oral anticoagulation indicated for recurrent thrombosis, or subcutaneous heparin. AT III concentrates should be used instead of heparin during pregnancy. Prophylactic anticoagulation or AT III concentrates should be given during perioperative periods.

Antithrombin concentrate should be used in patients with the inherited AT III deficiency suffering from severe thrombosis, heparin resistance or additional risk factors for the occurrence of thromboembolic complications.

In other cases treatment can be heparin based. However, it is necessary to apply the heparin dose required to keep PTT within the therapeutic range. In alliance with the recent understanding of this disorder, peroral anticoagulants should also
be introduced on the very first day of heparin administration. AT III should be administered when necessary and only until therapeutic range of prothrombin time has been achieved. Administration of AT III concentrate is reasonable in patients with AT III deficiency along with subcutaneous heparin administration in times of increased risk of thrombosis such as surgeries, traumas or infections.

Dose = (80 - AT III of patient) x patient’s b.w. A prophylactic dose is not known. However, it seems to be below normal level.

Warfarin should be avoided during the first three months of pregnancy because it might be teratogenic, and it also increases the risk of severe fatal hemorrhage. The best prevention for DVT in pregnancy is subcutaneous administration of heparin 20,000 to 45,000 IU/24h, twice daily. The heparin dose should be adjusted so that PTT is prolonged 5 to 10 sec compared with PTT 12 hours after the injection. During delivery or abortion, AT III level should be increased to reach normal values by administering AT III concentrate. There is no conclusive evidence on the efficiency of AT III concentrate administration in DVT prophylaxis.

In the treatment of lung embolism, embolectomy and infections of v.cave, thrombolytic therapy can be useful (urokinase and streptokinase administration). The fibrinolytic and thrombolytic effects of these preparations do not depend on the AT III level. In cases of acute thrombosis, AT III concentrate is an
adequate choice along with simultaneous heparin administration. At the beginning of therapy, the initial doses of heparin should be lower since there is a risk of hemorrhage. Low doses of heparin have minimum effect on TT and PTT (even if AT III level is two times higher than normal). The required daily dose of heparin is 30,000 IU, which does not prolong PTT considerably. There are no further hemorrhagic complications.

Experience shows that administration of AT III concentrate in patients with acute thrombosis does not increase the risk of bleeding, and it also has a beneficial antithrombolytic effect.

**PCD efficiency**
PC, a vitamin K-dependent protein that inactivates F Va and VIIIa in the clotting cascade, leads to a state of hypercoagulability due to the fact that F V and VIII are not properly inactivated and fibrinolysis is not stimulated. APC also stimulates fibrinolysis by neutralizing a major inhibitor of t-PA.

PC deficiency is the autosomal dominant inheritance of thrombophilia. There are two types of deficiency. Type I deficiency is due to decreased synthesis of the PC. Type II deficiency is due to production of an abnormally functioning preotein. About 75% of affected individuals have venous thrombosis, most often spontaneous. Neonatal purpura fulminans occurs in homozygotes with PC levels below 5%.
Inherited PC Deficiency
Clinical manifestations of PC deficiency are:
- deep venous thrombosis,
- pulmonal embolism,
- thrombophlebitis of the superficial veins,
- cerebral hemorrhagic infarctions, and
- skin necrosis.

Abnormalities in the PC molecule can vary: disorders in the binding site for Ca, inability of binding to thrombin-thrombomodulin complex or to PS, disorders in the fibrinolytic activation system, or disorders in cleavage of the natural substrates F Va and F VIIIa. Several different assays should be performed in order to determine the exact PC dysfunction.

Thrombosis does not necessarily occur in all patients with PC deficiency. Families with heterozygous members without thrombotic complications have been described, despite the fact that they were exposed to risk factors such as pregnancy, major surgeries or traumas. Homozygous PC deficiency is manifested with far more dramatic symptoms. Soon after birth, extensive thrombosis of visceral veins or skin necrosis with fatal consequences may occur (Branson et al, 1983). The PC level is very low (6 to 10%). PC deficiency is caused either by disorders in normal protein synthesis or by abnormal protein synthesis. In the majority of cases, patients have both functional defects and decreased antigen concentrations. Since patients with liver diseases and DIC may have decreased PC levels, these conditions
should be excluded prior to diagnosis of an inherited PC deficiency. The concentration of other vitamin K-dependent factors should be determined prior to diagnosis because the concentration is normal in inherited PC deficiency.

In 1981, Griffin first described a family with recurrent venous thromboembolism due to inherited PC deficiency with a PC level of around 50%. Prevalence of PC deficiency is similar to that of AT III deficiency. In the majority of cases, patients have half the normal level of PC. Inheritance is most frequently heterozygous through the autosomal gene of variable penetration. Children may inherit PC deficiency from both parents. Newborns with very low, even undetectable levels of PC, whose parents had 50% of the normal PC values, have been described in literature. Clinical characteristics of heterozygous and homozygous deficiencies are completely different.

In heterozygous deficiency, clinical signs are very similar to those of inherited AT III deficiency. Patients suffer from DVT or lung embolism, which appear prior to the age of 40, with recurrent thromboembolisms without any apparent external reasons. Thrombophlebitis of the superficial veins and cerebral hemorrhagic infarctions, rare in AT III deficiency, are typical characteristics of PC deficiency. Another characteristic is the occurrence of skin necrosis caused by progressive thrombosis in skin microcirculation at the beginning of oral anticoagulant therapy.
Acquired PC deficiency
Acquired PC deficiencies occur:
- in oral anticoagulant therapy,
- in liver diseases,
- in DIC,
- during neonatal period,
- postoperatively,
- in renal diseases, and
- in acute lymphocytic leukemia during treatment with L-asparaginase.

Since only few patients with PC deficiency suffer from thrombosis, no antithrombolytic therapy is administered only on the account of the discovered deficiency. Long-term prophylaxis is applied in patients with previous thrombotic episodes and a short-term prophylaxis in patients with PC deficiency when exposed to risk factors such as surgery, trauma, immobilization or pregnancy.

Clinical experience shows that OAT is useful in the prevention of thrombosis in patients with PC deficiency, despite the fact that those drugs inhibit anticoagulants as well. Skin necrosis can appear in some patients at the beginning of oral anticoagulant drug administration. Thus, the initial dose has to be low and be increased gradually with heparin administration in the first 7 to 10 days. Theoretically, even heparin can be a risk for patients with PC deficiency because it does not increase PC inhibitor activation, which has a potential prothrombotic effect. It is still
unknown whether this phenomenon in vitro has clinical implications. Replacement therapy using FFP is used prophylactically in prethrombotic conditions when oral anticoagulant therapy is a contraindication (pregnancy, delivery, surgery). FFP cannot be used in long-term prophylaxis due to a short PC half-life.

Therapy for PC deficiency (only for patients with clinical signs of disease).
- heparin first 7 to 10 days
- oral anticoagulant therapy
- FFP
- PC concentrate
- F IX complex
- steroid anabolics

The prothrombin complex concentrate (F II, F VII, F IX and F X) contains large amounts of PC (Manucci 1983), probably in its activated form as well. Its use, however, is not recommended in patients with PC deficiency since activated coagulation factors found in this preparation increase the risk of thrombosis in those patients. There are reports on the successful application of steroid antibiotics (stanozolol and dinasol; Mannucci, 1984). Their application in patients with PC deficiency increased PC levels to normal values. Reports about administration of PC concentrate and APC concentrate also came to a positive result.

Treatment of PC deficiency is similar to AT III deficiency. Because of the risk of warfarin-induced skin necrosis, oral an-
ticoagulation is given with concomitant heparin therapy; heavy loading doses of warfarin should be avoided. Neonatal purpura fulminans is treated by replacement of PC with either plasma infusions or PC concentrate.

PS Deficiency
PS is a vitamin K-dependent anticoagulant (MW 75 kDa) synthesized in hepatocytes, endothelium cells and megacariocytes under the control of two genes on the third chromosome. PS is required for the activity of APC in the inactivation of F Va and FVIIIa. However, APC and protein S have stronger activity in the presence of F V. PS and F V act synergistically as APC cofactors. PS in circulation binds to C4b complement component, and this way more than 50% of PS in circulation does not participate in hemostasis.

PS deficiency is an autosomal dominant inheritance of thrombophilia. Clinical features and therapy are similar to PC deficiency. A deficiency in PS, a vitamin K-dependent protein that is a cofactor for the anticoagulant activity of APC, is associated with hypercoagulability due to the fact that F V and VIII are not properly inactivated, thrombin generation is not inhibited, and fibrinolysis is not stimulated.

In 22.4% of heterozygotes with the lack of PS, presence of F V Leiden has been demonstrated. Double heterozygotes have more frequent venous thromboses at a younger age, which contributes to the statement that double disorders are not rare and that they increase the risk of thrombosis.
Thrombomoduline Disorder
Thrombomoduline plays an important role in PC activation. A thrombomoduline gene has recently been mapped and cloned on the 20th chromosome. So far, three thrombomoduline gene mutations have been discovered. The incidence of thrombomoduline gene mutation among 145 studied patients with venous thromboembolisms was 5%.

The Prothrombin 20210A
In 1996, Poort et al. published the discovery of a new genetic prothrombin disorder characterized by point mutation of a prothrombin gene where guanin located on the 20210 position is replaced by adenine. Prothombin G20210A increases prothrombin activity, and it is associated with arterial and venous thromboses.

The prothrombin 20210A allele is a term for a genetic defect in the gene for prothrombin (F II). This defect is associated with an elevated plasma level of prothrombin, which leads to a state of hypercoagulability.

Mutation of the 20210 prothrombin has been demonstrated in 2.3% of healthy people and 6.2% of patients with deep venous thromboses. However, in a selected group of patients with a personal and family history of venous thromboses, the incidence of this mutation is 18%. This points to the fact that mutation of the 20210 prothrombin is a relatively frequent thrombophilia, in comparison to lack of antithrombin, PC or PS, with a relative thrombosis risk of 2.8%.
In cases of arterial thromboses, such as myocardial infarction, cerebrovascular and peripheral arterial thromboses, the prevalence of 20210 prothrombin ranged from 1 to 7% (3.2% average), based on 10 studies published in the past two years where total of 1839 patients were investigated. However, in the control groups (total of 2750 investigated subjects), the prevalence of 20210 prothrombin ranged from 1 to 4.3% (1.5% average). These investigations have clearly pointed that prothrombin mutation increases the risk of myocardial infarction around 2.5 times, placing it among moderate risk factors for arterial thrombosis.

Inherited Resistance to APC / Factor V Leiden
Factor V Leiden / APC resistance is caused by a single point defect in the gene coding for coagulation F V. This genetic mutation, F V Leiden, is the most common cause of thrombophilia and is found in 4 to 6% of the U.S. population and 20 to 40% of patients with an episode of confirmed DVT. In patients with transient risk factors such as surgery or trauma, a F V Leiden defect can increase the risk of thrombosis eighty-fold.

APC comes from autosomal dominant inheritance and may be the most common inherited thrombophilia. Prevalence in general population is 25.5%. The risk of thrombosis is 5 to 10 times higher in heterozygotes and 50 to 100 times higher in homozygotes. Resistance to APC occurs due to point gene mutation that leads to F VIII and F V molecule alteration, termed as Leiden type, which is incapable of inactivation in the presence of APC and in the end results in thrombophilia. The disease is
most frequently manifested as venous thrombosis in younger subjects. By the age of fifty, 50 to 70% of patients with the resistance to APC develop thrombosis. Acute thrombosis in patients with the resistance to APC should be treated with heparin and oral anticoagulant therapy. Prophylactic anticoagulant therapy is need both in homozygotes and heterozygotes with resistance to APC as well as in patients with two or more disorders with higher risk of thrombosis.

Abnormality of Fibrinogen and Fibrinolysis
Dysplasminogenemia, inherited abnormality of fibrinogen, rarely may result in thrombosis. Inherited abnormality of fibrinolysis, hypoplasminogenemia (decreased level of t-PA, increased level of PAI-1), is also associated with thrombophilia. Treatment of acute thrombosis in these abnormalities is heparin ant oral anticoagulation.

Disorders of plasminogen and plasmin activation include two main types. The mechanism for hypercoagulability acts through either reduced fibrinolytic capacity due to increased activity (PAI-1) or decreased levels of plasminogen.

Dysfibrinogenemia has several possible mechanisms for increased thrombotic risk including fibrin resistance to plasmin proteolysis, the presence of fibrinogen that is not susceptible to normal fibrinolytic mechanisms, defective thrombin binding, enhanced platelet aggregation, increased blood viscosity, and an alteration of clotting architecture.
Homocysteinemia is defined as excess plasma and is most commonly caused by genetic defects, vitamin deficiency (folic acid, B6, B12), or renal failure.

Thrombomodulin disorder can be added to this list because of its significant role in the PC activation procedure.

Thromboembolic disease, which is thought to be initiated by damage to vascular endothelium, is the major cause of morbidity and mortality in patients with homocysteinemia.

Thrombophilia caused by antithrombin deficiency, PC and PS deficiencies occurs in only 5 to 15% of patients with thrombophilia. However, investigations demonstrated that resistance of F Va to APC is one of the most frequent mutations with a 25 to 50% prevalence in patients with thrombosis.

**Acquired Thrombophilia**

**Table IX.2**

<table>
<thead>
<tr>
<th>Acquired Risk Factors for Developing Thrombophilia</th>
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<tbody>
<tr>
<td>Antiphospholipid antibody syndrome</td>
</tr>
<tr>
<td>Lupus anticoagulant</td>
</tr>
<tr>
<td>Myeloproliferative disorders</td>
</tr>
<tr>
<td>Hyperviscosity syndromes</td>
</tr>
</tbody>
</table>
Antiphospholipid Antibody Syndrome (APS)
Antiphospholipid antibodies (e.g., anticardiolipin (aCL) antibodies) are directed against negatively charged cell membrane phospholipids and are present in approximately one-third of the patients with systemic lupus erythematosus (SLE).

Lupus Anticoagulants
The lupus anticoagulant is an IgG or IgM antiphospholipid antibody that attacks the phospholipid portion of platelets and endothelial cell membranes. The exact mechanisms of thrombosis formation are still unknown, but they are thought to include the inhibition of AT III and plasminogen to plasmin conversion, platelet damage leading to increased adhesiveness, thrombomodulin inhibition causing decreased PC activity, and vascular damage caused by the vasculitis commonly present in lupus patients.

Lupus is the most frequently acquired hypercoagulable state. Lupus-type anticoagulants are immunoglobulins, usually IgG or IgM, that prolong phospholipid-dependent in vitro coagulation assays but do not inactivate any of the known protein coagulation factors. aCL antibodies are related to, but are not identical with, antiphospholipid antibodies. The prevalence of lupus-type anticoagulants in the general population is 1 to 2%. About 60% of affected individuals have recurrent thrombosis.

Clinical manifestation of bleeding is very unusual unless there is associated hypoprothrombinemia, thrombocytopenia, or
platelet dysfunction. Both venous and arterial thromboses are associated with lupus-like anticoagulants. Recurrent fetal loss and intrauterine growth retardation in patients with SLE have been linked to the presence of lupus-like anticoagulants.

Most patients with lupus-like anticoagulants need no specific treatment. Prophylactic anticoagulant therapy should be considered during periods of high thrombotic stress. There are no controlled trials of the treatment of recurrent fetal loss, but high-dose glucocorticoids, aspirin, and adjusted dose subcutaneous heparin have all been reported to be useful.

Myeloproliferative Disorders (MPDs)
MPDs are commonly grouped into four diseases: polycythemia vera, myelofibrosis, thrombocythemia, and chronic myelogenous leukemia. Chronic MPDs are characterized by a high incidence of thromboembolic complications due to the hyperviscosity of blood and an inappropriate level of platelet activation including elevated levels of platelet-specific proteins, increased TA2 generation, and an over-expression of activation-dependent epitopes on platelet surfaces.

Hyperviscosity syndrome is associated with an increased concentration of whole blood constituents including fibrinogen, antibodies, and other proteins (e.g., Waldenstrom’s microglobulinemia). Treatment should be based on the elimination of risk factors and administration of heparin, oral anticoagulant drugs, fibrinolytics, antiaggregation therapy, and AT III.
Other Acquired Hypercoagulable States
Other acquired hypercoagulable states are: venous stasis, malignancy, hyperestrogenic states, prothrombin complex concentrate therapy, postoperative state, myeloproliferative diseases, paroxysmal nocturnal hemoglobinemia, hyperlipidemia, diabetes mellitus, homocystinuria, hyperviscosity syndrome, cigarette smoking, sickle cell anemia, and nephrotic syndrome.

If the excessive coagulation activation is higher than the protective biological inhibition, thrombosis occurs. The majority of the patients suffering from thrombosis has no identifiable hemostatic disorders, and thrombosis occurs in combination with some other disease.
Most patients with thrombosis have no clearly recognizable hemostasis disorder that contributes to the occurrence of thrombosis. However, in a number of patients, inherited or acquired he-

Figure X.1: Thromb in blood vessels
mostasis disorders have been demonstrated, and they probably contribute to the occurrence of thrombosis as well as recurrent thrombosis. Likewise, certain physiological conditions of diseases or certain drugs can lead to the appearance of thrombosis through numerous mechanisms.

The major risk factors for arterial thrombosis, myocardial infarction and cerebral infarction are hypertension, smoking, diabetes, obesity, and hypercholesterolemia.

Among the most common and frequent risk factors for venous thromboses are: tissue damage, stasis, trauma, surgical

Figure X.2: Venous stasis, vein wall injury or a hypercoagulable state are the components of Virchow’s triad and are thought to lead to the development of DVT, acquired and congenital risk factors.
procedures, immobilization, malignancy, pregnancy, and puerperium.

Risk factors common for both arterial and venous thrombosis are oral contraceptive drugs, hypercholesterolemia, lupus anticoagulants, and a high level of F I and F VIII. Risk factors can be inherited (see Thrombophilia) or acquired.

**Deep Venous Thrombosis (DVT)**

Venous thromboembolism (VTE) is a widespread clinical problem associated with significant morbidity and mortality. Pulmonary embolism (PE) is presently the leading cause of preventable death in hospitalized patients. Untreated DVT predisposes patients to episodes of recurrent VTE and the development of the post-phlebitic syndrome (PTS), which can involve a constellation of symptoms ranging from leg edema, pain, aching and tiredness to the development of skin discoloration, scarring and even open ulceration. Surgical patients, in particular, are at a high risk for DVT since surgery itself is very traumatic and is often accompanied with bed rest that increases venous stasis.

Prevention is the key to reducing death and morbidity from VTE. The key to appropriate prophylaxis is risk factor analysis (RFA). Prophylactic treatment should be given to patients at high or moderate risk for DVT whereas it may be unnecessary in patients at low risk for DVT.
Thrombosis is a disorder in which a coagulum is formed in the blood vessel due to an interaction of vascular, cellular and humoral factors. In normal conditions, there is a dynamic balance between activators and inhibitors with a slight prevalence of inhibitors; this is why blood normally remains fluid in circulation. When a blood vessel is injured, formation of a coagulum is beneficial for the organism since it blocks the defect. If a blood vessel is not injured, thrombus formation is a pathological process and harmful for the organism. Thus, a thrombus is an abnormal product of a normal mechanism. According to MacPharlane, “Thrombosis is hemostasis on the wrong site.”

If inhibitors prevail, hypocoagulability of blood and hemorrhagic syndrome occur. When activators prevail, hypercoagulability and thrombosis occur. Thrombosis is far more frequent than hemorrhagic syndrome. Around two thirds of the world population suffer and die due to thromboembolic complications. Thrombi are formed in all blood vessels: arteries, veins, capillaries and cardial cavities. Thrombi are divided into two groups: occlusive and parietal thrombi, depending on whether a thrombus completely or partially blocks the blood vessel lumen. Formation of an occlusive thrombus leads to a complete obliteration of the lumen and inhibition of blood circulation while a parietal thrombus narrows the lumen and slows down the blood flow.

Regardless of the site of thrombus formation, i.e., arterial or venous blood vessels, both primary (vascular and platelet) and
secondary (coagulation) stages of hemostasis play significant roles in the pathogenesis of thrombosis. A thrombus is formed in arteries primarily due to endothelium damage. Platelets adhere to damaged endothelium and open subendothelial tissue. Platelets from circulation stick to the adhered platelets, platelet aggregation occurs, and a platelet thrombus is formed that is consequently stabilized by fibrin fibers from coagulation activation. Due to the velocity of blood circulation in arterial blood vessels, circulation carries activated blood coagulation factors. Thus, a thrombus mainly consists of aggregated platelets in which fibrin tissue is interwoven.

A hypercoagulability state is either the result of increasing coagulation F I, F VIII, F XII or decreasing of inhibitors.

Decreased concentration of F XII (plasminogen activation) can be found as well as defective fibrinolysis in F XII deficiency (Hageman died from thrombosis). There is also a deficiency of natural inhibitors, both inherited and acquired. AT III inactivates thrombin, Xa, IXa, XIa, XIIa, kallikrein, plasmin and trypsin. PC and PS inactivate F VIIIa and F Va and release plasminogen activators from the endothelium. Deficiency is found in liver insufficiency, DIC, in newborns and postoperatively. Clinical manifestations are recurrent thromboembolisms.
In venous blood vessels, circulation is slower. This is why activated platelets and coagulation factors are deposited. The endothelium is usually intact, at least in the beginning. Activation of coagulation plays the most important role in coagulum formation. Later the endothelium is damaged because the factors that cause damage (antibodies, immune complexes, etc.) are deposited due to stasis. A thrombus formed in veins is similar to coagulum formed in vitro conditions, consisting of a small platelet-fibrin head and a long tail.

Significant factors in the pathogenesis of thrombosis are alterations in blood vessel walls, changes in blood circulation (stasis), and altered composition of blood. Normal, undamaged endothelium is the main barrier for the prevention of platelet adhesion and aggregation because it produces prostacycline, which is a powerful antiaggregation agent. Endothelial damage causes platelet adhesion to subendothelium and activation of coagulation. Endothelial damage may be caused by the following mechanisms:

Table X.1 (based on Bertina R.M. Thromb Haemost 2001.)

<table>
<thead>
<tr>
<th>Main Risk Factors for Venous Thrombosis</th>
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<tbody>
<tr>
<td><strong>Acquired Risk Factors</strong></td>
</tr>
<tr>
<td>Age</td>
</tr>
<tr>
<td>History of venous thrombosis</td>
</tr>
<tr>
<td>Surgery</td>
</tr>
<tr>
<td>Cancer</td>
</tr>
<tr>
<td>Hormonal treatment</td>
</tr>
<tr>
<td>Antiphospholipid syndrome</td>
</tr>
</tbody>
</table>
- stenosis (formation of a poststenotic whirlpool, mechanical endothelium damage),
- hypoxia and anoxia (cause swelling and oedema of endothelial cells, cleaving of endothelium, splitting of endothelial cells and revealing of subendothelium; reduction of t-PA synthesis),
- presence of antibodies against endothelial cells,
- presence of immune complexes,
- leukocytes adhered by toxic metabolic products,
- compounds of platelet granules (ADP, serotonin, cation proteins that pass through parts of the blood vessel wall with increased permeability, serotonin, fibrinopeptide B, and T A₂ cause blood vessel spasms and mechanically damage endothelium),
- hypertension (increased filtration pressure),
- hypercholesterolemia,
- smoking (endothelium cleavage by carbon dioxide formed from smoke that binds to hemoglobin and leads to hypoxia, which results in endothelium cleavage. Moreover, smokers’ platelets are sensitive to the effect of aggregation agents and less sensitive to the effect of prostacycline, which, in addition, is produced in smaller amounts), and
- viruses, endotoxins, bile salts, homocysteine.

Stasis itself does not lead to thrombus formation unless it is accompanied by blood hypercoagulability, decreased fibrinolytic activity, or deficiency of natural inhibitors. A thrombus is most frequently formed in veins of lower extremities and pelvis due to
the decreased tonus of blood vein walls of the lower extremities, particularly in pregnancy, and due to a decreased fibrinolytic activity in veins of lower extremities (decreased t-PA synthesis and increased formation of t-PA inhibitors). Furthermore, changes in blood composition are related to an increased concentration of coagulation factors, either inherited or acquired, in liver disorders and in pregnancy. Basic manifestations of these changes are slower blood circulation in the veins of the lower extremities, increased concentrations of F I, V, VII, VIII and X, decreased fibrinolytic activity, and increased t-PA inhibitor concentration.

**Risk Factors Associated with DVT**

Inherited Risk Factors
(See Thrombophilia.)

Acquired Risk Factors for the Development of DVT
(See Table X.2.)
After 40 years of age, the risk of developing a DVT increases rapidly. Firstly, venous stasis and blood pooling in the lower extremities occurs. Secondly, older people are more highly associated with co-morbid conditions such as surgery and malignancy, which are additional risk factors for DVT. There are increased levels of fibrinogen, prothrombin activation, fibrinopeptide A (FPA), and PC activation. Decreased activity of the fibrinolytic system, decreased levels of plasminogen activators, and increased levels of PAIs have been observed in older people.

- Prolonged use of a catheter predisposes patients to infection and sepsis; both increase the risk of catheter-related thrombogenesis thrombi due to intravenous catheters. The risk of developing DVT also increases with the duration of catheterization.

<table>
<thead>
<tr>
<th>Acquired Risk Factors for the Development of DVT</th>
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<tbody>
<tr>
<td>Acute spinal cord injury</td>
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<tr>
<td>Age</td>
</tr>
<tr>
<td>Central venous access</td>
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<tr>
<td>Congestive heart failure</td>
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<tr>
<td>Elective major lower extremity arthroplasty</td>
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<tr>
<td>Heparin-induced thrombocytopenia</td>
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<tr>
<td>Hip, pelvic, or proximal femur fracture</td>
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<tr>
<td>History of DVT or PE</td>
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<tr>
<td>Hormone replacement therapy</td>
</tr>
<tr>
<td>Homocysteinemia</td>
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<tr>
<td>Immobilizing plaster casts</td>
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<tr>
<td>Inflammatory bowel disease</td>
</tr>
<tr>
<td>Laparoscopic surgery</td>
</tr>
<tr>
<td>Major surgery</td>
</tr>
<tr>
<td>Malignancy</td>
</tr>
<tr>
<td>Minor surgery</td>
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<tr>
<td>Multiple trauma</td>
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<tr>
<td>Myocardial infarction</td>
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<tr>
<td>Obesity</td>
</tr>
<tr>
<td>Oral contraceptives</td>
</tr>
<tr>
<td>Pregnancy</td>
</tr>
<tr>
<td>Sepsis</td>
</tr>
</tbody>
</table>
- The frequency of VTE in patients with congestive heart failure is between 20% and 40%, which is similar to that in patients with acute myocardial infarction.

- Elective, major, lower extremity arthroplasty includes total hip or knee replacement surgery. Despite prophylaxis, the incidence of venographically detected DVT after total hip or knee replacement surgery remains high at approximately 15% and 30%, respectively.

- Hip, pelvic, or proximal femur fractures have a 35 to 60% incidence of DVT. History of DVT/PE as a risk factor for DVT recurrence has been confirmed by univariate and multivariate analyses.

- Hormone replacement therapy increases the risk of developing DVT by two to six-fold. Estrogen use leads to changes in the body’s hemostatic balance including an increase in coagulation factors II, X, VII, IX, VIII, fibrinogen, and vWF and a decrease in total and free PS, AT III, and fibrinolytic activity.

- Long leg casts lead to blood flow reduction and stasis due to immobilization.

- Inflammatory bowel disease is associated with having open ulcers in the bowel that allow toxic substances to be absorbed into the bloodstream.
- Laparoscopic surgery (>45 minutes) has an estimated post-operative DVT rate of 7 to 10%.

- Major surgery is considered to be an operative procedure greater than 45 minutes in length that is conducted under general anesthesia.

- Malignancy patients with clinically overt cancer have a high risk of developing a DVT. The etiology of thrombosis in malignancy is multifactorial and mechanisms include the release of procoagulants by tumor cells with the ability to cause coagulation dissemination, impaired fibrinolytic activity, activation of blood clotting factors (V, VIII, IX, X) and abnormal platelet vessel interactions (Fig. X.3).

- Multiple trauma patients who do not receive adequate prophylaxis have a 20%–90% incidence of DVT. Trauma exposes patients to multiple risk factors including immobilization, major surgery, central venous access and infection, depending on the severity of the trauma. Hypercoagulability injury to the vessel endothelium is accompanied by a loss of protective molecules and an expression of adhesive molecules. Furthermore, damaged endothelium has enhanced procoagulant activity, such as secretion of vWF to increase platelet adhesion and aggregation.

- A myocardial infarction can lead to increased activation of the coagulation system, immobilization, and venous stasis.
In addition, myocardial infarction is a reflection of underlying systemic artherosclerotic disease. In women, the use of oral contraceptives increases the risk of developing DVT two to six-fold. They lead to changes in the body’s hemostatic balance, including an increase in coagulation factors II, X, VII, IX, VIII, fibrinogen, and vWF and a decrease in total and free PS, AT III, and fibrinolytic activity.

Figure X.3: A schematic diagram illustrating the relations among tumor cell-secreted VEGF-A, vascular hyperpermeability, and extravascular fibrin deposition and degradation. H.F. Dvoral, F. Rickles, 2006
Obesity can cause increased immobility and is associated with reduced fibrinolytic activity.

Pregnant women and women who are less than one month postpartum have a risk of DVT. The greatest risk of DVT occurs during the third trimester of pregnancy. Due to increased levels of natural hormones, pregnancy is marked by increased coagulation factors and decreased fibrinolysis activity including elevated levels of F VII, F VIII, F IX, F X, and fibrinogen and significantly reduced levels of PS.

Sepsis is associated with the presence of foreign proteins, damaged cells, organisms, or toxins in the blood that can cause activation of the complement cascade, a state of hypercoagulability, and lowering of AT III levels in the blood.

Varicose veins can cause symptoms that mimic DVT (i.e., pain, edema, skin changes).

Lupus anticoagulant presence is also typical. Lupus anticoagulants are antibodies bound to platelet phospholipid, which prolong all blood coagulation tests in which TrF3 participates. These persons do not bleed, but they have thrombosis.

PNH (paroxysmal nocturnal hemoglobinuria) denotes increased sensitivity of red blood cells to complement erythrocyte hemolysis, release of tissue thromboplastin, and ADP from degraded erythrocytes.
- Acute promyelocyte leukemia is always followed by DIC induced by interleukin 1, although the detailed mechanisms are not known.

- In cases of hemolytic anemia, erythrocyte stroma acts thrombogenously.

- MPDs are commonly grouped into four diseases: polycythemia vera, myelofibrosis, thrombocythemia, and chronic myelogenous leukemia. The chronic MPDs are characterized by a high incidence of thromboembolic complications due to the hyperviscosity of blood and an inappropriate level of platelet activation, including elevated levels of platelet-specific proteins, increased TA2 generation and an over-expression of activation-dependent epitopes on platelet surfaces.

- Hyperviscosity syndrome is associated with an increased concentration of whole blood constituents including fibrinogen, antibodies, and other proteins (e.g., Waldenstrom’s microglobulinemia). Viscosity of blood is increased in polycytemia, paraproteinemia, cryoglobulinemia and thrombocytosis.

- Other risk factors that contribute to thrombosis occurrence are:
  - hypercholesterolemia
  - diabetes (diet, drugs)
  - hypertension (drugs)
- stress (any etiology)
- arteriosclerosis
- smoking.

**Diagnosis**

Most patients suffering from thrombosis have no visible signs of hemostasis disorders contributing to the development of thrombosis. Diagnosis of thrombosis is not easy. The most frequently disturbed laboratory parameters are the following:
- AT III - decreased
- PC - decreased
- fibrinolysis - slow
- fragment F$_{1,2}$ - present
- fibrinopeptide A - increased
- FDP - present
- b-thromboglobulin and TrF4 (specific markers of activated platelets) - increased
- platelet adhesion (Adeplat T) - increased
- spontaneous platelet aggregation - present
- aggregation induced by aggregating agents - increased (>100 %)
- platelet survival - shortened
- PTT - shortened
- coagulation factors (fibrinogen, F V and VIII in particular) - increased
Treatment

Treatment should be based on the elimination of risk factors and administration of heparin, oral anticoagulant drugs, fibrinolytics, antiaggregation therapy, and AT III. (See anticoagulant treatment.)

Conclusion

DVT and PE remain a genuine threat to many surgical and medical patients. If the risk factors are known, the disease is preventable. In the future, the designation of patients for venous duplex scanning may be based on RFA and D-Dimer testing in order to preserve valuable vascular laboratory resources.
ANTITHROMBOTIC THERAPY

Antithrombotic and thrombolytic agents are commonly administered in the intensive care unit. Over the last decade, major advances involving these agents have transpired.

The administration of heparin to patients with venous thromboembolic disease should be guided by the recently developed heparin monogram. The development of low molecular weight heparin (LMWH) may revolutionize the treatment of thrombotic and embolic disorders.

There has been a decreased incidence of stroke in patients with nonvalvular atrial fibrillation receiving oral anticoagulants. The international normalized ratio (INR) has standardized the monitoring of oral anticoagulation.
There has also been a decrease in vascular events and mortality of patients receiving aspirin. Thrombolytic trials involving acute myocardial infarction document improved survival of patients receiving thromboembolic agents.

This chapter highlights these advances and provides practical guidelines for the administration of antithrombotic and thrombolytic therapy.

**Antiplatelet Therapy**

During the past few years, antiplatelet drugs have played an ever-increasing role in the treatment and prevention of arterial thrombosis. The most frequently used drugs are aspirin, dipiridamol and sulphinpirason.

The need for efficient antiplatelet drugs is related to the role of platelets in the occurrence of arterial thrombosis and to the fact that arterial thrombosis is the underlying factor for numerous diseases that are at the top of morbidity and mortality lists in the most countries worldwide.
Apart from the above listed indications, certain antiplatelet drugs have demonstrated a significant efficacy in the prevention of diabetes retinopathy, in the treatment of preeclampsia in obstetrics, in the treatment of membrane proliferative glomerulonephritis in nephrology, in the treatment of erythromelalgia as a part of myeloproliferative diseases, as well as in the treatment of other diseases in which arteriolae are affected by pathological processes.

Antiplatelet drugs can be used individually or in a combination of two antiplatelet drugs having a different effect mechanism as well as combined with heparin, oral anticoagulants or thrombolytic therapy.

Table XI.1

<table>
<thead>
<tr>
<th>Antiplatelet drug indications are many and include:</th>
</tr>
</thead>
<tbody>
<tr>
<td>myocardial infarction, primary and secondary prevention, acute myocardial infarction supported with thrombolytic therapy</td>
</tr>
<tr>
<td>unstable and chronic stable angina pectoris</td>
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<tr>
<td>maintenance of aortocoronary bypass flow by venous grafts</td>
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<tr>
<td>prevention of restenosis following angioplastics</td>
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<tr>
<td>conditions following artificial heart valves implantation, along with oral anticoagulant therapy</td>
</tr>
<tr>
<td>peripheral arterial disease as well as conditions following venous graft bypasses of peripheral arterial occlusions</td>
</tr>
<tr>
<td>arterial-venous shunts</td>
</tr>
<tr>
<td>ischemic cerebrovascular insults and transitory ischemias of the central nervous system</td>
</tr>
<tr>
<td>conditions following endarterectomy of carotid arteries</td>
</tr>
</tbody>
</table>
Cyclooxygenase Inhibitors – Aspirins

Aspirin has an inhibitory effect on prostaglandin synthesis, which explains most of its anti-inflammatory, antipyretic and antiplatelet effects. Acetylsalicylic acid has been the most frequently used antiplatelet drug all over the world for a very long time.

Acetylsalicylic acid causes a permanent functional platelet defect by irreversible acetylation of cyclooxygenase (prostaglandin H synthesis), the key enzyme in the metabolism of arachidonic acid, leading to a decrease of synthesis of prostaglandin H₂ and T A₂. Aspirin causes hydroxyl serine group acetylation on site 529 due to which cyclooxygenase enzyme permanently loses its activity.

The antiplatelet action of aspirin is mediated by the irreversible inhibition of platelet cyclooxygenase (prostaglandin G/H synthetase). Platelet cyclooxygenase converts arachidonate to prostaglandin G₂; ultimately, T A₂ and prostaglandin H₂ are produced. T A₂ is a potent inducer of irreversible platelet aggregation. Furthermore, aspirin reduces eicosanoid production (prostaglandin E₂, prostacyclin, and T A₂) in different tissues, accounting for a variety of pharmacologic effects. Since platelets cannot synthesize new enzymes, the inhibition is permanent for the lifespan of the platelet. Aspirin rapidly disappears from circulation, but the effect is irreversible lasting up to 5 days, sometimes longer (up to 10 days).
With a dose of 300 mg of aspirin, bleeding time is prolonged and platelet aggregation is disturbed. Aspirin inhibits collagen-induced platelet aggregation and secondary aggregation to weak agonist, such as ADP and epinephrine. Aspirin can lead to bleeding in patients with gastrointestinal disorder. Administration should be avoided in patients treated with some other anticoagulant or hemorrhagic syndrome. Platelet concentrates are used for the management of bleeding episodes in order to compensate for the patient’s non-functioning platelets.

A laboratory measurable effect of aspirin, i.e., platelet aggregation inhibition and prolonged bleeding time, can be achieved using a dose that reduces platelet cyclooxygenase activity by over 95%. In order to achieve this effect, treatment should be initiated with the dose of at least 120 mg and followed with daily doses of 100 mg. The time interval between two aspirin doses can be 48 hrs if daily platelet production is 10 to 15%, i.e., if it is normal. If the production is higher, which is the case in the course of acute thrombotic incidents, the time interval between doses should be 24 hours.

Gastrointestinal side effects, in particular upper gastrointestinal tract bleedings, are dose dependant.

In addition to acetylsalicylic acid, other non-steroid anti-inflamatory drugs have an inhibitory effect on T A₂ synthesis through cyclooxygenase enzyme inhibition, including sul-
Phosphodiesterase Inhibitors – Dipyridamole

The most significant representative of this group of drugs is dipyridamol, a drug with vasodilatory and antiplatelet effects. The antiplatelet effect mechanism can be explained through the inhibition of cycle nucleotides phosphodiesterase and, to a lesser degree, PGI2 synthesis stimulation. Dipyridamole is used in combination with other antiplatelet drugs, most often with acetylsalicylic acid or oral anticoagulants, and primarily in patients with artificial prosthetic implants since its efficacy is considerably higher in these conditions as compared with its effect on biological surfaces.

Dipyridamole is a phosphodiesterase inhibitor with vasodilator effects. It increases platelet cAMP (cyclic adenosine monophosphate) levels or indirectly increases adenosine plasma levels. Dipyridamole does not inhibit aggregation of plateletrich plasma in vitro, but it does inhibit aggregation of platelets in the presence of erythrocytes, as measured by whole blood aggregometry.

Tienopyridines – Ticlopidinee

This group of antiplatelet drugs, including ticlopidine and clopidogrel, has an inhibitory effect on ADP-induced platelet
aggregability without any influence on arachidonic acid metabolism. None of these drugs have an effect on ADP-induced platelet aggregability in vitro; this is favorable because their efficacy in the organism requires transformation into an active metabolite. The most likely mechanism of their effect is the irreversible damage of the platelet ADP receptor, leading to the inhibition of adenyl cyclase activity. The optimal effect is achieved by using a 250 mg dose of ticlopidine twice a day while clopidogrel is administered once a day in doses ranging from 50 to 100 mg. In order to achieve a complete antiplatelet effect during the use of ticlopidine, at least 7 days of treatment are required. With clopidogrel administration, the antiplatelet effect begins faster. Normalization of platelet function is obtained 7 days after the administration of the last dose.

The most significant side effects of ticlopidine are neutropenia—occurring in around 1% of patients, skin rash, and gastrointestinal conditions such as nausea and diarrhea. In some countries, these severe side effects have limited the use of ticlopidine to patients allergic to aspirin. Advantages of clopidogrel compared with ticlopidine are less severe side effects, faster effect initiation, and single dose administration.

Ticlopidine prolongs the bleeding time and inhibits aggregation induced by shear high force and by ADP. Its mechanism of action is unclear; it may inhibit the ability of ADP to activate GPIIb/IIIa.
Unlike aspirin, the thienopyridines do not affect arachidonic acid metabolism in platelets or prostacyclin metabolism in the vasculature. Maximal platelet inhibition occurs 3 to 5 days after initiating ticlopidine and dissipates within a week after discontinuing the drug.

Ticlopidine is effective only when it is given orally. Its effects are irreversible for the lifespan of the platelet. Recommended dose is 250 mg. Adverse effects include diarrhea and rash. Neutropenia may occur, usually in the first 3 months of treatment, and may be severe but is reversible. Ticlopidine should not be administered as a first-line antiplatelet agent. It should be reserved for patients who do not tolerate aspirin.

**GPIIb/IIIa Antagonists**

It has been noted that the expression of the functionally active GPIIb/IIIa on platelet surface is the final mutual act in the process of platelet activation. Regardless of the causative agonist, this glycoprotein has become the target of investigation in the field of new antiplatelet drugs.

Investigations performed more than a decade ago, using the animals' arterial thrombosis model, demonstrated that the blockade of GPIIb/IIIa prevents the development of acute thrombosis after the blood vessel damage. The blockade increases degradation of the existing thrombus when used in combination with a thrombolytic drug and heparin. The GPIIb/IIIa blockade also facilitates
the thrombolytic drug effect on the platelet rich thrombus, which is usually resistant to thrombolysis, and prevents reocclusion of blood vessel following reperfusion with thrombolytic drugs.

GPIIb/IIIa receptor inhibitors act in a way that, by binding to this receptor, disables activated platelets to bind with adhesive proteins, such as fibrinogen and vWF. This altogether results in the inability of platelets to aggregate and form thrombus. In addition GPIIb/IIIa have a profibrinolytic effect. Reducing the release of dark and alpha platelet granules content, they decrease the local concentration of fibrinolysis inhibitor and prevent the release of platelet growth factor, ADP, and serotonin, which take part in mitogenesis and migration of smooth muscle cells. Finally, GPIIb/IIIa decrease the local thrombin formation.

The first GPIIb/IIIa inhibitor was a mouse monoclonal antibody. Due to its immunogenicity, chimerized mouse-human 7E3 antibody was soon synthesized. An antibody dose affecting GPIIb/IIIa, sufficient to block around 80% of receptors, almost completely leads to loss of platelet aggregation ability and is associated with only a moderate prolonged bleeding time. Only with a 90% blockage of receptors does bleeding time become noticeably prolonged. A bolus dose of 0.25 mg/kg of b.w. leads to a receptor blockade exceeding 80% and reduces aggregability of platelets induced by ADP to less than 20%, so that the maximum effect is achieved 2 hours after administration of the bolus dose. The platelet function recovery, i.e., returning of the bleeding time to normal values, occurs after 12 hours. If, after
the administration of a bolus dose, treatment is continued with an infusion of 10 microgram/min, receptor blockade, platelet aggregation inhibition, and prolonged bleeding time can be maintained throughout the infusion.

Through the activity of GPIIb/IIIa antagonist, inhibitory effect on platelets could considerably increase the possibility to achieve and maintain coronary reperfusion during thrombolytic therapy, which has so far been demonstrated by clinical investigations. The first results obtained in the TAMI-8 study demonstrated that the use of GPIIb/IIIa antagonist following thrombolytic therapy combined with aspirin and heparin had been efficient. Murine monoclonal antibodies against GPIIb/IIIa have been potent inhibitors of platelet function and thrombus formation in animal models.

**Platelet Receptors for Collagen**

The main signal receptor for platelet activation by collagen is GPVI. Platelets have two direct and one indirect (through vWF) receptors for collagen. Two possible therapies are available: GPVI inhibition on platelets using peptide collagen analogue or blocking the binding site to GPVI on the collagen molecule.

**Thromboxane Synthetase Inhibitors**

Regardless of possible theoretical advantages, clinical investigations of this group of drugs have produced no satisfactory results.
**TA2 and Prostaglandin H2 Receptor Inhibitors**

Long and strong effect TP receptor (thromboxane A2 receptor protein) antagonists have been synthesized: ifetroban and sulotroban. However, they showed disappointing results in the 2nd and 3rd stages of clinical investigation. Unfortunately, they did not fulfill the expectations.

**Other Antiplatelet Drugs**

- **Prostanoids** (iloprost and beraprost)
- **Phosphodiesterase inhibitors III** (cilostazol and serotonin antagonist, sarprogrelat)
- **α-tacoferol**
- **Flavonoids**
- **Persantin** inhibits phosphodiesterase, which degrades cAMP, increasing cAMP level (antiaggregating effect); recommended dose is 400 mg daily.
- **Trental** is pentoxyphilin, which decreases blood viscosity and affects red blood cells, increasing tendency towards deformities. It inhibits phosphodiesterase; recommended dose is 600 mg daily.
- **Prostacyclin (PGI2)** is an inhibitor of platelet aggregation as well as potent vasodilator.
- **Anturan** (sulphinpirason) is a weak inhibitor of platelet function that reversibly blocks cyclooxygenation; optimal dose is 400 mg daily.
- Dextran infusions inhibit platelet function by mechanisms that are not yet clear but may involve alteration of platelet membranes or plasma proteins.

**Follow Up of Antiplatelet Therapy Effects**

In the follow up of the effect of antiplatelet therapy, the following laboratory analyses are used.
- bleeding time
- determination of the degree of induced platelet aggregability along with the addition of various inductors, ADP most frequently
- assessment of the platelet cyclooxygenase activity (measuring the level of thromboxane B2 in the serum, determination of the urinary excretion of thromboxane B2 metabolite, and determination of malondialdehyde level in plasma)
- measurement of the inhibition of fibrin binding with platelets
- determination of β thromboglobulin level as the indicator of platelet activation

Laboratory control is based on platelet aggregation tests.

Clinical indications for antiplatelet therapy are: ischemic heart disease, valvular heart disease, atrial fibrillation, cerebrovascular disease, peripheral vascular disease, myeloproliferative disorders, complications of pregnancy ( eclampsia and pre eclampsia), microangiopathic states, and venous thrombosis.
Anticoagulant Therapy

Anticoagulant therapy includes heparin, oral anticoagulant therapy and fibrinolytic therapy.

Heparin

Heparin is a heterogeneous glycosamine glycan with a molecular weight ranging from 4,000 to 40,000 daltons. 60 to 90% of its structure consists of sulfate disaccharide sequences. The sulfate group lacks certain sequences at position 6 but contains uricopoesis and D-glycosamine residuals. The binding sites for antithrombin are pentasaccharides, which provide the energy. Compared with other regions of the heparin molecule, the structures that antithrombin binds to are very stable. The biological activity of heparin can be modified chemically or by enzymes. Elimination of sulfate groups, for example, decreases the anticoagulant activity of heparin.

Figure XI.1: Anticoagulant therapy
Heparin is formed in mastocytes and basophile granulocytes. Normally it is not found in circulation. It induces anticoagulant effects in parenteral application only. Heparin half-life is short, lasting only around 60 minutes; 4 hrs after administration it is no longer detectable in circulation. Heparin is inactivated in circulation by heparinase, an enzyme formed in the liver and eliminated afterwards through RES. Heparin also inactivates platelet factor that is released from activated platelets. Thus, the heparin effect is increased in patients with thrombocytopenia.

The anticoagulant effect of heparin results from its ability to bind to AT III and to increase AT III effects several times during the inactivation process of serine proteases (thrombin, Xa, XIIa, IXa, XIa, kallikrein, plasmin and trypsin). The binding site for heparin in the AT III molecule is a lysine amino acid. Heparin binding to AT III results in certain conformational alterations of the molecule. It revolves around the AT III molecule, and its binding sites become available for binding with thrombin and other serine proteases.

Heparin and AT III form a reversible complex. After fulfilling its function, heparin can be released from the complex, and the same heparin molecule can then be bound to other AT III molecules. Heparin can bind directly to thrombin; although in this case, its affinity is 50 times lower than that towards antithrombin. Heparin binding to thrombin does not require specific polysaccharide sequences, which are necessary for binding with antithrombin. The effect of heparin on the interac-
tion between AT III and serine proteases depends on the heparin molecular weight.

Fractionation of heparin can be carried out by various methods (affinity chromatography, gel filtration, or precipitation with alcohol). LMWH fractions have a stronger effect on the increase of the reaction between AT III and F Xa while higher molecular weight heparin fractions have a stronger antithrombin effect. The effect on F IXa and XIa also depends on the heparin molecular weight and is similar to the effect induced in thrombin - activity increases with the increase of molecular weight. In the case of F XIIa, the effect is similar to the effect found in F Xa. Clinical application of heparin is most frequent in the following situations:

− prophylaxis of venous thromboses: low doses subcutaneously
− treatment of thromboses; standard doses intravenously
− prophylaxis of recurrent thromboembolisms: standard doses subcutaneously
− as anticoagulant for extracorporeal circulation, during renal dialysis and cytapheresis
− treatment of thrombosis during pregnancy

The therapeutic intention of heparin use is very significant. Various roles of heparin in the prophylaxis and treatment of diseases should be considered in relation to the central role of thrombin. When heparin is used as a prophylaxis, thrombin formation is prevented by the increased activity of AT III. The
heparin-AT III reaction in the inhibition of F Xa is probably much more significant in the context of prophylaxis compared with the ability of AT III to neutralize thrombin. This is because F Xa is a much more potent thrombogenic agent than thrombin itself. However, in cases of acute thrombosis, there is an additional need, apart from preventing new thrombin formation, to neutralize already formed thrombin. Thus, effective doses preventing DVT usually amount to 10,000 IU administered subcutaneously in daily single doses. For the control of diagnosed venous thromboembolisms, higher daily doses, ranging from 25,000 to 30,000 IU administered intravenously, are necessary. In heparin administration, therapeutic values with minimum risk of bleeding should be determined.

Continuous IV infusion of heparin is preferable to repeated IV boluses because of a higher bleeding risk with boluses. An acceptable schedule for full-dose IV heparin is a loading bolus of 5,000 IU followed by an infusion of 30,000 to 40,000 IU per 24 h. For subcutaneous treatment, an initial IV bolus of 5,000 IU can be followed by 17,500 IU subcutaneously every 12 h.

In this context, the results of heparin fractionation are of considerable use, particularly in prophylaxis. For example, LMWH fractions (4,000 to 9,000 daltons) specifically increase F Xa and induce relatively low effects on other factors compared with unfractionated heparin. The use of LMWH is suggested for the prevention of venous thrombosis, in particular postoperative DVT.
For laboratory monitoring of heparin therapy, an aPTT test is most frequently used. It should be performed daily and should be prolonged 1.5 to 2 times compared with the aPTT of normal citrate plasma. No laboratory monitoring is required for routine prophylactic subcutaneous heparin therapy at doses of 5,000 IU twice a day.

LMWHs have an average molecular mass of 6,000 daltons and possess higher anti-Xa activity than antithrombin activity. aPTT is not prolonged by LMWH. Therapy with LMWH may be monitored by chromogenic anti-Xa assays. Usually administered once daily, the dosage and anticoagulant profile depends on the particular LMWH preparation. Accumulating evidence suggests that LMWH are generally as safe and as effective as standard heparin. It is not known if protamine reverses LMWH. Heparin-induced thrombocytopenia has been reported to be caused by LMWH. Warfarin therapy is usually begun simultaneously with heparin.

Complications of Heparin Therapy

During heparin administration some complications might occur.
- Alopecia
- Anaphylactic reactions
- AT III decrease
- Bleeding
- Heparin induced thrombocytopenia (HIT) – Types I and II
- Increase of transaminases
- Osteoporosis
- Skin necrosis

**Bleeding**

The most significant and also the most frequent complication that might occur at any moment during heparin administration is bleeding. It not only occurs when the aPTT value is more than 2.5 times higher than the control aPTT value but also in cases when prolonged aPTT is within the optimal therapeutic range. Heparin induced bleeding episodes are also significantly influenced by the following factors: patient’s age, recent surgical procedures or injuries, thrombotic brain stroke that might have occurred within the previous 14 days, ulcer or renal insufficiency, and previous gastrointestinal or genitourinary bleeding as well as recently applied thrombotic treatment.

Bleeding is more frequent in older patients, in patients using other drugs (due to drug interactions) and in patients who had hemostasis disorders prior to the beginning of heparin treatment. Bleeding is rarely found in prophylactic use of heparin with low doses administered subcutaneously. The highest risk of bleeding is found in patients who recently submitted to surgical procedures and in patients with thrombocytopenia, uremia, a previous history of bleeding, administration of intramuscular injections, and antiplatelet drugs. The most frequent bleedings are hematomas, ecchymosis, hematuria, gum bleeding, and bleeding into perioperative regions as well as any other forms of bleeding.
In order to control mild bleeding episodes, it is sufficient to omit the next dose of heparin or to reduce the prescribed daily dose (because of the short heparin T1/2). In more severe forms of bleeding, the heparin effect should be neutralized by the administration of protamine sulfate (1 mg of protamine sulfate neutralizes 100 IU of heparin).

**Heparin Induced Thrombocytopenia**

Thrombocytopenia is a very significant complication of heparin therapy that occurs after subcutaneous as well as intravenous heparin administration.

According to various authors, the effect of heparin on platelets can vary - increased aggregation, decreased aggregation or no effect on aggregation at all. Those variations are probably caused by the use of different test systems and different heparins. According to Salzman et al. (1980), heparin induces and increases platelet aggregation in citrate platelet-rich plasma, and it increases secretion of serotonin caused by other agents. High molecular weight heparin fractions (over 20,000 daltons) react with platelets better than low molecular ones (7,000 daltons). Salzman suggests that there might be two types of binding sites on the heparin molecule - one for AT III or platelet binding (with higher affinity for AT III binding) and the other for platelet binding primarily. A complex formed between AT III and heparin protects platelets from aggregation induced by heparin.
Some authors have investigated fibrinogen receptors on platelets after stimulation with ADP and found that unfractionated heparin increases binding of fibrinogen with platelets treated with ADP. Low molecular weight heparin fractions, however, do not increase this binding capacity. It has been found that heparin prepared from bovine lungs causes thrombocytopenia more frequently than heparin prepared from porcine mucosa. Transient mild forms of thrombocytopenia occur on the second or the third day of treatment in 5 to 30% of patients, without clinical significance. Postponed thrombocytopenia, occurring after one week or later, is a very serious complication in which patient’s plasma contains antibodies, probably antiheparin ones, that induce platelet aggregation in the presence of heparin. A sudden fall of the platelet count on the 8th day, or later, of heparin use is an indication for immediate interruption of heparin administration. Once these antibodies are formed, they persist for at least six weeks. If heparin is administered during that period, thrombocytopenia occurs again.

Heparin administration may also lead to platelet damage caused by complements and thrombocytopenia. Heparin-induced thrombocytopenia is frequently associated with intravascular thrombosis. This so called “white clot” syndrome is often found in older patients and patients with myeloproliferative diseases. It is connected with high mortality and probably results from antiheparin antibodies binding to platelet membranes in predisposed patients. Thus, platelet count should be determined daily in patients treated with heparin.
Among possible heparin induced complications, HIT, classified as types I and II, deserves particular attention. Type II HIT is often associated with thrombotic syndrome.
Type I HIT is induced by direct platelet activation under the influence of heparin. Platelet activation is associated with intensive platelet reversible aggregation and enhanced consumption. It appears during the first few days of the initiation of heparin administration. The decrease in platelet count is usually moderate and often remains above 100 x 10^9/l. Although type I HIT occurs in as many as 20% of the patients treated with heparin, it withdraws spontaneously despite continued heparin administration. It does not require any particular procedure.

Type II HIT is less frequent but a far more serious disorder occurring 5 to 14 days following the initiation of heparin administration in almost 5% of patients. A decrease of the platelet count below 100 x 10^9/l, or more than 50% compared with the initial value, is the detection criteria for this disorder, if and when all other possible causes of thrombocytopenia are excluded. The immune mechanism forming the pathogenic basis of this type of thrombocytopenia has been completely clarified.

Under the influence of heparin, platelet factor 4 (PF 4) is first released from thrombotic alpha granules. Released PF 4 is then bound with heparin, and the formation process of antibodies against the newly formed heparin-PF 4 complex is initiated. The formed antibodies most frequently belong to the IgG class. Later on, these antibodies are bound with heparin-PF 4 complex on the platelet surface and with the Fc platelet receptor (FcγRIIa) at the same time. Binding of the newly formed immune complex with the Fc receptor causes platelet activation.
through the stimulation of the GPIIb/IIIa receptor, inducing platelet aggregation and the release process of their granules constituents. Thus, the coagulation system is activated, and the process of thrombin formation is initiated. Immune complexes also stimulate the activation of endothelial cells, thus, leading to the release of endothelium tissue factor and PAI. Through these processes, significant thrombogenic potential is formed in the vascular pool with similar frequency in both venous and arterial blood vessels, which causes thrombosis in around 50% of patients with type II HIT. Venous thrombosis is usually manifested by the enhancement of the existing thrombus or the appearance of thrombosis in a new venous segment. If arterial thrombosis occurs, it is usually found in cerebral, coronary or lower extremity arteries.

Occurrence of severe thrombotic complications in type II HIT sometimes precedes a dramatic decrease in platelet count, which, of course, makes it more difficult to recognize the etiology of these complications.

Once type II HIT is detected and confirmed, heparin administration is interrupted; it is usually NF heparin. Antithrombotic treatment is continued using direct thrombin inhibitors, oral anticoagulants, a combination of heparinoid glycosaminoglycans, heparan sulfate, dermatan, condroitin sulfate (Dana-paroid), or in some cases 3M heparin, which causes a minimal degree of cross reactivity with the patient’s blood. There are data in favor of desirable effects of antithrombotic drugs such
as Dextran, aspirin or prostacyclines in the treatment of this severe complication occurring during heparin administration.

Osteoporosis

Osteoporosis with spontaneous spine and rib fractures is considered a complication of prolonged heparin therapy. Prophylactic use of heparin administered subcutaneously (20,000 IU daily) more than 25 weeks may result in demineralization of bones.

Allergic reactions

Allergic reactions may occur during heparin treatment and are manifested by high temperature, rash, rhinitis, asthma and hypotension.

Alopecia

Transient alopecia may appear 3 to 4 months after the beginning of heparin treatment.

Oral Anticoagulant Therapy (OAT)

Therapy with oral anticoagulant drugs is most frequently used in the prevention of venous thrombosis. It is also applied to prevent arterial thrombosis, particularly in mitral valve and prosthesis implantations. Treatment of myocardial infarction is less
successful, probably due to thrombus morphology in arteries with proportionally less fibrinogen than platelets.

Derivatives of 4-hydroxycoumarin are the most frequently used oral anticoagulants. Indandions may cause hypersensitive reactions and thus are administered less often. Oral anticoagulant drugs are vitamin K antagonists. Their effect is based on the competitive action with vitamin K-dependent coagulation factors (II, VII, IX and X) as well as on the decrease of the level of PC anticoagulant and its cofactor PS. Coagulation factors II, VII, IX and X are found in circulation in the form of inactive precursor substances. Vitamin K is the catalyst of glutamine acid carboxilation on specific sites in vitamin K-dependent factors as well as in PC and PS. \(\gamma\)-carboxy-glutamine acid is responsible for calcium binding and important for the functional activity of vitamin K-dependent factors.

Oral anticoagulants block glutamine acid carboxilation. Coagulation factors without the \(\gamma\)-carboxyl group are identical to normal factors in terms of antigenicity, although they do not have any functional activity since, due to the lack of the \(\gamma\)-carboxyl group, they do not bind calcium. These functionally inactive molecules are termed PIVKA (Proteins Induced by Vitamin K Antagonists). Oral anticoagulants do not immediately exert their effect due to different coagulation factors’ half-lives (F VII: 6 hrs; F IX: 24 hrs; F X: 40 hrs; F II: 60 hrs). The decrease in the concentration of vitamin K-dependent factors develops gradually, and hypocoagulability is reached in 2 to 4 days.
Drug Interaction with OAT

If taken simultaneously, many drugs may lead to difficulties in monitoring oral anticoagulant therapy. This has to be compensated by regular laboratory testing and modification of the anticoagulant dosage rate. Drugs that potentiate the effect of oral anticoagulants are antimicrobial substances, analgetics, oral antihypoglycemics, anti-hyperlipid preparations, anabolic steroids, antiarrhythmics, immunosuppressive drugs and alcohol. Drugs that reduce the effect of oral anticoagulants are hypnotics, anti-epileptics, preparations containing vitamin K (blood and plasma), corticosteroids, antihistaminics and oral contraceptive drugs.
### Table XI.2

#### Drugs Inducing *Synergistic Effects* With OAT

<table>
<thead>
<tr>
<th>Category</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antibiotics</td>
<td>synthetic penicillin, cephalosporines, tetracyclines, macrolides, sulphonamides, metronydazole</td>
</tr>
<tr>
<td>Analgetics</td>
<td>except paracetamols</td>
</tr>
<tr>
<td>Nonsteroid antirheumatics</td>
<td></td>
</tr>
<tr>
<td>Oral hypoglycemics</td>
<td>sulphonyle-urea preparations</td>
</tr>
<tr>
<td>Antihyperlipemic drugs</td>
<td>cholestramine, clophibrat</td>
</tr>
<tr>
<td>Corticosteroids</td>
<td></td>
</tr>
<tr>
<td>Antacids and laxatives</td>
<td></td>
</tr>
<tr>
<td>Antiaggregation drugs</td>
<td>aspirin</td>
</tr>
<tr>
<td>Cardiovascular system (CVS) disease drugs</td>
<td>diuretics (diazoxid)</td>
</tr>
<tr>
<td>Drugs affecting CNS</td>
<td>anti-depressives</td>
</tr>
<tr>
<td></td>
<td>chlorpromazine (largactil)</td>
</tr>
<tr>
<td></td>
<td>chloralhydrate</td>
</tr>
<tr>
<td>Cytostatic &amp; immunosuppressive therapy</td>
<td>endoxane, methotrexate</td>
</tr>
</tbody>
</table>

#### Drugs Inducing *Antagonistic Effects* With OAT

<table>
<thead>
<tr>
<th>Category</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypnotics</td>
<td>tegretol</td>
</tr>
<tr>
<td>Antiepileptics</td>
<td>fenobarbiton primidon</td>
</tr>
<tr>
<td>Vitamin K</td>
<td></td>
</tr>
<tr>
<td>Oral contraceptive drugs</td>
<td></td>
</tr>
<tr>
<td>Use of blood and plasma</td>
<td></td>
</tr>
</tbody>
</table>
Administration and Loading Doses

Since these drugs have a suppressive effect on PC anticoagulant, whose half-life is shorter than the half-life of coagulation factors (PC half-life is similar to F VIII half-life, around 6 to 8 hrs), an increased tendency towards thrombosis may be expected at the beginning of oral anticoagulant treatment (skin necrosis is a typical effect) (Fig. XI.4).

Figure XI.4: Pathogenesis of coumarin-induced venous limb gangrene complication. Warkentin TE, 2001.
For this reason, heparin is administered at the beginning, 7 to 10 days, of a long-term oral anticoagulant treatment. Originally, this treatment was started with higher initial doses, which were gradually reduced. However, it was noted that in treatment started with higher doses the risk of bleeding was increased because F VII and F IX concentrations were reduced faster than those of F X and F II. For this reason, gradual achievement of hypocoagulability by administration of low doses, increased gradually if necessary, is now recommended.

Oral anticoagulant drugs are most frequently used in 6 to 9 month intervals for the treatment of deep venous thrombosis and lung embolism (during the first few months there is the highest risk for new thrombosis). In prophylaxis this treatment lasts 3 to 6 months. Long-term therapy is applied in recurrent thromboembolisms. Anticoagulant drugs are also administered in cases of myocardial infarction, artificial valve replacement, and coronary bypass. With cerebrovascular infarction particular care should be taken because of the risk of cerebral hemorrhage. Inherited deficiency of natural inhibitors (AT III and PC) with clinical thrombosis and the presence of lupus-like anticoagulants with clinical thrombosis are possible indications for a long-term oral anticoagulant therapy.

Oral anticoagulant drugs are used to prevent and control thromboembolisms. There are various opinions in the indications for their use.
Medium and short-term treatment (3 to 12 months)
- prophylaxis of deep venous thrombosis including high risk of surgery
- myocardial infarction
- deep venous thrombosis
- artificial valve replacement
- lung embolism
- coronary arterial bypass graft, up to 2 months

Long-term treatment with OAT
- recurrent venous thromboembolism
- embolic complications induced by rheumatoid cardiac disease and arterial fibrillation
- artificial valve and arterial graft implantation

Possible indications for a long-term treatment with OAT
- congenital AT III deficiency with clinical thrombosis
- congenital deficiency of PC and PS with clinical thrombosis
- transient cerebral artery ischemia, including basilar and vertebral arterial syndrome
- lupus-like anticoagulant antibodies with clinical thrombosis

Contraindications for OAT
- previous hemostatic defect (with the exception of lupus inhibitors where oral anticoagulation is indicated)
- cerebrovascular episodes (except from embolism)
- mental diseases - non-compliant patients
- chronic alcoholism - severe hypertension
Dosage of anticoagulant drugs depends on the biological assay induced by the coagulation defect. This is measured by prothrombin time, which actually presents recalcified plasma time after the addition of tissue thromboplastin. Thromboplastin is obtained from various sources and produces different prothrombin times for the same test plasma because of different potentials and different responses to the deficiencies of vitamin K-dependent factors. Prior to the introduction of OAT, liver function should be investigated. High initial doses of warfarin for adults (10 mg on the first and second days) are no longer recommended.

Estimated doses should be reduced in the following conditions:
- prolonged base prothrombin time,
- abnormal test results of liver function,
- congestive heart disease,
- parenteral nutrition,
- less than the average body weight, or
- persons over 80 years.

The dose has to be diminished if these patients undergo surgery. When PT reaches 40 to 50%, surgery can be performed without
risk of bleeding. Oral anticoagulant treatment should be withdrawn gradually. Disposition towards thromboembolic disease may occur if there is abrupt termination. When the levels of vitamin K-dependent factors range from 15 to 35%, the risk of thrombosis is sufficiently reduced, and there is a minimum risk of bleeding. Since this is a long-term therapy, patients must be checked regularly to make sure that stable therapeutic values have been reached.

**OAT in Pregnancy**

Vitamin K antagonists pass through the placenta during pregnancy. Due to the teratogenic effect, OAT is contraindicated in the first trimester of pregnancy (warfarin embryopathy). The risk of fetal hemorrhage increases with the progress of pregnancy. If anticoagulant therapy is necessary, heparin should be administered. In the second and third trimester, patients treated with oral anticoagulant drugs should be checked more frequently, with careful dose determination and maintenance. Long-term OAT may induce maternal osteoporosis. OAT must be replaced with heparin in the 36th gestation week. Oral anticoagulant drugs can be administered 24 to 48 hours after delivery. The decision to administer OAT during pregnancy must be made carefully with consideration of the benefit-risk ratio for mother and fetus.
Laboratory Control

PT is the test most frequently used for OAT control. However, when PT is expressed directly in seconds or in percentages read from the curve, the obtained results can vary from one laboratory to another due to the use of different reagents and considerable variations in sensitivity of thromboplastin reagents to hemostatic defects caused by OAT. Thus, the results depend on the type of reagent. Thromboplastin of different origins (rabbit, bovine, human) can induce various degrees of sensitivity. Due to the possible risk of HIV transmission, WHO does not recommend the use of human thromboplastin.

The WHO recommended a calibration scheme for thromboplastin in order to enable international standardization of OAT monitoring. This scheme expresses PT results in international normalized ration (INR). Patient’s PT is easily turned into INR. The patient’s PT is divided by the PT of the normal plasma pool, and prothrombin ratio (PR) is obtained. This PR is then graduated on the international sensitivity index (ISI), and the obtained INR result is expressed as the following equation:

\[
\text{INR} = \text{PR}^{ISI}
\]

ISI is the sensitivity of each thromboplastin in relation to the international reference preparation (IRP). The WHO Committee for biological standardization marked one series of lyophilized human thromboplastin as IRP (67/40). ISI is obtained by cali-
bration of commercial thromboplastin preparations in relation with IRP, whose ISI is defined as 1.0. Each manufacturer has to denote the exact ISI on the reagent kit for each series. INR can be determined by calculation or by using a table. Therapeutic ranges for INR are from 2.0–4.5. Calculating results using INR offers more realistic therapeutic values and improves cooperation among laboratories. Besides, INR offers a direct comparison of PR results regardless of reagent and equipment. When new reagents or equipment for PT are used, transition to INR will minimize differences that otherwise occur. It should be emphasized that INR must be applied in monitoring patients on a stabilized long-term OAT. INR varies in the initial therapy phase. This is why results obtained in transition into INR in the first ten days of therapy initiation are not precise. It should be stressed that the test must be performed on citrate plasma stored at 13° to 20°C during 4 hours of blood collection.

**Side Effects - Overdose of OAT**

Bleeding may be due to a simple overdose of oral anticoagulant, to a potentiation by other drugs, or to an alteration in patient’s sensitivity to treatment by an intercurrent infection, liver disease, alcohol or change in diet. Some patients can bleed from an existing lesion, such as peptic ulcer or carcinoma of the bladder, while their prothrombin time ratio is within the accepted range.

Minor spontaneous bleedings, such as epistaxis, menorrhage, microscopic hematuria, subconjunctival hemorrhage, and ecchy-
moses, are common. Life-threatening episodes include massive gastrointestinal hemorrhage, bleeding from a ruptured follicular cyst, severe recurrent hemarthroses, and subdural, subarachnoid and retroperitoneal hemorrhage. Patients may also bleed from recent surgery sites, vein punctures and catheter sites.

If the patient’s coagulation times are within the therapeutic ranges at the time of bleeding, a full coagulation screen and platelet count should be determined in order to exclude liver disease, DIC, and/or thrombocytopenia. Many patients with minor bleeding manifestations are on non-steroidal anti-inflammatory drugs which interfere with platelet release reaction. Minor problems are dealt with by reducing the dose of the oral anticoagulant or by interrupting therapy for 24 to 48 hours and then continuing at a lower dose. More severe bleeding episodes are arrested by administration of 1 to 2 units of FFP or a small dose (1 to 2 mg) of vitamin K, orally or intravenously.

Life-threatening hemorrhage may require repeated infusions of FFP, larger doses of vitamin K (2.5 to 10 mg), or an infusion of F II, IX and X concentrate. Vitamin K must be administered repeatedly in patients who have taken massive overdoses. Both vitamin K and FFP or concentrate are given to patients with suspected cerebrovascular bleeding.
**Thrombolytic Therapy**

A blood clot (thrombus) developed in the circulatory system can cause vascular blockage leading to serious consequences including death. A healthy hemostatic system suppresses the development of blood clots in normal circulation but reacts extensively in the event of vascular injury to prevent blood loss. Outcomes of a failed hemostasis include stroke, pulmonary embolism, DVT and acute myocardial infarction.

Pathologies involving hemostasis failure and the development of a clot require clinical intervention consisting of intravenous administration of thrombolytic agents. Streptokinase is

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**Figure XI.5**

![Plasminogenesis Diagram](image)
one such clinically important thrombolytic agent. Its production from micro-organisms will be discussed in detail in this book.

Thrombolytic drugs are used in the treatment of occlusive vascular diseases in order to remove thrombi formed in blood vessel lumen without surgical procedure. In many cases of ischemia, residual thrombi, rethrombosis or the underlying blood vessel disease may be responsible for the moderate effect of this therapy. Several endogenous and exogenous fibrinolysis activators are in clinical use for the treatment of various forms of thromboembolic diseases. Exogenous plasminogen activators are in wide use since they have significant therapeutic value.

Hemostasis failure and consequent formation of blood clots in the circulatory system can produce severe outcomes such as stroke and myocardial infarction. Pathological development of blood clots requires clinical intervention with fibrinolytic agents such as urokinase, t-PA and streptokinase.

Thrombolytic Agents
- s-PA (streptokinase plasminogen activator)
- u-PA
- APSAC (acylated plasminogen-streptokinase activator complex)
- t-PA
- recombinant scu-PA (single-chain urokinase plasminogen activator)
- recombinant t-PA
**Streptokinase**

The extracellular enzyme streptokinase (ec 3.4.99.22.) is produced from various strains of β-hemolytic streptococci. The enzyme is a single-chain polypeptide that indirectly exerts its fibrinolytic action by activating the circulatory plasminogen. Streptokinase has a molar mass of 47 kDa and is made up of 415 amino acid residues. The protein exhibits its maximum activity at a pH of approximately 7.5; its isoelectric pH is 4.7. The protein does not contain cystine, phosphorous, and lipids. Streptokinase produced by different groups of streptococci differ considerably in structure.

Streptokinase is the most thoroughly investigated and clinically used plasminogen activator. It was first isolated in 1933 from the filtrate of β-hemolyticus streptococcus culture, Lancefield’s group C. In 1957, the interaction between streptokinase and plasminogen was described as well as the formation of plasminogen activator complex. Streptokinase is a single stranded glycoprotein containing amino acid sequences similar to serine protease from Streptomyces griseus and from human trypsin. It is stable at pH from 6 to 8 and inactivated at pH less than 5 or more than 9. In contrast to other plasminogen activators, it has practically no intrinsic enzyme activity. In order to gain catalytic activity, it has to form a 1:1 noncovalent stoichiometric complex with one of the various plasminogen forms. These are glu-plasminogen, lys-plasminogen and B chain plasminogen. During formation of streptokinase-plasminogen
complex activator, the active site of the molecule is exposed, without cleavage of peptide bonds, enabling conversion of both complexes and free plasminogen to plasmin by the activator complex. In the course of complex formation, streptokinase molecules are cleaved into at least 6 fragments of molecular weight from 10 to 44 kDa.

Potency of the activator complex varies directly depending on the size of the streptokinase fragments and indirectly depending on size and form of the plasminogen it forms a complex with. Streptokinase-plasmin B chain complex has the greatest potency, lys-plasminogen has a lower potency and glu-plasminogen the lowest potency. The in vivo-potency of activator complex modifies several components from the fibrinolytic and coagulation system. FDP Y and E are bound to plasminogen increasing the potency of the activator complex. If α₂AP is bound to the complex, it acts as an inhibitor.

Streptokinase activity is determined by coagulum lysis or using the chromogen substrate method. In this manner, the Christensen unit has been defined as the amount of streptokinase required for the lysis of coagulum of defined weight within 10 minutes. This unit is equivalent to the international unit (IU).
Structure of Streptokine and Its Mechanism of Action

Physiochemical properties of streptokinase

Streptokinase is a bacterial protein secreted by several strains of hemolytic streptococci. It is a single-chain polypeptide with a molecular weight of 43,000, containing 414 amino acids, a few carbohydrates and no disulfide bonds. Streptokinase has neither peptidase nor amidase activity. Some of the early streptokinase preparations were relatively impure containing only around 10% of the active drug while current preparations have a purity exceeding 95%.

Pharmacokinetic properties of streptokinase

Streptokinase fibrinolytic activity in vivo is cleared through the RES with a half-life of 18 to 25 minutes. The level of antistreptokinase antibodies, possibly resulting from the previous infections with β-hemolytic streptococci, varies significantly among individuals. Around 350,000 units of streptokinase are necessary to neutralize the circulating antibodies in 95% of the healthy population while individual requirements range from 25,000 to 300,000 units. Several days following administration of streptokinase, the antistreptokinase titer rises rapidly to 50 to 100 times the value preceding the infusion and remains high for at least 4 to 6 months. During that period, renewed thrombolytic streptokinase treatment or treatment based on compounds containing streptokinase becomes impracticable.
Plasminogen activation mechanism

Streptokinase activates plasminogen indirectly turning it into plasmin following a three step mechanism. In the first step, streptokinase rapidly and stoichiometrically reacts with plasminogen forming a streptokinase-plasminogen complex where plasminogen undergoes a conformational change that results in the exposure of an active site. In the second step, the active site catalyzes the activation of plasminogen to plasmin. In the third step, plasminogen-streptokinase molecules undergo conversion to plasmin-streptokinase complex preserving the activity of the activator. Fibrin does not contain specific binding sites for streptokinase.

Streptokinase induces a “systemic fibrinolytic state” in blood characterized by plasminogen activation in plasma (known as hyperplasminemia), by depletion of α₂AP, and by breakdown of fibrinogen, factor V and factor VIII. The breakdown FDPs formed also impede blood coagulation. Besides, plasmin degrades components of the extracellular matrix including fibronectin and laminin. Plasmin activates latent tissue collagenases capable of degrading interstitial collagens, types I, II and III, or basement membrane collagen-type IV.

Streptokinase consists of multiple structural domains (i.e., α-, β- and γ-domains) with different associated, functional properties. Scanning calorimetric analysis suggests that the protein is composed of two distinct domains. The N-terminal domain,
residues 1 to 59, has been found to complement the low plasminogen activation ability of the 60-414 amino acid residue domain of the protein (Nihalani et al. 1998). Recently, the crystal structure of streptokinase complexed with human plasmin light chain has been studied (Wang et al. 1998).

Streptokinase is known to activate plasminogen both by fibrin-dependent and fibrin-independent mechanisms. Streptokinase interacts with plasminogen though multiple domains. At least two independent plasminogen binding sites of streptokinase had been identified by 1995 (Nihalani and Sahni 1995). The C-terminal domain of streptokinase is involved in plasminogen substrate recognition and activation.

The coiled region of the streptokinase γ-domain is said to be essential for plasminogen activation (Wu et al. 2001). The streptokinase β-domain is involved in forming the streptokinase-plasminogen complex responsible for activating the plasminogen (Robinson et al. 2000).

Elimination half-life is 18–25 minutes, and clearance is performed mostly through the RES. The half-life of streptokinase-plasminogen activator complex is much shorter (1.5–2.5 minutes); clearance is also performed through the RES. Due to a very short half-life, rethrombosis may occur several minutes after streptokinase infusion. Infusion of streptokinase causes a significant decrease in the level of circulating plasminogen and fibrinogen. Due to the increased fibrinogen catabolism, fi-
brinogen and fibrin degradation products appear in circulation. Decreased fibrinogen concentration is responsible for a decrease in blood viscosity, red blood cell sedimentation, and platelet aggregation. After the infusion of streptokinase, euglobulin lysis is accelerated.

Production of streptokinase - Producing microorganisms

By 1919, Streptococcus sp. had been classified into alpha, beta and gamma variants based on the distinct types of hemolytic reactions the variants produced on blood agar plates.

In 1933, Lancefield used serologic distinctions to further differentiate the β-hemolytic streptococci into groups A to O (Lancefield). Most of the streptokinases are obtained from β-hemolytic streptococci of the Lancefield groups A, C and G. Group C is preferred for producing streptokinase as it lacks erythrogenic toxins. The group C strain Streptococcus equisimilis H46A (ATCC 12449), isolated from a human source in 1945, has been widely used for producing streptokinase. The strain H46A was selected from more than a hundred fibrinolytic isolates because it yielded the most active streptokinase.

S. equisimilis H46A does not produce erythrogenic toxins. The H46A isolate can be grown on semisynthetic media to secrete large quantities of streptokinase. H46A is also the main source of the streptokinase gene that has been expressed in various other microorganisms (Wong et al. 1994).
Recombinant producers

Mutant streptokinase with improved stability has been prepared (Shi et al. 1998). Two of the major sites of the proteolytic action of plasmin on streptokinase are Lys 59 and Lys 386 (Wu et al. 1998). It has a longer functional half-life. Recombinant streptokinase produced in Pichia pastoris yeast is glycosylated, and this appears to enhance its resistance to proteolysis (Pratap et al. 2000).

Plasmin-resistant, long-life variants of protein-engineered streptokinase have been produced in a protease-deficient recombinant bacillus subtilis WB600 (Wu et al. 1998). It appears that the streptokinase domains responsible for activity, stability and immunogenicity have considerable overlap.

The streptokinase gene from S. equisimilis H46A was sequenced by Malke et al. (2000). The transcriptional control of this gene has been studied (Gase et al. 1995), and the functional analysis of its complex promoter has been reported (Grafe et al. 1996). Therefore, considerable information exists on how to effectively use this gene to safely produce streptokinase in non-pathogenic bacteria.

Studies of the streptokinase gene isolated from various sources indicate it is polymorphic (Malke 2000). The streptokinase gene (skc) cloned from S. equisimilis H46A has been expressed in several grams positive and grams negative bacteria, including B. subtilis WB600 (Wong et al. 1994) and E. coli
(Yazdani and Mukherjee 2002). Production in Pichia pastoris has been reported (Pratap et al. 2000).

The ability to produce recombinant streptokinase greatly enhances the possibilities for beneficial structural modifications of this protein and enhanced production of the desired recombinant streptokinase.

A recombinant streptokinase was produced in E. coli using LB medium at 37°C (Lee et al. 1997a). The plasmid used imparted ampicillin resistance to the bacterium, and ampicillin provided the selection pressure for plasmid retention. The production of streptokinase was induced by adding 1mM isopropyl-β-d-thiogalactopyranoside (IPTG) to the medium. The plasmid encoded a streptokinase that lacked the 13 N-terminal amino acid residues of the normal protein. This enhanced productivity of the recombinant protein and enabled secretion into the extracellular medium (Lee et al. 1997a,b). At least a part of the N-terminal domain is known to be functionally relevant in streptokinase (Young et al. 1995; Nihalani et al. 1998; Azuaga et al. 2002; Mundada et al. 2003), but this may not include the first 13 residues (Lee et al. 1997b). Other reports have also described IPTG-induced production of recombinant streptokinase (Yazdani and Mukherjee 1998, 2002). The specific productivity of the recombinant protein was substantially enhanced by feed batch cultivation compared to batch fermentations of IPTG-induced E. coli (Yazdani and Mukherjee 1998). Some work on kinetic analysis and modeling of streptokinase fermentation has been reported (Stuebner et al. 1991).
Streptokinase fermentation

Recently, work has focused on elucidating the fermentation conditions for producing streptokinase from mutants of the wild-type streptococci and other genetically engineered microorganisms. Hyun et al. (1997) produced copious quantities of streptokinase using a mutant streptococcus. The culture medium consisted of casein or serratio peptidase hydrolyzed casein, glutamine, cysteine, and yeast extract. The mutant was cultured at pH 6.8-7.2 between 35 to 38°C in broth aerated at 0.1 to 1.0 vvm. The titer of streptokinase exceeded 8500 units per milliliter.

The S. equisimilis streptokinase gene expressed in E. coli has led to a 10-fold greater streptokinase titer than values obtained in cultures of group C streptococci such as S. equisimilis (Estrada et al. 1992). Work has been reported on localizing the core promoter region of the streptokinase gene, skn (Malke et al. 2000). The plasmid pSK100 has enabled high level secretory expression of streptokinase in E. coli cultured in ampicillin-containing LB medium. Expression titers of about 5000 units per milliliter have been attained (Ko et al. 1995). The LB medium consists of Bacto-tryptone, yeast extract, sodium chloride, and 50 μg·ml⁻¹ ampicillin at pH 7.3. Other media have been described for producing recombinant streptokinase in E. coli (Narciandi et al. 1996).
Purification of streptokinase

Several schemes have been described for recovery and purification of streptokinase from either commercially available crude preparations or fermentation broths of various streptococci.

Repeated chromatography was necessary to remove the last detectable traces of impurities. This required a combination of ion exchange chromatography (DEAE-Sephadex A-50) and gel permeation chromatography (Sephadex G-100). A highly pure streptokinase was recovered from the relatively crude commercial Kabikinase.

Ammonium sulfate fractionation was first used to obtain a crude precipitate of streptokinase. This was redissolved and subjected to gel permeation chromatography. The eluted streptokinase fraction was further purified using column chromatography on DEAE-cellulose or DEAE-Sepharose.

Several affinity chromatography methods have been discussed for purifying streptokinase (Liu et al. 1999). One affinity purification used a monoclonal antibody ligand (Andreas 1990). Rodriguez et al. (1994) used a combination of two affinity matrices for chromatographic purification of recombinant streptokinase. The affinity ligands were human plasminogen and monoclonal antibody against streptokinase. Both ligands were bound to Sepharose as the chromatographic matrix. This
puriﬁcation method produced a preparation with about 50,000 units of activity per mg of protein and a purity of >93%.

Use of immobilized p-nitrophenyl p-guanidinobenzoate (NPGB) acylated plasminogen for afﬁnity puriﬁcation was further reported by Liu et al. (1999). A solution of urea was the eluent. The speciﬁc activity of the puriﬁed material was 74,000 units/mg. Hernandez-Pinzon et al. (1997) recovered streptokinase by cross-ﬂow ultraﬁltration.

Streptokinase has been puriﬁed from the ﬁltrate of a streptococcal fermentation broth using hydrophobic interaction chromatography on a phenil- or octyl-Sepharose column. A gradient elution with 21% ammonium sulfate was used to recover the streptokinase. Further puriﬁcation involved gel permeation and ion exchange chromatography steps.

Generalized schemes for the recovery and renaturation of inclusion body recombinant proteins have been published (Chisti 1998a).

Enhancing streptokinase - Side effects

Transitory hypotension and bradycardia occur often during streptokinase therapy, which might be related with histamine and/or bradykinin release. Clinically signiﬁcant hypotension has been noted in only a small percentage of treated patients. However, the incidence of hypotension does not seem to be related with the steroid premedication.
Fever as a complication occurs in around 5 to 30% of patients, most often with a delay of several hours before onset.

Streptokinase administration can induce serious allergic reactions, such as urticaria, bronchospasm, angioneurotic or periorbital edema. In the ISIS-3 trial, the incidence of streptokinase related allergic reactions was only 3.6%, and major anaphylactic reactions to streptokinase treatment were quite rare (0.3%).

Shivering, chills, rashes, flushing, nausea and musculoskeletal pain have been reported in 10% of patients during the course of or soon after streptokinase infusion. In addition serum sickness symptoms have occasionally been reported related to streptokinase treatment. Likewise, hemorrhages into the iliopsoas muscles have been reported. Strong, lower thoracic back pain and severe lower abdominal pain have repeatedly been described during or after streptokinase infusion.

If repeated between 5 days and 6 months after the initial infusion, streptokinase administration may be clinically ineffective as a result of a high titer of circulating antibodies. Repeated
streptokinase administrations should, therefore, be avoided after 5 days and up to 1 to 2 years after the first administration. If, however, repeated thrombolysis is necessary during this time interval, nonantigenic drugs such as urokinase and alteplase are the treatment of choice (Collen and Verstidete 1994).

**Hemorrhage**

Minor bleeding that does not require transfusion occurs in 3 to 4% of patients. Serious or major bleeding, possibly life threatening and requiring transfusion, occurs in 2% of patients treated with streptokinase. In the ISIS-3 trial, the bleeding incidence was 1%.

Cerebral bleeding is the most serious complication, occurring in 0.1 to 0.5% of patients. Ischemic stroke during the course of streptokinase administration with myocardial infarction occurs in 0.8 to 1.5% of patients. The risk of intracranial bleeding seems to be greater with hypertension and in older patients.

**Immunogenicity of streptokinase (anti-streptokinase antibody)**

As a foreign protein, streptokinase is an antigen that can cause anti-streptokinase antibody formation. A considerable number of persons have those antibodies in various titers due to previous streptococcal infections. Thus, prior to the therapy, AST (anti-streptokinase antibody titer) should be determined. If a patient already has anti-streptokinase antibodies, higher doses are required for neutralizing them. The mean dose required for the
elimination of those antibodies is 200,000 IU, but it can be much higher, reaching 1,000,000 IU. After infusion of streptokinase, the number of antibodies increases rapidly during the first 7 to 10 days with a peak after two or three weeks and disappearance after 4 to 6 months.

During the administration of streptokinase, hypersensitivity reactions are frequent, either immediately, caused by IgE antibodies, or postponed, caused by IgG antibodies. Temperature increases in 15 to 20% of patients although it is often less than 40°C. Nausea, urticaria, pruritus, headache and rash occur in less than 15% of patients and hypotension in less than 5%.

Immunogenicity of streptokinase and its relatively short half-life in circulation limit the therapeutic potential of this protein. Streptokinase in circulation is proteolytically degraded by plasmin. Consequently, research has focused on structurally modifying streptokinase to extend half-life, reduce or eliminate immunogenicity, and improve plasminogen activation. Structurally modified streptokinases have been produced several ways including genetic mutation, recombinant DNA technology, and chemical or enzymatic modification of the native streptokinase.

Soon after the discovery of streptokinase in the 1930s, its immunogenicity became known (Tillett and Garner 1933). Early studies showed that blood from patients with recent streptococcal infections inactivated streptokinase because of the presence
neutralizing antibodies. Because streptococcal infections are common, a detectable level of antibodies against streptokinase appears in most populations. Many different anti-streptokinase, platelet-activating antibodies are known to exist and occur widely (Regnault et al. 2003).

The different domains of streptokinase differ in immunogenicity (Reed et al. 1993). New antigenic domains were identified as recently as 2001 (Coffey et al. 2001).

Recombinant streptokinases with reduced immunogenicity have been produced (Ojalvo et al. 1999b). A mutant streptokinase that lacked the C-terminal 42 amino acids was found to be less immunogenic than the native molecule. One chemical modification has involved complexing streptokinase with PEG (polyethylene glycol), primarily to reduce immunogenicity.

Because of its antigenic characteristics, streptokinase can induce allergic reactions. Likewise, it can cause hypotension, which is undesirable in acute myocardial infarction conditions. Due to its antigenic characteristics, streptokinase causes antibody formation, and for that reason it can be used successfully for up to seven days at the most. If after the elapse of 7 days there is a need for thrombolytic drug administration, t-PA should be used since it has no antigenic characteristics.
Administration of streptokinase

Table XI.3: Administration of Streptokinase – Doses

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Doses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutralization of Anti-streptokinase</td>
<td><strong>250,000 IU for 30 min</strong></td>
</tr>
<tr>
<td>Antibodies</td>
<td></td>
</tr>
<tr>
<td>Acute Myocardial Infarction</td>
<td><strong>1,500,000 IU for 3 - 60 min</strong></td>
</tr>
<tr>
<td>DVT</td>
<td><strong>250,000 IU for 25 - 30 min</strong></td>
</tr>
<tr>
<td></td>
<td>and <strong>100,000/ hour for 24 - 72 hours</strong></td>
</tr>
<tr>
<td>Acute Pulmonary Embolism (APE)</td>
<td><strong>3,500,000 IU for 24 hours</strong></td>
</tr>
</tbody>
</table>

**Streptokinase dosage (Collen and Verstraete)**

A standard, initial, intravenous dose of 500,000 units can be administered over 10 to 30 minutes followed by a continuous maintenance dose of 100,000 units per hour for one or more days. A high dose of 1,500,000 units is used routinely in patients suffering from acute myocardial infarction.

Local infusion of 2000 to 4000 units of streptokinase per minute over 60 to 120 minutes is the treatment of choice in cases of pulmonary and limb arteries occlusions.

An intracoronary infusion of streptokinase with an initial dose of 10,000 to 20,000 units followed by 5000 units per minute (range: 2000 to 6000 IU) over the course of 60 minutes was used at the beginning of the 1980s.
Streptokinase can be administered by *systemic infusion* with numerous colloid or crystalloid solutions, including physiological solution, dextrose and commercial gel solutions. Doses range from 250,000 to 1,250,000 IU. High doses are administered if anti-streptokinase antibodies are present. In this case, systemic fibrinolysis may be postponed for several hours. The ideal standard dose is determined according to the distribution of streptococcal resistance in local population. Infusion should be performed within 30 minutes. *Local infusions* are used in many cases with the advantage of high activator concentration in the thrombus itself and small systemic effect. It is applied in limited venous thrombosis, lung embolism, peripheral arterial thromboses, and in acute myocardial infarction. Small doses (5,000 IU/h) combined with systemic heparinization have a considerable effect in pulmonary and arterial thrombolysis. Large doses are administered in cases of coronary artery thromboses (3,000 to 5,000 IU/min). Infusion should last 60 to 90 minutes. Advantages of *intermittent administration* of streptokinase have not been proven yet. A dose of 600,000 IU is usually followed by 250,000 to 600,000 IU every 12 or 14 hours.

In order to obtain optimal thrombolysis prior to, during or after streptokinase therapy, pharmacological or physical ways of removing stenosis and blood vessel spasm can be applied, including infusion of nitroglycerol during angiography or recanalization by catheter. Arterioplasty or arterial coronary bypass can be implanted soon after successfully performed thrombolytic therapy. For the prevention of new thrombosis, all forms
of thrombolytic therapy have to be followed by administration of heparin or oral anticoagulant drugs. Heparin should be administered at the end of streptokinase infusion, a few days before introduction of oral anticoagulant therapy. Along with the streptokinase standard dose, a small dose of heparin, 15,000 to 20,000 IU/h, is administered. Although there is no clear evidence for the efficiency of oral anticoagulant therapy in combination with streptokinase, it is widely accepted in practice. Aspirin administration (160 mg daily for one month) along with 1,000,000 IU of streptokinase within 60 minutes after myocardial infarct significantly decreases mortality in these patients.

Indications for streptokinase administration

1. DVT
   The advantage of thrombolysis is rapid dissolution of thrombus and maintenance of circulation. Thrombus dissolution eliminates the source of the next embolisation and alleviates hemodynamic disorders caused by thrombosis. Thrombolysis prevents further valvular damage in deep veins as well as damage that frequently occurs during thrombectomy. Hypertension in lower extremities and other side effects are also prevented (postthrombotic syndrome).

2. APE
   Streptokinase administration eliminates large thrombi in the pulmonary tree as well as the underlying source of lung embolization, i.e. peripheral thrombi. Lysis of residual microemboli in
lung capillaries improves microcirculation and tissue perfusion. This way of eliminating thrombi is far simpler compared with surgical procedures. Therapy can be initiated immediately, which is particularly significant in a fulminate embolism.

3. Arterial Occlusions
Thrombolytic therapy is an alternative to thrombectomy in cases of arterial occlusions. It is recommended in peripheral occlusions where surgery is not indicated or occlusion cannot be attained as well as in patients in which surgical procedures have not been successful (10 days after surgery).

4. Acute Myocardial Infarction
Temporary intravenous infusion of high doses of streptokinase is indicated in the treatment of acute myocardial infarction (1,500,000 IU/h) resulting in rapid recanalization of coronary arteries, immediate disappearance of pain and prevention of further infarct boosting. Thrombolysis is a rather simple and non-invasive therapy, which requires neither specially trained staff nor expensive equipment. Therapy can be introduced immediately (around one hour earlier than intracoronary techniques) and can be applied anywhere.

Application of Streptokinase in Acute Myocardial Infarction

In the early eighties, the first thrombolytic drug, streptokinase, was applied in the form of intracoronary infusion directly into
the occluded infarctial artery of a patient suffering from the acute myocardial infarction. The streptokinase dose used at the time was 4000 IU/minute.

Although intracoronary use of streptokinase infused directly into occluded blood vessel enabled control of the efficiency of the drug, its use was feasible only in a small number of patients treated in specialized institutions with professional staff available on 24 hours basis. This was soon abandoned because it was demonstrated by Schroder et al. that almost the same effect could be achieved with higher doses, i.e., 1,500,000 IU of streptokinase. The new dosage offered a wide range of routine use for this drug in all patients with acute myocardial infarction, unless counter indications were present.

Streptokinase was the first thrombolytic drug administered in the treatment of acute myocardial infarctions. A single chain protein originating from group C beta hemolytic streptococcus, it has both the antigen qualities and strong systemic fibrinolytic activity. It binds with plasminogen forming streptokinase-plasminogen complex, which only then becomes active and through this activation turns plasminogen into plasmin. Plasmin is a nonspecific enzyme that induces fibrinolysis when formed on fibrin surface through the activation of plasminogen bound to fibrin. In this way, both artery and venous thrombi can be lysed to a degree depending on the intensity of fibrinolysis based on thrombus age, its structure and the content of plasminogen in the thrombus.
In the case of acute myocardial infarction, an intravenous infusion of streptokinase is used in 1,500,000 IU doses for 30 to 60 minutes. The highest concentration is achieved after 2 to 3 minutes, and the optimal fibrinolytic activity is reached after around 30 minutes. Streptokinase is eliminated from plasma in two ways: first through its binding with circulating antibodies with a half life of around 18 minutes and formation of streptokinase-plasminogen complex with a half life of around 80 minutes. Thus, clearance depends on the effect of the circulating antibody values on streptokinase as well as on the applied drug dose. It is further metabolized by proteolysis into low molecular weight fragments.

Unlike streptokinase, t-PA does not cause antibody formation, thanks to its human origin, but the systemic effect of t-PA is small. t-PA is metabolized quickly in the liver and has a plasmatic half life of around 30 minutes. The initial dose is administered as bolus and is then followed by an infusion.

With acute myocardial infarction, t-PA is administered in 100 mg doses in the form of an infusion over the course of 3 hours or in the form of a 15 mg bolus followed by an infusion of 0.75 mg/kg over the course of 30 minutes. The remaining part of the initial 100 mg dose is administered over the course of 60 minutes.

Results of the GISSI-1 study, which included 1100 patients from coronary units throughout Italy, demonstrated that on the 21st day the mortality of patients treated with streptokinase,
doses of 1.5 ml IU/60 minutes, was significantly lower compared with the control group (10% vs. 13%). Namely, there were 23 saved lives for every 1000 treated patients. At the same time, it was noted that bleeding complications were relatively rare (0.3% of patients). The most severe complication, i.e., cerebral bleeding, was not significantly higher than in the control group (0.92% versus 0.77%). Data regarding the timing of the use of streptokinase also showed that the best effect was achieved when drug was used within the first three hours. A statistically significant drop of mortality was reported when the drug was used within a 3- to 6-hour-interval. The highest efficiency of streptokinase therapy was noted in patients treated with streptokinase during the first hour following infarction. Thus, the final conclusion of the GISSI-1 study was: the earlier the drug is administered the better the clinical effect. This has been confirmed on numerous occasions since.

Another significant study, ISIS-2, gave new insight into the significance of antiaggregation therapy along with thrombolytic therapy. The goal was to decrease the frequency of reocclusion in patients. In 17,000 randomized patients divided into 4 groups, the effects were observed and followed: streptokinase (1.5 ml IU / 50 min), aspirin (160 mg), streptokinase plus aspirin, and placebo.

Results were as convincing as in the GISSI-1 study: aspirin caused reduced mortality similar to the reduction noted with the use of streptokinase. Furthermore, the effect of these two drugs on mortality was additive and independent. Five weeks after the
use of therapy, mortality was 12% in the placebo group, 9.2% in the streptokinase group, and only 8% in the group treated with both aspirin and streptokinase. The most significant decrease of mortality was achieved in the group of patients treated with the thrombolytic drug of each therapeutic group within the first 4 hours. Complications were not particularly significant with the use of the streptokinase–aspirin combination. There were 0.3% systemic and 0.1% intracerebral bleedings.

<table>
<thead>
<tr>
<th></th>
<th>Intracoronary Streptokinase</th>
<th>Intracoronary Urokinase</th>
<th>Streptokinase</th>
<th>Urokinase</th>
<th>rt-PA</th>
<th>APSAC</th>
<th>Scu-PA</th>
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</thead>
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<tr>
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<td>600 IU/min</td>
<td>1.5 million IU/min</td>
<td>3 million IU/min</td>
<td>200 mg</td>
<td>30 U</td>
<td>80 mg</td>
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<td>1.5-3 hr</td>
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<td>0.3%</td>
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<td>3+</td>
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</tr>
</tbody>
</table>

Comparison of the use of t-PA on one hand and streptokinase on the other demonstrated that there were no significant differences of mortality among studied groups.

The most significant factor in the use of thrombolytic therapy is the timing; the other factors have a far less significant effect. Patients treated with thrombolytic therapy within the first hour following infarction showed the most significant decrease of mortality (50% according to GISSI-1 study). Mortality decreases each consecutive hour, so that 12 hours following myocardial infarction, thrombolytic therapy becomes ineffective.

Aspirin and heparin
Following the initial effect of the thrombolytic drug, reocclusion occurs in a number of patients. Platelet activation plays an important role in the process of reocclusion; thus, the administration of aspirin is indicated in the first minutes of the acute myocardial infarction. Due to its well known effect in the prevention of platelet formation, heparin is administered as an addition to thrombolytic therapy, especially during or after the use of t-PA. Hirudin, likewise, has a similar effect.

Thrombolytic therapy in older population
It is a well known fact that myocardial infarction in the older patient population carries higher risks due to previous damages of cardial muscle, other diseases, and major alterations in coronary blood vessels.
Thrombolysis and “open artery” theory
Despite large amounts of data supporting the fact that thrombolytic therapy is the therapy of “condition sine qua non”, modern treatment of myocardial infarction requires consideration of criteria for its use, i.e., consideration of contraindications, which can either be absolute (acute internal bleeding, recent surgical procedure, head trauma or neoplasma, cerebral hemorrhage, noncompressible vascular puncture, pregnancy, treatment for diabetes, high blood pressure (>200/129)) or relative (active ulcer, liver insufficiency).

In order to minimize the risk and make an appropriate decision regarding when and who should be treated with these drugs, American and European Cardiology Associations have made recommendations for the use of thrombolytic therapy in the treatment of acute myocardial infarction: clinical history containing data on anginous pain lasting more than 30 minutes and electrocardiogram (ECG) alterations demonstrating ST elevations higher than 1 mm in two or more outputs, or newly formed left branch block within the first 6 hours. Myocardial infarctions associated with ST depression are not indicated for the use of thrombolytic therapy since the mortality in such cases is higher with than without thrombolytic treatment. Individual therapy that takes into consideration all necessary data is crucially importance.
Combined use of thrombolytic drugs and GPIIb/IIIa receptor inhibitors

New antiplatelet drugs belonging to the group of GPIIb/IIIa receptor inhibitors, affecting the final platelet aggregation pathway, have the potential of a much higher expressed platelet inhibition and, thus, an even greater clinical efficiency.

Experimental and clinical studies show that combined use of thrombolytic drugs and GPIIb/IIIa receptor inhibitors can increase the speed and the degree of coronary artery opening without increasing the risk of bleeding. Fibrinolytic drugs eliminate only the surface part of the thrombus while the platelet core remains intact. Likewise, platelets create PAI-1, the natural inhibitor of thrombolysis, as well as T A₂, which causes local vasoconstriction. Viewed as a whole, it forms the basis of “thrombolytic resistance” of fibrinolytic drugs.

Two other significant limiting factors related with the use of thrombolytic drugs are reocclusion of coronary artery and myocardial reinfarction occurring in the early stage of thrombolysis. The reocclusion phenomenon can be explained by the prothrombotic effect of thrombolytic therapy. Namely, after the influence of a thrombolytic drug, thrombin is released from lysed thrombus, which has an autocatalytic effect on its own production. Thrombin is the most potent activator of platelet aggregation, which in association with other prothrombin factors released from the residual fissure site or from the atherosclerotic structure rupture, stimulates rethrombosis. Besides, in
patients with substantial residual blood vessel stenosis, there is a tendency towards stasis and thrombus formation.

In the TIMI-14 study, based on randomized group of patients, the combined effect of lower doses of thrombolytic drugs (t-PA or streptokinase) and GPIIb/IIIa receptor inhibitors (abciximab) compared with the use of thrombolytic drugs only (t-PA in standard doses of 100 mg) or abciximab alone was investigated. The best angiographic results were achieved through the combined effect of 50 mg of t-PA and abciximab. Namely, TIMI-3 flow after 90 minutes was achieved in 77% of patients treated this way compared with 62% of patients treated with the standard dose of t-PA. Other regimes showed far more moderate results. In all groups of patients, the percentage of major bleedings was the same and the percentage of intrahospital mortality was quite similar.

The TIMI-14 study, as well as numerous studies currently underway, offers the possibility of a “real thrombolysis” resulting in lysis and inhibition of platelets, thrombin, and fibrin components of the occlusive thrombus.

Unfavorable side effects of thrombolytic therapy
Though extremely efficient, thrombolytic therapy is associated with the risk of unfavorable side effects. Numerous studies have shown that side effects are relatively rare and that the benefits resulting from the use of thrombolytic therapy surpass the impact of side effects. Side effects are divided into five groups:
intracranial hemorrhage, systemic hemorrhage, hypotension, immunological reactions and reperfusion arrhythmias.

The most severe side effect is intracerebral hemorrhage occurring in 0.1 to 0.7% of patients. Older patients (>65 years old), low body weight patients (<70 kg), as well as patients with higher values of arterial pressure have a higher risk of hemorrhage compared with younger patients. When there is moderate bleeding, therapy should be interrupted for a few hours. Severe hemorrhages require a complete stoppage of therapy and the introduction of antifibrinolytic therapy (tranexamic acid, epsilon aminocapronic acid, aprotinin). Aspirin is not responsible for the increase of systemic hemorrhage frequency.

Hypotension appears in 10 to 12% of patients undergoing streptokinase treatment and in 6% of patients treated with t-PA. One half of all patients require medical treatment for hypotension.

Allergic reactions, usually mild, appear in 3 to 5% of patients treated with streptokinase or APSAC. Severe allergic reactions are rare. Mild allergic reactions can be controlled with corticosteroids, and in cases of severe reactions, streptokinase infusion should be stopped immediately. Adrenaline and high doses of antihistamines and corticosteroids should be administered. Prophylactic application of corticosteroids decreases the frequency of such reactions. Upon administration of streptokinase, higher titers of antibodies appear after 3 to 7 days and persist for one year, so that normal dose streptokinase treatment
becomes ineffective during this period. A high temperature is another possible side effect.

Reperfusion arrhythmias can occur following reperfusion. The most frequent arrhythmia is idioventricular tachycardia, and to a lesser degree, ventricular fibrillation can occur. That is why thrombolytic therapy is available in intensive care units as well as in the form of prehospital thrombolytic treatment.

During DVT treatment, embolic complications can occur. Streptokinase infusion should be continued in such cases in order to dissolve the embolus. In case of myocardial infarction after recanalization of coronary arteries, reperfusion arrhythmias can occur. They, however, can be alleviated with anti-arrhythmics.

Contraindications
Systemic use of streptokinase is contraindicated
- in patients with bleeding manifestations or recent bleeding episodes
- in all conditions of blood hypocoagulability, especially if fibrinolysis is accelerated
- in patients with tendency towards local bleeding of gastrointestinal or urogenital tracts
- cerebral hemorrhages
- after surgeries, traumas, deliveries or abortions
- in pregnancy
- in severe cases of hypertension
- severe forms of diabetes mellitus
- in severe liver and kidney damage
- in acute pancreatitis and acute bronchitis.

Streptokinase therapy is also contraindicated in cases of recent streptococcal infections that resulted in high titers of anti-streptokinase antibodies (acute rheumatic fever, acute glomerulonephritis) as well as after recent streptokinase therapy. In all conditions, except for hemorrhages, recent polytraumas and cerebrovascular insults, contraindications do not exclude local application of low doses of streptokinase requiring, however, special attention due to possible systemic effects.

### Comparison of Thrombolytic Agents

<table>
<thead>
<tr>
<th></th>
<th>STK</th>
<th>t-PA</th>
<th>APSAC</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Doses</strong></td>
<td>1,500,000 IU/30-60 min</td>
<td>100 mg/90 min</td>
<td>30 mg/5 min</td>
</tr>
<tr>
<td><strong>antigenic characteristics</strong></td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td><strong>intracranial bleeding</strong></td>
<td>0.3%</td>
<td>0.6%</td>
<td>0.6%</td>
</tr>
<tr>
<td><strong>Flow through coronary artheria after 90 min</strong></td>
<td>40%</td>
<td>79%</td>
<td>63%</td>
</tr>
</tbody>
</table>

*Table XI.5: Thrombolytic therapy in acute myocardial infarction, Vasiljevic Z., Bilten, 2000*

Of the three major available thrombolytic agents: t-PA, u-PA, and s-PA, the microbial sourced streptokinase is the least ex-
pensive, but it is also immunogenic. Human t-PA and u-PA are immunogenically benign but are short-lived in vivo and therefore of limited therapeutic effectiveness. Comparative clinical trials and cost-effectiveness considerations suggest that streptokinase is the drug of choice for thrombolytic therapy, but this is debatable. Streptokinase is a nonhuman protein, and its introduction into the circulatory system can illicit a severe anaphylactic response, including death (Lee 1995; Jennings 1996). The risk of this immune response is dependent on the level of the anti-streptokinase antibodies present in circulation. This im-

![Figure XI.6: Comparison of Thrombolytic Drugs](image-url)
munogenicity restricts multiple treatments with streptokinase (Jennings 1996).

The plasmin produced through the streptokinase mediated activation of plasminogen breaks down streptokinase. This limits the in vivo half-life of streptokinase to about 30 min. Although streptokinase survives in circulation significantly longer than t-PA does (a half-life of about 5 min.), the survival length is too short for efficient therapy (Wu et al. 1998).

Recombinant forms of normal human plasminogen activators t-PA and u-PA are used in clinical intervention. Another commonly used plasminogen activator is streptokinase (s-PA), a bacterial protein that does not occur naturally in human circulation. s-PA, t-PA and u-PA do not have a direct fibrinolytic activity, so their therapeutic action is via the activation of blood plasminogen into clot dissolving plasmin (diagram). Unlike t-PA and u-PA, which are proteases, streptokinase possesses no enzymatic activity of its own. Streptokinase acquires its plasminogen activating property by complexing with circulatory plasminogen or plasmin. The resulting high-affinity 1:1 stoichiometric complex (i.e., the streptokinase-plasminogen activator complex) is a high-specificity protease that proteolytically activates other plasminogen molecules into plasmin (Bajaj and Castellino 1977; Castellino 1984). Thus, the plasminogen activating action of streptokinase is fundamentally different from the proteolytic activation brought about by t-PA and u-PA.
Although streptokinase is the best known microbial plasminogen activator, it is not the only one. Staphylokinase (SAK), sourced from Staphylococcus sp., is a potential alternative plasminogen activator. Recombinant staphylokinase has been produced in bacteria such as Escherichia coli and has been shown to induce fibrin specific clot lysis in human plasma milieu in vitro.

Another activator in clinical use is APSAC, in which human plasminogen with an acylated active site has been complexed with bacterial streptokinase. APSAC has an extended therapeutically effective half-life in circulation relative to streptokinase.

Urokinase
Urokinase is a protease similar to trypsin and is responsible for fibrinolytic activity in human urine. Urokinase activates plasminogen by cleaving arg-560-val bond. Activated enzyme appears in two forms: S2 form with 54 kDa molecular weight consisting of two polypeptide chains connected with disulphide bonds and S1 form with 31 kDa molecular weight, otherwise proteolytic derivative. A single urokinase chain, probably a proenzyme, is also isolated and converted into a double, fully active chain after limited digestion with plasmin. The urokinase gene has been cloned, so recombinant urokinase is now available. Recombinant urokinase (double chain) and recombinant pro-urokinase (single chain) have been compared with the natural urokinase in biological and thrombolytic studies. Preliminary reports suggest that pro-urokinase has a higher specific throm-
bolytic activity, i.e., it has higher binding ability to fibrin in comparison with the double chain urokinase.

Urokinase for clinical application used to be prepared by purifying human urine. Since urine contains small amounts of urokinase, 1500 liters of urine were required to obtain a dose for one thrombolytic treatment. Urokinase is now produced in large amounts from tissue cultures of human fetal kidney cells (preparations are a mixture of high and low molecular weight urokinase) or by recombinant technology. Commercial preparations of urokinase are prepared from urine, tissue culture, or by recombinant technology; they all have similar thrombolytic activity.

Urokinase is administered intravenously; oral application has no effect. After administration, urokinase is rapidly distributed within the body, quickly reaching the liver and kidneys. After 18 hours, the largest amount is found in kidneys, most probably as metabolites. Degradation and elimination are performed by the liver in two stages. $T_{1/2}$ is 13 to 20 minutes in the first stage and 3 to 10 hours in the second stage (eliminated as biexponential curve). Urokinase $T_{1/2}$ in circulation is 9 to 16 minutes (similar to streptokinase), and rethrombosis can occur 15 to 30 minutes after the infusion. Systemic fibrinolytic effect can be measured through the decrease of fibrinogen, plasminogen, and $\alpha_2$AP or through an increase of FDP. All plasma inhibitors, $\alpha_2$MG, $\alpha_2$AP, $\alpha_1$ antitrypsin and ithr, slowly inhibit urokinase. In contrast to streptokinase, urokinase is no antigen, and it does not cause antibody formation.
Urokinase is administered by continuous intravenous infusion. Recommended doses vary (250,000 to 500,000 CTA units [Committee on Thrombolytic Agents]). If a dose of 30,000 to 80,000 Ploug units (approximately 45,000 to 120,000 CTA units) is applied, fibrinolytic activity is low. For a beneficial effect, 250,000 Ploug units (375,000 CTA units) are required. A urokinase infusion, similar to streptokinase, is usually followed by anticoagulation with heparin during the first few hours of the therapy. 7500 units of heparin should be administered along with the continuous infusion of 250,000 CTA units of urokinase.

APSAC
Anistreplase or APSAC was designed to prolong the half-life of streptokinase and to make treatment more convenient by allowing an intravenous bolus injection of the drug. With acute myocardial infarction, the recommended standard dose is 30 units of anistreplase, administered as a bolus injection containing around 1,200,000 units of streptokinase.

The incidence of allergic reactions to APSAC is around 5%. Serious allergic reactions such as anaphylaxis and bronchoconstriction are rare (0.2 to 0.4%). Fever, flushing or chills are more common (5 to 10%). Cerebrovascular accidents in relation with anistreplase treatment have been reported in around 0.6% of patients. Anistreplase treatment causes immunization; the antibody titer increases up to 60-fold after 2 to 3 weeks and remains very high for three months following the administration.
APSAC is a recently defined thrombolytic agent derived by streptokinase-plasminogen-activator-complex modification. This agent is a noncovalent equimolar complex of human plasminogen and streptokinase, modified by covalent acylation of plasminogen, catalytic site ser-740, located on pro-B chain. Its molecular weight is 131 kDa. By the acylation of this catalytic site, fibrinolytic activity and $\alpha_2$AP molecule inactivation are prevented. Since the pro-A plasminogen chain remains unmodified, APSAC preserves its ability to bind to fibrin.

Thrombolytic activity of APSAC is 10 times higher than that of streptokinase. The half-life of APSAC is 120 minutes, compared with a streptokinase half-life of 15 to 20 minutes. In the presence of fibrin, t-PA increases APSAC fibrinolytic activity. Soon after administration, considerable decreases in fibrinogen, plasminogen and $\alpha_2$AP are observed while FDP increases, lasting 24 to 48 hours. Although F V and F VIII are substrates for plasmin, a decrease in their level and prolongation of coagulation tests cannot always be seen after the use of APSAC. Similar to streptokinase, APSAC is an immunogen that leads to formation of antibodies that are still present three months after the application. The thrombolytic effect depends on the presence of anti-streptokinase antibodies. Doses for patients without antibodies are less than 10 IU, for patients with low antibody titers doses are less than 50 IU, and for patients with high antibody titers doses are over 100 IU. APSAC is administered to patients with myocardial infarction.
Recombinant scu-PA

scu-PA, or pro-urokinase, is a human protein. It was first identified in cell culture fluid and later produced using DNA technology. The human gene responsible for its synthesis is located on chromosome 10.

scu-PA is a physiological urokinase precursor. Contrary to urokinase, scu-PA demonstrates specificity for clot and increases plasminogen activator activity on the thrombus surface. It is obtained by recombinant technology from E. coli. It is not immunogenic, and $T_{1/2}$ is 16 minutes. Its fibrinolytic effect depends on the dose. The usual dose is 40 mg i.v. For myocardial infarction, 80 mg are administered.

Recombinant t-PA

t-PA was identified in 1947. Since 1980, it can be prepared in sufficient quantities from melanoma cells. In 1993, a recombinant preparation was made. Currently, there is insufficient data concerning the latter preparation. There are several rt-PA preparations varying in single and double chains. They are administered intravenously since absorption cannot be obtained with oral administration, and if administered intramuscularly, absorption is very low.

Studies based on the investigation of a small series of patients with coronary thrombosis, treated with t-PA and scu-PA, showed that the combination of both preparations administered in a 1:3 molar ratio resulted in thrombolysis three times higher compared with the separate use of these two preparations.
Laboratory Control

During thrombolytic streptokinase therapy, alterations in coagulation and fibrinolytic tests occur. Testing is significant for streptokinase treatment follow-up since dose variations can lead to systemic effects. TT is most frequently used. A prolonged TT test (more than 60 seconds) indicates decrease of fibrinogen concentration and presence of circulating FDP. When usual doses of streptokinase are administered, TT is prolonged for 3 to 6 hours after the introduction of the therapy, lasting 2 to 4 times longer than normal. If TT is significantly prolonged, streptokinase has been overdosed, and therapy should be interrupted for several hours. A standard dose of 100,000 IU/h should be reintroduced.

With minimum TT, the administered dose is either insufficient or the patient has a high titer of anti-streptokinase antibodies that cannot be treated with the usual dose. Apart from TT, three additional tests are required.
- AST should be performed prior to theapy introduction; dose is determined according to the obtained result.
- Shortened euglobulin lysis time is a confirmatory test for thrombolysis, particularly useful if TT is short.
- Finally, screening tests for the determination of fibrinogen are sometimes necessary and always needed with bleeding episodes.

New, specific tests for FDP caused by the effect of plasmin on cross-bound fibrin are useful in the follow-up of thrombo-
lytic therapy. Apart from TT, other screening tests such as bleeding time, platelet count, PT and PTT should be performed to detect possible hemostatic disorders during thrombolytic therapy. If therapy is followed by heparin administration, TT should be performed every 24 hours. PT should be determined for the first time 4 days after introduction of oral anticoagulant therapy and followed by weekly checks up to the point when the therapeutic level is reached. PTT is prolonged due to the decreased concentration of F V and F VIII and the presence of heparin.

**Assaying Streptokinase**

Characterization of streptokinase produced by fermentation and assessment thereof require methods for assaying the streptokinase.
- Fibrin plate method
- Heated fibrin plate method
- Solid-phase chromogenic assay for plasmin
- Highly sensitive and reproducible enzyme-linked immunosorbent assay (ELISA) for antibodies against streptokinase has been developed (Leonardi et al. 1983).

**Concluding Remarks**

Native streptokinase is useful for cost-effective thrombolytic therapy in clinical practice, but its use is not risk free. Large quantities of streptokinase can be produced inexpensively via bacterial fermentation. Cloning of the streptokinase gene in nonpathogenic micro-organisms has allowed the production of recombinant streptokinase that eliminates any risk of inad-
vertent inoculation of patients and production personnel with potentially pathogenic streptococci.

Various chemical modifications of streptokinase have been used to extend its half-life in circulation, improve plasminogen activation, and reduce or eliminate immunogenicity. These objectives have been attained to various degrees by producing mutated and engineered streptokinases. The streptokinase domains responsible for antigenicity, stability and plasminogen activation appear to overlap to some degree. Consequently, it may be impossible to produce a modified streptokinase that combines every hoped for, functional trait at the desired level.

Despite significant limitations, streptokinase remains the drug of choice particularly in poorer economies because of its relatively low cost.
Diagnosis of hemorrhagic disorders can be made based on:
- history of the disease
- clinical check-up
- laboratory tests
  1. screening tests for vascular and platelet disorders
  2. screening tests for coagulation disorders
  3. specific tests.

Screening tests are necessary in day-to-day routine practice of all clinical and laboratory transfusion medical services.

**History of the Disease**

A patient’s history indicates whether the bleeding is from one or more sites. It is a fact that hemostasis will never manifest in bleeding from one site only. The following questions should be asked:
- Was the bleeding spontaneous, or was it provoked by tooth extraction or some other surgical procedure?
- Did it occurred immediately or 3 to 4 days after the procedure?
- Has there been a susceptibility towards bleeding since early childhood (inherited hemostasis disorders)?

Negative family history does not exclude the existence of an inherited disorder. This is due to possible mutations. Diagnosis of a hemorrhagic syndrome is sometimes made based only on an adequate history and a detailed clinical check-up.

**Clinical Check-Up**

Clinical tests can indicate whether the problem is result of blood vessel, platelet, or blood coagulation disorders. Hemorrhagic syndrome in patients with vascular disorders, such as thrombocytopenia or platelet function disorder, manifests in bleeding into skin or mucous membranes, petechias and hematomas. Bleeding usually starts immediately after injury and lasts for hours. When it stops, however, it does not occur again. In patients with defective coagulation, deep hematomas in muscles and joints occur. Posttraumatic bleeding does not occur immediately after injury but several hours later, and sometimes it can reappear during the next 4 to 5 days. Laboratory testing is necessary in order to determine the type and intensity of the disorder as well as for therapy monitoring.
Laboratory Analysis

In order to diagnose vascular and platelet disorders, bleeding time and platelet count should be determined. Determination of bleeding time is the most easily and most frequently performed test. Normal values range from 1 to 3 minutes (Duke). It is prolonged in blood vessel wall structure disorder, decreased platelet count, disorders of adhesion and aggregation, disturbance of platelet reaction release, and decreased TrF3. Normal platelet count ranges from 150 to 350x10^9/l, according to Brecher and Cronkite’s direct method.

- Tourniquet Test
- Bleeding Time
- Inspection of Blood Film
- Platelet Count

Bleeding time can be prolonged due to:
- abnormal vessel constiction
- low platelet count
- abnormal platelet adhesion
- abnormal platelet release of ADP or T A₂
- abnormal platelet aggregation
- vWD

TT, PT and aPTT are determined in order to diagnose coagulopathies. These tests include disorders of the intrinsic, extrinsic and common activation pathways, both individual and associated defects of several coagulation factors.
Figure XII.1: Clotting Cascade

The Clotting Cascade

Intrinsic Pathway

Surface Contact Collagen FXII activator

F XII → F XIIa

F XI → F Xa

F IXa

F VIIa (Activated Factor 3)

Factor F X

TT

Prothrombin

F Xa → Thrombin

F Xa → F XIII

F XIIIa

Fibrin polymers

Fibrin monomers

Fibrinogen

Extrinsic Pathway

Tissue/Coll Defect

F VIIa

F VII

F VIII

F VIIa

Factor F X

F III (Tissue Thromboplastin)

Ca²⁺
Thrombin Time (TT) measures the time of fibrinogen transformation into fibrin under the influence of thrombin. Thus, all other factors are avoided. TT is prolonged in hypo- and afibrinogenemias, in the presence of inhibitors, when fibrinogen changes into fibrin, such as heparin, and in the presence of FDP and soluble fibrin monomers. Normal time ranges from 16 to 20 seconds.

Figure XII.2
Figure XII.3

Abnormal

Thrombin

1. Hypofibrinogenemia
   A fibrinogenemia

2. Inhibitors of Fibrinogen-Fibrin:
   - Heparin
   - Fibrin/Fibrinogen degradation products

Platelet-free Plasma
Prothrombin Time (PT) determines extrinsic and common coagulation pathways and, thus, is prolonged in deficient F II, F V, F VII, F X and fibrinogen. This test is necessary in peroral anticoagulant therapy control, as well as in the investigation of liver function. Normal values range from 70 to 100%.

Figure XII.4

The Quick (PT)-Test
Figure XII.5

**Abnormal Prothrombin Time**

1. Deficiencies of Factors
   VII, X, V, Prothrombin & Fibrinogen
2. Inhibitors of Fibrinogen
   - Fibrin
   - Heparin
   - Fibrin/Fibrinogen degradation products
Partial Prothrombin Time (PTT) determines the internal mechanism of blood coagulation and is prolonged in deficiencies of all plasma coagulation factors: F I, F II, F V, F VIII, F IX, F X, F XI and F XII, except in F VII and F XIII deficiency. It is prolonged in prekallikrein and in HMWK deficiency.

Figure X II.6
PTT is prolonged in the presence of heparin and inhibitors. It is particularly prolonged in hemophilia and in the presence of heparin. This test has completely replaced the coagulation time test from venous blood. A coagulation time test from capillary blood is not safe because of the presence of tissue thromboplastin; thus, it shows normal values in all coagulopathies, even in hemophilias. Normal time ranges from 33 to 45 seconds.
Table XII.1: Screening Tests In Hemorrhagic Disorders

<table>
<thead>
<tr>
<th>Hemorrhagic Disorder</th>
<th>Bleeding Time</th>
<th>Platelet Count</th>
<th>PT</th>
<th>PTT</th>
<th>TT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vasculopathy</td>
<td>P</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>P</td>
<td>P</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Hemophilia</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>P</td>
<td>N</td>
</tr>
<tr>
<td>vWD</td>
<td>P</td>
<td>N</td>
<td>N</td>
<td>P</td>
<td>N</td>
</tr>
<tr>
<td>Vitamin K deficiency</td>
<td>N</td>
<td>N</td>
<td>P</td>
<td>P or N</td>
<td>N</td>
</tr>
<tr>
<td>Liver disease</td>
<td>P or N</td>
<td>P or N</td>
<td>P</td>
<td>P</td>
<td>P</td>
</tr>
<tr>
<td>Massive blood loss</td>
<td>P or N</td>
<td>P</td>
<td>N</td>
<td>P</td>
<td>N</td>
</tr>
<tr>
<td>DIC</td>
<td>P</td>
<td>P</td>
<td>P</td>
<td>P</td>
<td>P</td>
</tr>
<tr>
<td>Primary pathological fibrinolysis</td>
<td>N</td>
<td>N</td>
<td>P or N</td>
<td>P or N</td>
<td>P or N</td>
</tr>
<tr>
<td>Renal disease</td>
<td>P or N</td>
<td>P or N</td>
<td>N</td>
<td>P</td>
<td>N</td>
</tr>
<tr>
<td>Oral anticoagulants</td>
<td>N</td>
<td>N</td>
<td>P</td>
<td>P or N</td>
<td>N</td>
</tr>
<tr>
<td>Heparin</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>P</td>
<td>P</td>
</tr>
</tbody>
</table>

N – normal; P – pathological

Specific tests, both in diagnostics and therapy monitoring, are performed in specialized laboratories:
- assay of individual coagulation factors
- determination of platelet function
- vWF specific test
- inhibitor determination tests
- specific tests for DIC
- fibrinolysis disorder control tests.
Table XII.2

<table>
<thead>
<tr>
<th>Laboratory Test</th>
<th>Bleeding Disorder</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood count &amp; smear</td>
<td>anemia, leukemia, DIC</td>
</tr>
<tr>
<td>Platelet count</td>
<td>thrombocytopenia</td>
</tr>
<tr>
<td>Bleeding time</td>
<td>platelet-vessel wall interaction</td>
</tr>
<tr>
<td>PT</td>
<td>warfrin use; deficiency factors I, II, V, VII and X</td>
</tr>
<tr>
<td>Activated PTT</td>
<td>heparin use; deficiency of all coagulation factors-</td>
</tr>
<tr>
<td></td>
<td>especially VII &amp; IX, except VII</td>
</tr>
<tr>
<td>TT or fibrinogen</td>
<td>heparin use, fibrin degradation products,</td>
</tr>
<tr>
<td></td>
<td>hypofibrinogenemia or dysfibrinogenemia</td>
</tr>
</tbody>
</table>
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ABBRIVATIONS & ACRONYMS

ACL .............. Anticardiolipin (antibody)
ADP .............. Adenosine Diphosphate
AMCA ............ Tranexamic Acid (trans-4-aminomethyl cyclohexanoic acid)
APC .............. Activated Protein C
APCC ............ Activated Prothrombin Complex Concentrate
APE .............. Acute Pulmonary Embolism
APL .............. Acute Promyelocytic Leukemia
APS .............. Antiphospholipid Antibody Syndrome
APSAC .......... Acylated Plasminogen-Streptokinase Activator Complex
aPTT .......... Activated Partial Thromboplastin Time
AST .......... Anti-streptokinase Antibody Titer
AT III .......... Antithrombin
ATP .............. Adenosine Triphosphate
b.w. .............. body weight
BU .............. Bethesda Unit
Ca .............. Calcium
cAMP .......... Cyclic Adenosine Monophosphate
CHD .......... Coronary Heart Disease
CHF .......... Congestive Heart Failure
CNS  ..............Central Nervous System
CPU  ..............Carboxypeptidase U
CSF  ..............Colony Stimulating Factor
CTA  ..............Committee on Thrombolytic Agents
CVS  ..............Cardiovascular System
DDAVP  ...........Desmopression (1-deamino-8-d-arginine vasopressin)
DIC  ..............Disseminated Intravascular Coagulation
DVT  ..............Deep Venous Thrombosis
E. coli ..........Escherichia coli
EACA  ..........Epsilon Aminocaproic Acid
ECG  ..........Electrocardiogram
ELISA  ..........Enzyme-linked Immunosorbent Assay
F a ..............Factor (plasma coagulation) activated
FDP  ..........Fibrin Degradation Products
FEIBA  ..........Factor Eight Bypassing Activity
FFP  ..........Fresh Frozen Plasma
FPA  ..........Fibrinopeptide A
GP ..........Glycoprotein
HAV ..........Hepatitis A
HBV ..........Hepatitis B
HCV ..........Hepatitis C
HDN ..........Hemorrhagic Disease of the Newborn
HELLP ..........Hemolysis, Elevated Liver Enzymes, Low Platelet Count
HIT ..........Heparin Induced Thrombocytopenia
HMWK ..........High Molecular Weight Kininogen
IBD ..........Inflammatory Bowel Disease
IgG (E) ........Immunoglobulin G (E)
IgM ..........Immunoglobulin M
IL-1 ..........Interleukin-1
INR ..........International Normalized Ratio
IPTG ..........Isopropyl-β-d-thiogalactopyranoside
IRMA ..........Radioimmunoassay
IRP ..........International Reference Preparation
ISI ..........International Sensitivity Index
ISTH ..........International Society on Thrombosis and Hemostasis
ITI ..........Immune Tolerance Induction
ITP ............Idiopathic Thrombocytopenic Purpura
KIU ..........Kallikrein Inhibitor Unit
LETS ..........Leiden Thrombophilia Study
LMWH ..........Low Molecular Weight Heparin
MHC ..........Major Histocompatibility Complex
MPD ..........Myeloproliferative Disorders
MW ..........Molecular Weight
NO ..........Nitrogen Oxide
NPBG ..........p-nitrophenyl p-guanidinobenzoate
OAT ..........Oral Anticoagulation Therapy
OC ..........Oral Contraceptive
OLT ..........Orthoptic Liver Transplantation
PAI ..........Plasminogen Activator Inhibitor
PC ..........Protein C
PCC ..........Prothrombin Complex Concentrate
PCI ..........Protein C Inhibitor
PCR ..........Polymerase Chain Reaction
PE ..........Pulmonary Embolism
PEG ..........Polyethylene Glycol
PF ..........Platelet Factor
PGI2 ..........Prostaglandin (Prostacyclin)
PIS ............Postphlebitic Syndrome
PIVKA ..........Proteins Induced by Vitamin K Antagonists
PNH .............Paroxysmal Nocturnal Hemoglobinuria
PR .............Prothrombin Ratio
PS ............Protein S
PT .............Prothrombin Time
PTT ..........Partial Thromboplastin Time
PZ ..........Protein Z
r ..........Recombinant
RES ..........Reticulo-endothelial System
RFA ..........Risk Factor Analysis
RIPA ..........Ristocetin Induced Platelet Aggregation
scu-PA ..........Single-chain Urokinase Plasminogen Activator
SLE ..........Systemic Lupus Erythematosus
s-PA ..........Streptokinase Plasminogen Activator
ST ..........A segment in an ECG.
TA2 ............Thromboxane A2
T ..........Thromboxane
T1/2 ..........Half-life
TAFI ..........Thrombin-Activated Fibrinolysis Inhibitor
TIMI ..........Thrombolysis in Acute Myocardial Infarction
TF ..........Tissue Factor
TFPI ..........Tissue Factor Pathway Inhibitor
TGF-β ..........Transformed Growth Factor β
THR ..........Total Hip Replacement
TKR ..........Total Knee Replacement
TIMI ..........Thrombolysis in Myocardial Infarction
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>TM</td>
<td>Thrombomodulin</td>
</tr>
<tr>
<td>TNF</td>
<td>Tumor Necrosis Factor</td>
</tr>
<tr>
<td>TP</td>
<td>Thromboxane A&lt;sub&gt;2&lt;/sub&gt; Receptor Protein</td>
</tr>
<tr>
<td>t-PA</td>
<td>Tissue Plasminogen Activator</td>
</tr>
<tr>
<td>TrF3</td>
<td>Platelet Phospholipid</td>
</tr>
<tr>
<td>TT</td>
<td>Thrombin Time</td>
</tr>
<tr>
<td>u-PA</td>
<td>Urokinase Plasminogen Activator</td>
</tr>
<tr>
<td>VTE</td>
<td>Venous Thromboembolism</td>
</tr>
<tr>
<td>vWD</td>
<td>von Willebrand’s Disease</td>
</tr>
<tr>
<td>vWF</td>
<td>von Willebrand Factor</td>
</tr>
<tr>
<td>vWF:Ag</td>
<td>von Willebrand Factor Antigen</td>
</tr>
<tr>
<td>vWF:RCo</td>
<td>von Willebrand Factor Cofactor Activity</td>
</tr>
<tr>
<td>WFH</td>
<td>World Federation of Hemophilia</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
</tr>
<tr>
<td>ZPI</td>
<td>Protein Z-related Protease Inhibitor</td>
</tr>
<tr>
<td>α&lt;sub&gt;2&lt;/sub&gt;AP</td>
<td>α&lt;sub&gt;2&lt;/sub&gt; Antiplasmin</td>
</tr>
<tr>
<td>α&lt;sub&gt;2&lt;/sub&gt;MG</td>
<td>α&lt;sub&gt;2&lt;/sub&gt; Macroglobulin</td>
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