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Department of Biological Chemistry

HEMOSTASIS SYSTEM. COAGULOLOGICAL SYNDROMES

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INTRODUCTION

The writing of the tutorial on hemostasis was dictated by production needs. The experience of communicating with doctors of clinical laboratory diagnostics, with clinicians, with clinical residents and interns has shown that the hemostasis system causes the greatest number of difficulties associated, first of all, with the interpretation of laboratory test results, as well as with the correct choice and conduct of tests.

Currently, not a single laboratory can do without at least a minimal set of hemostasis tests, since disorders of the blood coagulation system are found in almost all types of pathology, and the use of drugs that affect coagulation has entered the practice of most medical institutions.

Existing monographs on hemostasis, a large amount of scientific literature describe this system in full and in detail, however, they are replete with a large number of mechanisms and schemes and are difficult for an unprepared reader to perceive.

The purpose of this manual is to summarize in the most accessible form the mechanisms of hemostasis and give the main approaches to the diagnosis of disorders of the blood coagulation system within the standard hemostasiogram.

This manual is intended for an initial study of the hemostatic system and

Recommended for doctors of clinical laboratory diagnostics, for clinicians, for clinical residents, interns and medical students.

THEORETICAL PART

MECHANISM OF HEMOSTASIS.
SYSTEM OF HEMOSTASIS. BASIC CONCEPTS

The blood coagulation system (hemostasis) is one of the most complex systems in the body. This is a biological system, the main function of which is to maintain a balance between antagonistically acting mechanisms that ensure the preservation of the liquid state of the blood, prevent blood loss by maintaining the integrity of the vascular wall and the formation of blood clots at the sites of damage to the vessel, dissolving the blood clot and restoring blood flow, and ensuring primary wound healing. The interaction of various links allows the hemostasis system to be kept within the physiological fluctuations between hypocoagulation (decreased blood clotting) and hypercoagulation (increased clotting).

The structural groups of the hemostatic system include:

- intima of blood vessels,
- blood cells (platelets, erythrocytes, leukocytes, macrophages),
- plasma enzyme systems of the blood.

Under the conditions of life of an organism (in vivo), hemostasis is a single system, all links of which are closely interconnected. For ease of study, the hemostasis system is conventionally divided into two parts: primary vascular-platelet hemostasis and secondary plasma-enzymatic coagulation hemostasis, consisting of coagulation and fibrinolytic links.

The polysystem of hemostasis also includes the kallikrein – kinin system and the complement and immunity system.

The processes of blood coagulation and fibrinolysis in the human body go on constantly from birth to death. The cause may be damage to the endothelium, cell death, release of adrenaline, etc. However, these processes are local in nature. The generalization of the coagulation process is impeded by the close interaction of the links of hemostasis. Disruption of adaptation mechanisms leads to the generalization of the process, which is expressed by the development of disseminated intravascular coagulation syndrome (DIC).

To stop the bleeding that has arisen, the concentration of blood clotting factors at the site of injury is required. The accumulation of factors is collagen, tissue factor and phospholipids of platelet membranes and some other cells.

Primary vascular platelet hemostasis.

The task of primary hemostasis is the initial stopping of bleeding by the formation of a platelet plug at the site of vessel damage. Endothelial cells of

blood vessels, subendothelium, vascular muscle fibers and platelets are involved in primary hemostasis (Table 1).

Endothelial cells contain both inhibitors (anticoagulants) and activators (procoagulants) of hemostasis. A healthy endothelial cell secretes substances with anticoagulant activity into the lumen of the vessel. These substances include antiplatelet agents and vasodilators: heparan sulfate, nitric oxide NO, prostacyclin and other compounds. All active substances are concentrated within the glycocalyx of the endothelial cell. Glycocalyx is a molecular layer on the outside of the endothelial cell, which consists of glycoproteins, proteoglycans, glycolipids. In it, parietal metabolic processes take place. Sialic acids of glycocalyx form a negative charge on the cell surface, which prevents adhesion (adhesion) of negatively charged blood cells, including platelets, to the endothelium.

When endothelial cells are damaged, procoagulants enter the lumen of the vessel, in particular, von Willebrand factor, tissue factor, PAI-1 fibrinolysis inhibitor, and the subendothelium layer is exposed.

The subendothelium of the vascular wall is formed by polymeric proteins: collagen, elastane, etc. Collagen forms an elastic vessel wall and is a substrate for platelet adhesion. It has a pronounced thrombogenic effect. Subendothelial cells (macrophages, fibroblasts, leukocytes) contain on their surface a tissue factor (formerly tissue thromboplastin), which activates the plasma link of hemostasis.

Table 1.

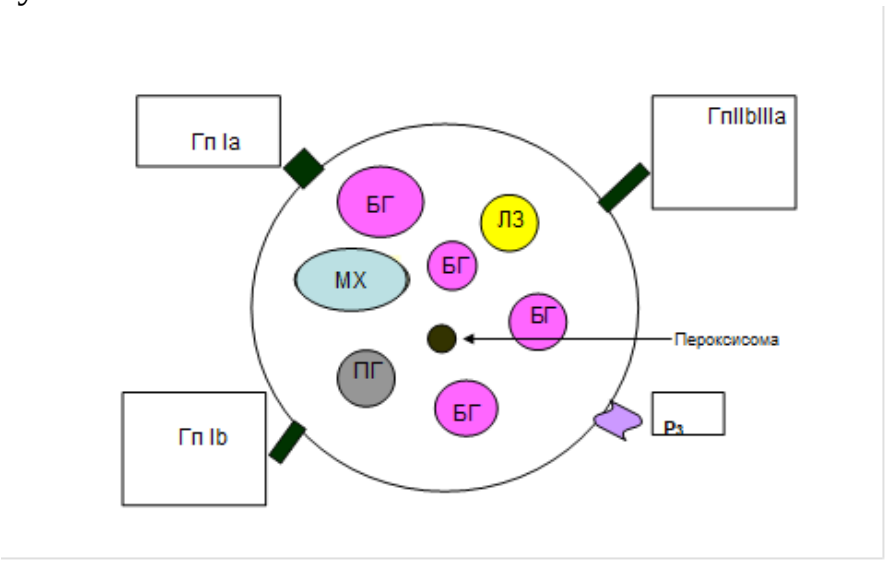
Elements and functions of components of primary hemostasis

The elements	Function
Endothelium	Providing thromboresistance. Obstruction of the release of blood cells and contact activation of platelet and plasma hemostasis. Damage is the release of activation factors for all links of hemostasis.
Subendothelium	Clotting activation. Isolation of tissue factor, platelet stimulants, collagen activation of platelets and coagulation factors, including von Willebrand factor.
Smooth muscles	Providing vascular tone. Stopping bleeding by contraction and overlap of the vessel lumen.
Platelets	Primary thrombus formation. Secretion of vasoactive substances, adhesion and aggregation of platelets, release of platelet coagulation factors

Platelets (platelets) are small non-nuclear cells with a diameter of 2-4 microns. Formed in the bone marrow from megakaryocytes and utilized in the spleen. Platelet life cycle is 9-10

days. The platelet pool includes mature plates with a diameter of 2-3 microns (80-95%), young forms - macrothrombocytes (1-10%) and microplatelets ending their life (3-15%). Approximately 2/3 of platelets circulate in the blood plasma, and 1/3 are in the spleen. The platelet membrane has a complex structure and consists of outer and inner layers, which differ in the composition of phospholipids. Microtubules, together with fibrillar proteins, form the platelet cytoskeleton. In the cytoplasm of platelets, there are subcellular structures and granules in which various substances accumulate, the so-called storage pools. On the surface of platelets there are receptors necessary for active interaction with other cells, plasma proteins and non-protein substances (Fig. 1).

For example, the platelet membrane receptor glycoprotein Ia (GP Ia) is sensitive to collagen, glycoprotein IIbIIIa (GP IIbIIIa) is the main receptor for platelet aggregation, glycoprotein Ib (GPIb) is involved in von Willebrand factor-mediated and thrombocyte adhesion to thrombocyte adhesion. External stimulants of von Willebrand factor are the antibiotic ristomycin (ristocetin) and the snake venom protein botocetin. Congenital or acquired deficiency of platelet receptors leads to impairment of their function (thrombocytopathy) and manifests itself in the form of hemorrhagic syndrome.



MC - mitochondria, LZ - lysosome, PG - dense granules, BG - protein granules, glycoprotein (GP) Ia - platelet receptor for collagen, GP Ib - platelet receptor for von Willebrand factor and ristocetin, GPIIb IIIa - platelet receptor for platelets and fibrinogen P3 - platelet phospholipid factor.

Platelets contain a number of specific platelet factors. Platelet factors are designated by the letter P (for platelet). One of them is platelet factor 3 (abbreviated as P3, PF3). He

represented by acidic phospholipids of the platelet membrane: phosphatidylserine (PS), phosphatidylethanolamine (PE) and phosphatidylinositol (PI). When platelets are activated, their inner membrane is inverted and the amount of phospholipids on the outer platelet membrane increases. Phospholipids form the surface necessary for fixation, activation and interaction of plasma proteins of hemostasis.

The cytoplasm of platelets contains mitochondria (MC), which provide the cell with energy, peroxisomes containing catalase, lysosomes with a set of acidic hydrolases, dense granules and protein granules. The platelet cytoplasm also contains XIII (fibrin-stabilizing) plasma coagulation factor and glycogen.

In dense granules, non-protein substances are concentrated: ATP, ADP, Ca ++, serotonin, adrenaline, histamine, etc. Once in the bloodstream, they cause a vascular reaction and platelet aggregation.

Protein granules store up to 30 different proteins, most of which have been synthesized in megakaryocytes. The granules store proteins such as von Willebrand factor, fibrinogen, factor V, protein S, high molecular weight kininogen (HMC), platelet factor 4 P4 and others. With a standard staining of a peripheral blood smear, the protein granules acquire a lilac hue, due to which platelets are easily identified.

The main functions of platelets:

1. Formation of the primary platelet plug by adhesion and aggregation of platelets.
2. Participation in plasma hemostasis:

- release of phospholipid factor P3 as a matrix for the concentration of plasma factors,
- release of procoagulants from the platelet storage pool,
- vasoconstriction,
- retraction of a blood clot

3. Angiotrophic - nutrition of the endothelium through the transfer of cytoplasm.
4. Reparative - release of platelet growth factor, which causes migration and division of fibroblasts and macrophages.

The formation of a primary platelet plug in case of damage to a vessel conditionally takes place in 3 stages:

1. Adhesion of platelets to the subendothelium.
2. Activation of platelets with the release of mediators from the storage pool.
3. Aggregation of platelets.

Platelet adhesion occurs directly through adhesion receptors and through adhesion mediated by von Willebrand factor (vWF) and other adhesion molecules (fibronectin, vitronectin, laminin, thrombospondin). Von

Willebrand factor is one of the highest molecular weight glycoproteins in blood plasma. This factor is synthesized in the vascular endothelium and megakaryocytes. Protein is heterogeneous in composition and includes polymers of various molecular weights. Part of the protein enters the blood, part remains in the cells in the storage pool, and part of it binds to the membranes of the subendothelium. The most high molecular weight and active vWF is found in platelet granules. The vWF features include:

- ensuring the adhesion of platelets to the damaged vascular wall, especially in conditions of strong blood flow (artery, arteriole),
- binding factor VIII and protecting it from degradation by protein C.

Platelet activation is manifested in a change in shape (the appearance of pseudopodia, platelet spreading), the appearance of an excess of acidic phospholipids (P3) on the outer membrane, the secretion of substances from storage pools, adhesion and aggregation of platelets. When exposed to weak stimulants (ADP, adrenaline, etc.), activation can be reversible. The platelet returns to an inactive state and can function again. Under the action of strong stimulants (collagen, thrombin, etc.) and the release of proaggregants from platelets, irreversible aggregation occurs. Such a platelet is tightly bound to other cells; it has lost the contents of the storage pools and cannot return to its original state. In the blood stream, it cannot function and is quickly eliminated from the circulation by the spleen.

Aggregation is the attachment of activated platelets to each other. It is mediated by fibrin and von Willebrand factor. Aggregated and permeated with fibrin platelets form the basis of a platelet (white) thrombus, which forms within a few minutes. The resulting thrombus is compacted due to platelet myosin fibrils, i.e. retraction (compression) of the clot occurs.

Arachidonic acid, which is found both in platelets and in endothelial cells (EC), plays an important role in vascular-platelet hemostasis. However, its role in the above structures is different. Upon platelet activation, arachidonic acid is released from membrane phospholipids. From it, with the participation of the enzyme cyclooxogenase 1 (COX-1), a powerful procoagulant of the prostaglandin series, thromboxane A2, is formed, which has a vasoconstrictor effect and promotes platelet adhesion. In endothelial cells from arachidonic acid with the participation of the same enzyme COX-1, prostacyclin is formed, which belongs to prostaglandins and has a pronounced antiplatelet effect. Various mechanisms of the conversion of arachidonic acid in platelets and EC are physiologically justified, since the task of platelets is to form a clot, and EC is to prevent its formation.

Plasma (coagulation) hemostasis

Currently, 2 systems of plasma hemostasis are conventionally distinguished: the plasma coagulation system (coagulation) and the system

fibrinolysis. Both systems closely interact with each other and with platelet hemostasis.

Coagulation link of hemostasis.

The plasma coagulation system is an enzymatic proteolytic system that forms fibrin plugs at the sites of vessel damage. It consists of enzymes, protein catalysts (cofactors), plasma clotting inhibitors, and some non-protein substances. Historically, plasma proteins that are part of the blood coagulation cascade (factors) are usually denoted by Roman numerals. Recently, a number of factors have been excluded from the nomenclature: factor VI, since it turned out to be activated by factor V; factor IV, calcium ions that are not proteins; tissue factor (formerly tissue thromboplastin, factor III), which is not a plasma protein and enters the blood from tissues. The newly discovered factors do not have Roman symbols (Table 2). Almost all factors in the blood plasma are inactive. They are activated by proteolysis. To designate an activated factor, the letter a is added to its name (for example, Xa).

Table 2.

Plasma coagulation factors

Symbol	Name	Synthesis site	Plasma concentration	Hemostatic minimum
I	*Fibrinogen*	Liver	2-4 g/l	0,5-1 г/л
II	Prothrombin	Liver	100-150 mg/l	40%
V	*Proaccelerin*	Liver	7-10 mg/l	10-15%
VII	Proconvertin	Liver	0,4-0 mg/l	5-10%
VIII	*Antihemophilic globulin A*	Liver	0,7 nmol / l	30-35%
IX	Antihemophilic globulin B	Liver	3-5 mg / l	20-30%
X	Stuart-Prower factor	Liver	8-10 mg / l	10-20%
XI	Antihemophilic globulin C	Liver	3-6 mg / l	10-20%
XII	Hageman factor	Liver	25-35 mg / l	< 1%
XIII	Fibrin stabilizing factor	Macrophages, megakaryocytes	20-30 mg / l	2-3%
PK	Prekallikrein (PC, f. Fletcher)	Liver	30-50 mg / l	< 1%
hmwk	High molecular weight kininogen (VMK, F. Fitzgerald)	Liver	60-80 mg / l	< 1%

Note: bold in the column of symbols denotes vitamin K- and at the same time Ca ++ - dependent factors, in italics - proteins, but not enzymes.

Most of the factors are enzymes. Serine proteases include factors II, VII, IX, X, XI, XII, PK; transglutaminase - factor XIII. Factors V, VIII and IUD are cofactors of a protein nature. Their role is to accelerate coagulation reactions.

Some of the factors are vitamin K-dependent and at the same time Ca-dependent. These include factors II, VII, IX, X, proteins C and S. These proteins are synthesized in the liver and have a similar molecular structure. To obtain full-fledged factors, a γ-carboxylation reaction is required

glutamine, which requires the presence of an activated form of vitamin K. Ca ++ contacts vitamin K-dependent factors with membrane phospholipids, which makes these proteins functionally active. The factors formed in the absence of vitamin K are called PIVKA factors (Protein Induced by Vitamin K Absence). They are functionally defective.

The last two factors, PK and IUD, are glycoproteins and belong to the kallikrein-kinin system. The kallikrein-kinin system is a proteolytic system that is involved in the regulation of the activity of cascade proteolytic systems of blood plasma: kininogenesis, hemocoagulation, fibrinolysis, complement and renin-angiotensin system, which provide the processes of adaptation and protection of the body.

The activity of proteolytic systems is regulated by kinins, which in blood plasma are represented by inactive precursors - kininogens, in particular, IUDs. The formation of active kinins from kininogens occurs under the action of trypsin-like serine proteinases - kallikreins, localized in the blood plasma (plasma kallikrein) and tissues of some organs. They are also dormant in their precursor form. Prekallikrein is a precursor of blood plasma kallikrein. Recently, the participation of the components of the kallikrein-kinin system in the activation of the contact phase of blood coagulation has been well studied. It was found that four proteins are involved in contact activation: factors XII, XI, precallikrein, and IUD.

The scheme of blood plasma coagulation with the participation and activation of all factors is presented in detail in most monographs. Without constant use, it is quickly forgotten, and when studied, it becomes difficult. This, apparently, is connected both with the complexity of the system and with the relatively random numbering of factors due to the sequence of their discovery.

Ultimately, blood plasma clotting consists in the conversion of soluble fibrinogen protein into insoluble fibrin protein (Fig. 2). The latter forms a fibrin network in which blood cells get stuck, and a red blood clot forms. Thrombus formation time is about 30 minutes. The conversion of fibrinogen to fibrin requires the presence of active thrombin in the plasma. It is he who, under physiological conditions, starts the process of fibrinogen polymerization. In blood plasma, thrombin (IIa), like other blood factors, is in an inactive state in the form of a precursor, prothrombin (factor II). The presence of active thrombin in the plasma leads to the obligatory coagulation of blood plasma. Therefore, thrombin is called the main coagulation factor, and the condition of a patient with free thrombin is called thrombinemia. The presence of thrombinemia is always associated with hypercoagulability and the risk of thrombosis.

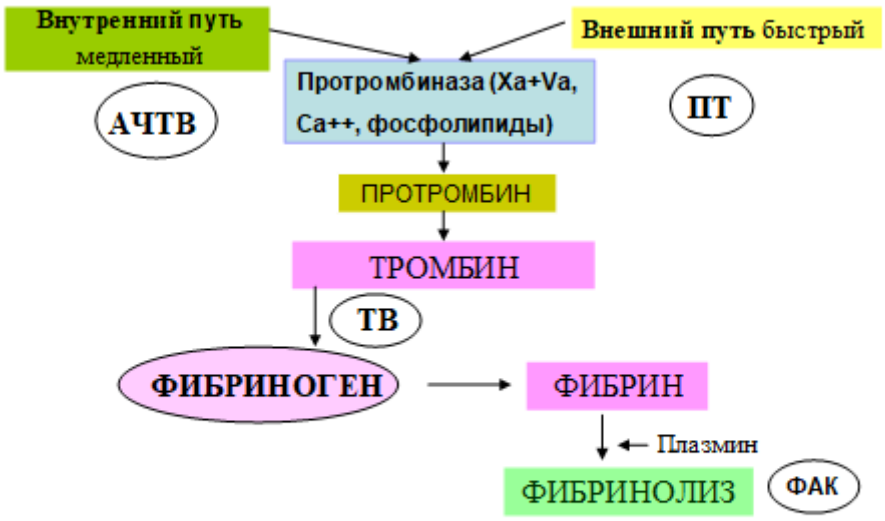


Рис. 2. Схема коагуляционного гемостаза

For the formation of thrombin (f. IIa) it is necessary to activate prothrombin (f. II). Prothrombin is activated by prothrombinase. Prothrombinase includes activated factors Xa + Va, and factor Xa has enzymatic activity, and factor Va is a protein coenzyme that accelerates the reaction tens of thousands of times. The conversion of prothrombin to thrombin occurs on acidic phospholipids of membranes, where plasma factors and calcium ions are concentrated, which are necessary for the interaction of factors with phospholipids. Ca ++ is found in plasma and comes from platelets when they are activated. In the human body (in vivo), phospholipids are represented by the third factor of platelets (P3) and phospholipids of the membranes of some other cells. In a laboratory study of hemostasis for phospholipid-dependent tests, the kit must include phospholipids of plant or animal origin. Tests that require phospholipids are called phospholipid-dependent tests (PT, APTT, protein C).

Prothrombin is activated in two ways: slow internal (10-15 minutes) and fast external (10-15 seconds). Various factors are involved in the activation of prothrombin through the internal and external pathways. The activation mechanisms also differ. Prothrombin activation is a multistep cascade process in which the activation of one molecule of the previous level leads to the activation of tens or hundreds of subsequent molecules. Protein cofactors play an important role in signal amplification. In the body, both pathways are closely interconnected. For the convenience of evaluating the research results, they are conditionally

separated and evaluated by different tests. Activated partial (partial) thromboplastin time (APTT, APTT) is used to assess the internal pathway, and prothrombin time (PT, aka prothrombin test - PT) is used to assess the external pathway (Table 3).

Activation of plasma clotting through the intrinsic pathway is so called because all the factors necessary for plasma clotting are in the blood plasma. The formation of prothrombinase along the internal pathway is carried out by contact activation. Factors of the contact phase include factor XII (Hageman), factor XI, and IUD with an activating enzyme PC. It is believed that activation of coagulation begins with the contact of the Hageman factor with a negatively charged surface of a solid. Under the conditions of the body, this can be collagen, cell membranes, non-physiological surfaces, for example, artificial valves or prostheses. Under in vitro conditions, kaolin, ellagic acid and some other substances are used as an activator of the contact phase. Further, factor IX (IXa) and its cofactor VIII (VIIIa) are activated. Factors IXa + VIIIa form a tenase complex (ten - ten), which activates factor X. Factor X is an enzyme, and factor V is its cofactor. The sum of Xa + Va factors form prothrombinase, the activity of which is manifested on phospholipids of platelet membranes (P3) in the presence of Ca ++. All four factors together form a prothrombinase complex (Xa + Va, P3, Ca ++).

Table 3.

Prothrombin activation pathways

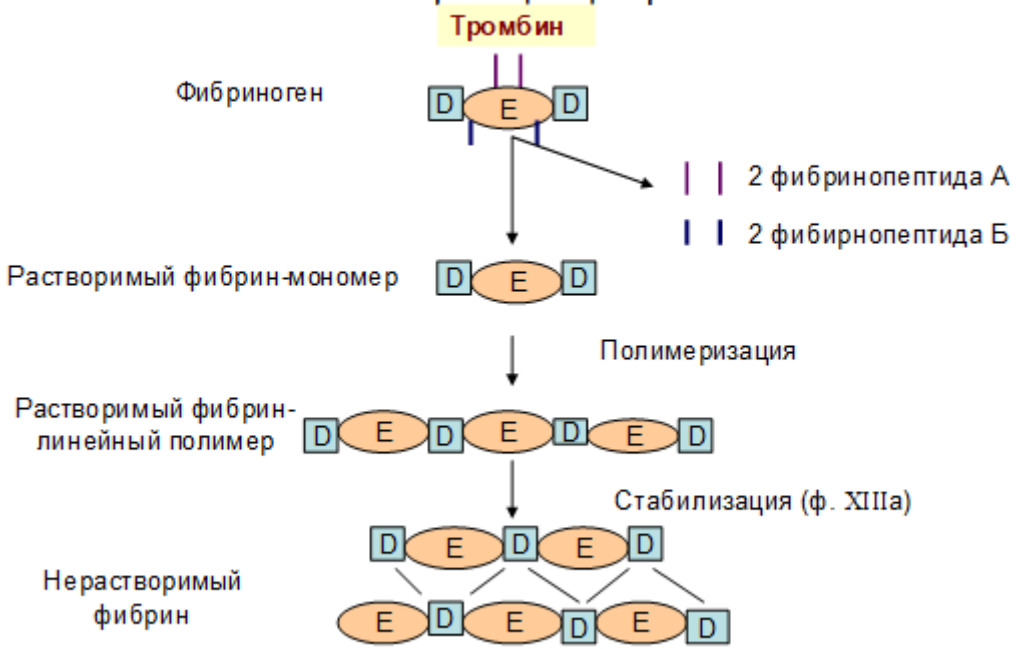
The way	The Activators	The factors	The time
Inner path (slow)	. Contact with collagen, cell membranes, CEC, etc.	XII, XI, BMK, PIK IX+VIII **Xa+Va, P3, Ca^{++}**	15 min
Outer path (fast	Tissue factor (TF, cell membrane glycoprotein).	VII **Xa+Va, P3, Ca^{++}**	15 sec

Note: the prothrombinase complex is indicated in bold.

The activation of prothrombin by the external pathway is carried out by tissue factor TF (previously, tissue thromboplastin), which is located outside the vascular bed, therefore the pathway is called external. Particularly rich in TF EC, lungs and brain tissue. The source of TF can be phagocytes, tumor cells, atherosclerotic plaques. TF contacts factor VII and converts it to factor VIIa. The latter activates factor X (Xa), which, with the participation of factor Va, phospholipids and calcium, forms a prothrombinase complex that activates prothromin and the conversion of fibrinogen to fibrin. Thus, the result of the activity of both internal and external pathways of plasma hemostasis is the formation of the same prothrombinase complex. However, the activation process along the external path is much faster and includes 1 factor and 1 activation stage instead of 5 factors and 3 stages

activation along the inner path. It is known that all plasma coagulation factors are inactive in the blood. The exception is factor VII, 1-2% of which is activated in blood plasma. All of the above explains the rapid coagulation of blood through the external pathway, which is currently considered the main physiological mechanism for triggering the plasma coagulation process.

Рис. 3. Полимеризация фибриногена



It was previously indicated that the basis of blood plasma coagulation is the conversion of fibrinogen to fibrin under the action of thrombin (f. IIa). Fibrinogen is a protein that has a unique ability to polymerize in the bloodstream. It is synthesized in hepatocytes and possibly a little in megakaryocytes. A high concentration of fibrinogen in blood plasma (2-4 g / l) is due to its function. The protein consists of 3 pairs of polypeptide chains (2α, 2β, 2γ), which form 3 domains: central E and two peripheral D. The conversion of fibrinogen to fibrin under the action of thrombin goes through several stages (Fig. 3):

a) Formation of fibrin monomers as a result of cleavage of two fibrinopeptides A and two fibrinopeptides B (2 FPA + 2 FPV).

b) Spontaneous polymerization of fibrin monomers with the formation of soluble fibrin-monomeric complexes (fibrin fibers, soluble fibrin, fibrin S - soluble, since it dissolves in a 5-7 M solution of urea and monochloroacetic acid).

c) Stabilization of fibrin fiber f. CIIIa with the formation of cross-isopeptide bonds. A network of interacting fibrin fibers is formed that can hold platelets at the site of injury

(insoluble fibrin, fibrin i - insoluble). F. XIIIa is activated by thrombin.
d) Retraction (reduction) of the clot occurs due to the contractile proteins of platelets. In the absence of platelets or a defect in the GP IIbIIIa receptor, retraction decreases, the thrombus is rapidly lysed (hyperfibrinolysis), and the likelihood of a thrombus detachment with subsequent thromboembolism of the removed vessels increases.
During fibrinogen polymerization, intermediate products are formed, some of which are not included in the composition of insoluble fibrin. They are present in blood plasma in the form of fibrin monomers and soluble fibrin monomeric complexes (RFMK). In a healthy person, there are practically no fibrin monomers, and RFMK are present in an insignificant amount, since the blood coagulation process is ongoing, but is of a local nature.

Inhibitors of enzymes of the hemostasis system (anticoagulants).

To prevent the generalization of the blood coagulation process, which is constantly going on in the human body, inhibitors of the enzymes of the hemostasis system are present in the plasma. Their nature and mechanism of action are different, but the goal is the same: to keep blood coagulation processes within the physiological norm.
The following inhibitors are known: antithrombin (formerly antithrombin III, AT), protein C system (read "si"), heparin cofactor II - neutralization of thrombin, C1 inhibitor - control of the contact phase of the internal pathway of hemostasis, α1-antitrypsin, α2-macroglobulin. In the last decade, a tissue factor pathway inhibitor or an extrinsic pathway inhibitor (IPTP, TFPI) has been discovered. However, AT and the protein C system are of the greatest importance for hemostasis.
Antithrombin (AT) is a glycoprotein that is synthesized in the liver. It forms a stable complex with serine proteases and inhibits several enzymes, however, it is most active in relation to thrombin and ph. Ha. At the same time, antithrombin can bind to heparin. Under physiological conditions, heparin is produced by mast cells and, if necessary, is secreted into the bloodstream. The activity of AT is enhanced by heparin thousands of times. Heparin has a catalytic effect on AT, causing AT conformational changes. After the formation of the thrombin-antithrombin complex (TAT), heparin can be released to organize other complexes. Despite the fact that AT is present in plasma in excess, with a decrease in its level to 60%, the risk of thrombosis increases.
The protein C system inactivates cofactors of enzymes Va and VIIIa, located on phospholipid membranes, by lysis in the presence of Ca ++. The system includes protein C, which is synthesized in the liver, is a vitamin-K-dependent serine protease and is activated by thrombin, its cofactor protein S, membrane protein thrombomodulin,

protein C receptor and C4 binding protein. Protein S is a vitamin K-dependent protein that is synthesized in the liver. 40% of the protein in plasma is in a free state, and 60% is bound to the complement component C4b. Only free protein S can act as a cofactor.
The lack of factors of the protein C system leads to the risk of developing venous and arterial thrombosis - thrombophilia. The protein C system actively responds to inflammatory processes in the body, especially those caused by gram-negative microbes. Inflammatory mediators suppress and inactivate components of the protein C system, causing hypercoagulability.
In addition to physiological anticoagulants, which are also called primary, secondary anticoagulants are present in the hemostasis system. These include RFMK and other fibrin / fibrinogen degradation products (FDP). Their role increases with massive thrombosis and disseminated intravascular coagulation.

Fibrinolysis system.
In the process of the formation of a hemostatic plug, mechanisms are activated aimed at limiting the growth of a thrombus, its dissolution and restoration of blood flow. These functions are performed by the fibrinolysis system, which belongs to one of the five proteolytic systems of blood plasma. Fibrinolysis is the main endogenous mechanism that prevents thrombus formation.
The key enzyme of the fibrinolysis system is plasmin. In blood plasma, it is present in the form of an inactive zymogen plasminogen, a single-chain glycoprotein that is synthesized in the liver, a little in the kidneys, eosinophils and the cornea. The main task of plasmin is to dissolve fibrin. However, with high activity, it can break down fibrinogen, some blood coagulation factors and components of the complement system. Under physiological conditions, plasmin activity is limited to the thrombus formation zone. In pathological conditions, fibrinolysis can be generalized (hyperfibrinolysis, active fibrinolysis, fibrinolytic state). As a result of hyperfibrinolysis, fibrin / fibrinogen degradation products (PDP) accumulate; clinically, the patient experiences bleeding.
The level and activity of plasmin in the body is tightly regulated and includes activators and inhibitors of fibrinolysis. Their interaction allows you to limit the growth of a thrombus and lyse it. Fragments of fibrin formed during clot lysis are then utilized by leukocytes and macrophages (the cellular component of fibrinolysis).
Plasminogen activation, similar to the activation of prothrombin, is carried out through the internal and external pathways.

The internal pathway represents contact activation, in which the previously mentioned contact activation factors are involved: XII, XI, IUD, PC. Internal activators include urokinase (u-PA), which is synthesized and secreted by cells of various organs, including kidneys, fibroblasts, macrophages, and endothelial cells (EC).

The main pathway of plasminogen activation is external under the action of tissue-type plasminogen activator (t-PA, TAP) coming from endothelial cells. Studies have shown that the main stimulator of TAP secretion is bradykinin, which is formed from the IUD when it is cleaved by kallikrein.

Nonphysiological plasminogen activators can be hemolytic streptococcus streptokinase and enzymes of other bacteria.

Plasmin in the bloodstream is rapidly inactivated by natural inhibitors. There are two types of inhibitors:

- antiplasmins (α2-antiplasmin, α2-macroglobulin, α1-antitrypsin, etc.),
- inhibitors of plasminogen activators (PAI-1, PAI-2, PAI-3).

The main plasmin inhibitor is α2-antiplasmin. It is synthesized in the liver and belongs to serine protease inhibitors.

Among the inhibitors of plasminogen activators, PAI-1 is of the greatest importance. It inhibits t-PA, u-PA and streptokinase. Produced by EC, smooth muscle cells, megakaryocytes and mesothelial cells; deposited in platelets. The synthesis of PAI-1 is stimulated by bacterial endotoxins, proinflammatory cytokines, thrombin and other factors; therefore, its level increases in many pathological conditions. The main task of PAI-1 is to limit fibrinolysis to the thrombus site by inhibiting t-PA. This is easily accomplished due to the high content of PAI-1 in the vascular wall.

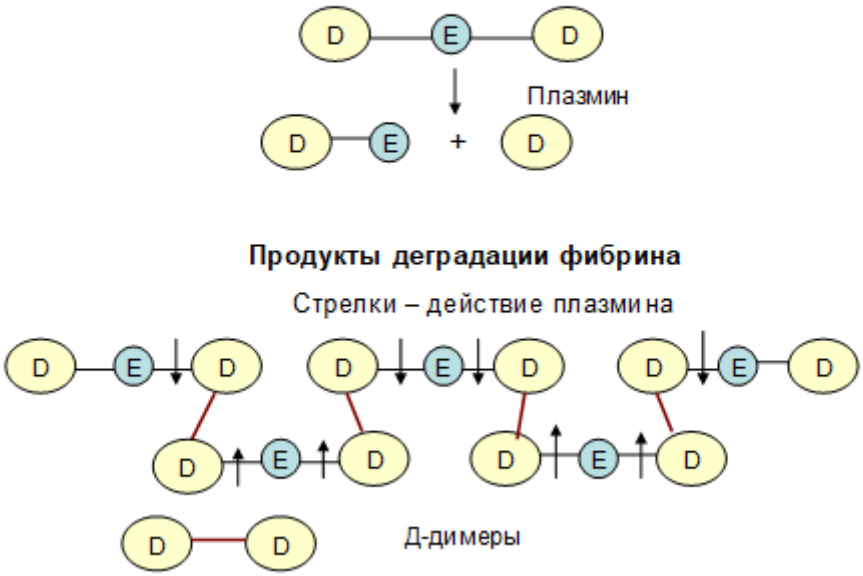
Lysis of a fibrin clot.

Plasmin is an active, but rather nonspecific, serine protease. Although its main function is fibrin lysis, plasmin also cleaves fibrinogen well, especially when its content is high, plasmin is activated or its inhibitors are lacking (Fig. 4).

When fibrin and fibrinogen are degraded, low molecular weight products are formed, which are called fibrin / fibrinogen degradation products (FDP) and are secondary anticoagulants. When fibrinogen degrades, its molecule is split into D, E and DE fragments. When insoluble fibrin, which has cross-linked isopeptide bonds, is cleaved, DD fragments are formed, which are called D dimers. Thus, in the presence of a fibrin clot, the level of D-dimers increases, which is typical for thrombosis and thromboembolism, including pulmonary embolism (PE). Other low molecular weight products form soluble fibrin-monomeric complexes (RFMC). The growth of RFMK can be observed when

activation of blood coagulation processes, with a high level of fibrinogen, with increased fibrinolysis and fibrinogenolysis and some other conditions. The level of fibrin monomer in healthy people and in most types of pathology does not increase, since it quickly polymerizes or breaks down. An increase in fibrin monomers is observed practically only in DIC syndrome.

Рис.4. **Продукты деградации фибриногена**



Features of hemostasis in women during menstruation and pregnancy.

In the childbearing period, women have monthly menstruation - physiological bleeding from the uterus. Bleeding is supported by two main mechanisms: inhibition of platelet aggregation by prostaglandins and activation of fibrinolysis. During menstruation, endothelial cells secrete large amounts of tissue plasminogen activator (t-PA). A high content of prourokinase was also found in the blood from the uterus. T-PA and urokinase, by activating plasminogen, destroy fibrin formed in menstrual blood, and a clot does not form. Increased fibrinolysis is a local process limited to the uterus.

During pregnancy, changes in the hemostatic system appear from 2-3 months and continue with a progressive increase until delivery (Table 4). In pregnant women, hypercoagulability and a decrease in fibrinolysis develop, which are most noticeable in the 3rd trimester. A decrease in fibrinolysis is associated with an increase in the content of inhibitors of plasminogen activators of the 1st and 2nd types (PAI-1 and PAI-2).

During pregnancy:

• the activity of factors VII, VIII, IX, X and the level of fibrinogen increases,

• the content of von Willebrand factor and platelet aggregation increase,
• decreases fibrinolytic activity,
• decreases anticoagulant activity (decrease in AT III, protein S),
• the velocity of venous blood flow decreases and blood viscosity increases due to the increase in the content of fibrinogen and immunoglobulins,
• the tone of the venous wall decreases due to hormonal changes in the body.

Table 4

Some indicators of hemostasis in pregnant women

Indicators	Not pregnant	Pregnancy 1 trimester	Pregnancy end of the 2nd trimester	Pregnancy end of the 3rd trimester
Fibrinogen g / l	2-4	2-5	3-5	4,5-6
APTT sec	41,5 ± 3,8	39,2 ± 4,1	36,5 ± 2,1	34,1 ± 2,5
PTI%	85,5 ± 3,4	89,3 ± 4,5	95,4 ± 5,3	108,8 ± 3,3
PDP μg / ml	до2			5,7 ± 0,9
AT III g / l	0,25 ± 0,02	0,22 ± 0,03	0,17 ± 0,02	0,15 ± 0,02
Thrombocytes thousand / ml	295 ± 32	302 ± 15	288 ± 12	250 ±14

Hypercoagulation during pregnancy is a physiological condition that allows the implantation of the egg, the proper connection of the placenta with the uterus, and the arrest of bleeding during childbirth. At the same time, pregnancy increases the risk of venous thrombosis.

In some cases, disorders in the blood coagulation system lead to the development of fetal loss syndrome (FSS). In case of severe disorders in the hemostasis system, it is necessary to exclude the hereditary factor. Hemorrhages in pregnant women are extremely rare and are usually associated with hereditary defects in coagulation factors. Thrombophilia is much more common, and their main cause is the presence of antiphospholipid syndrome (APS).

PATHOLOGY OF HEMOSTASIS

Studies have shown that the vast majority of diseases are accompanied by disorders in the hemostatic system. The pathology of hemostasis is expressed either by hypocoagulation or hypercoagulation, in some cases by mixed disorders. Hypocoagulation is relatively rare. Diagnosis of the causes of hypocoagulation is not always possible within the framework of a standard, even extended hemostasiogram. As a rule, like

patients are examined in specialized medical institutions. A large number of patients suffer from hypercoagulability. It manifests itself in patients in the form of thrombosis, thromboembolism or thrombophilic condition. Diagnosis, treatment and monitoring of therapy are usually carried out in general hospitals.

Hypocoagulation. Hemorrhagic diseases.

Hypocoagulation is clinically manifested by hemorrhagic syndrome - various kinds of hemorrhages or bleeding, therefore, diseases are called hemorrhagic. They may be related

- with pathology of platelets,
- with a fall below the coagulation threshold of blood coagulation factors,
- with inhibition of blood coagulation factors,
- with hyperfibrinolysis,
- with pathology of the structure of the capillaries.

Diseases can be congenital or acquired.

Platelet pathology.

Platelet pathology is expressed either by thrombocytopenia (a decrease in platelet count), or thrombocytopathy - a violation of their function. With these types of pathology, at least the platelet-vascular link of hemostasis changes. This pathology, as a rule, is accompanied by microcirculatory petechial-bruising type of bleeding, in which there is the appearance of petechiae and small bruises on the skin of the limbs and trunk, nosebleeds, menorrhagia, hematuria. Disorders can be hereditary or acquired. Thrombocytosis is an increase in the level of platelets. It can be accompanied by hemorrhages or thrombosis, depending on the preservation of platelet function. Thrombocytosis is only acquired in nature.

Thrombocytopenia - a decrease in the platelet count below 150 thousand / ml. The main hereditary thrombocytopenias: Fanconi, Wiskott-Aldrich syndromes, May-Hegglin anomaly.

Acquired thrombocytopenia can be associated with:

- with impaired platelet formation in systemic hematological diseases,
- with increased consumption of platelets (disseminated intravascular coagulation, angiomatosis).
- with increased destruction of platelets (hypersplenism, HIV infection, systemic lupus erythematosus, administration of drugs, including heparin, repeated blood transfusions, etc.). In most cases, this type of thrombocytopenia is associated with immune pathology.

If thrombocytopenia is suspected, platelets are counted, their size is estimated, and bleeding time is determined. With autoimmune

processes are characterized by the appearance of giant platelets. The bleeding time in most patients with thrombocytopenia is prolonged.
Thrombocytopathies are a large group of heterogeneous diseases. A large place in this group is occupied by congenital platelet dysfunctions: defects in platelet receptors (for example, HP Ib defect - Bernard-Soulier syndrome, HP IIbIIIa defect - Glanzmann thrombasthenia), storage pool granule abnormalities, arachidonic acid metabolism disorder.
Von Willebrand disease is associated with a mutation of vWF and is the result of qualitative or quantitative disorders of von Willebrand factor. Strictly speaking, it does not belong to thrombocytopathies, but an abnormal factor disrupts platelet adhesion and leads to the appearance of a hemorrhagic syndrome, the manifestations of which depend on the type of disease. Both men and women are ill.
Dysfunction of platelets, a decrease in their thromboplastic activity and, as a result, increased bleeding can be caused by drugs, systemic hematological diseases, uremia, paraproteinemia and dysglobulinemia.
Available methods for assessing the platelet link of hemostasis are: counting the number of platelets, determining their size, assessing the morphology of platelets and determining the time of bleeding. The number of platelets in the pathology of platelets can be normal or reduced, the bleeding time is often lengthened. In thrombocytopathies associated with a defect in the storage pool of protein granules, the staining of these granules in the blood smear is impaired (“gray” platelet syndrome). In some thrombocytopathies, APTT and PT are lengthened (prothrombin in% according to Quick, respectively, decreases). In a specialized medical institution for differential diagnosis, the content of von Willebrand factor, factor VIII is additionally determined, platelet aggregation with adrenaline, ADP, collagen, ristocetin, arachidonic acid and other aggregation inducers is assessed.

Hemorrhagic coagulopathy.
This group of pathology includes diseases caused by a deficiency or inhibition of blood coagulation factors and hyperfibrinolysis.
Factor deficiency can be congenital, genetically determined, or acquired. Congenital deficiency of factors is accompanied by bleeding of the hematoma type. With this type of bleeding with small bruises, hemorrhages occur in the cavity of the joints, abdomen, under the fascia, etc. Patients experience delayed (after 1-2 hours) bleeding from the wound after surgery, prolonged bleeding from the hole after tooth extraction. Sometimes there is a mixed hematoma-microcirculatory or microcirculatory type of bleeding.
Congenital deficiency of factors is caused by genetic disorders. Currently, diseases associated with defects in all plasma coagulation factors have been

identified, although they are extremely rare. The most common are hemophilia A (lack of factor VIII, incidence of 100 patients per million population), and hemophilia B (deficiency of factor IX, incidence of 20 patients per million population). Both types of hemophilia are sex-linked, so only boys are affected. The disease was first described in the Babylonian Talmud in the 5th century in connection with the presence of cases of fatal bleeding in boys after circumcision. Hemophilia is a severe blood clotting disorder with hemorrhages in the subcutaneous tissue, joints and muscles and subsequent damage to these organs and nerves. Bleeding in hemophiliacs without treatment is fatal.

Acquired hypocoagulation can be associated with the consumption of factors (DIC syndrome), the presence of inhibitors to factors, with hyperfibrinolysis, with treatment with anticoagulants, with liver pathology and with some other diseases.

The most common and clinically important cause of hypocoagulation is deficiency of vitamin K-dependent factors. Vitamin K is partly synthesized in the intestines and partly supplied with food. It belongs to fat-soluble vitamins, therefore, for its absorption, the presence of bile acids of bile as emulsifiers of fats and pancreatic lipase is necessary. With a deficiency of vitamin K, congenital or acquired, the liver disrupts the synthesis of vitamin-K-dependent factors - IIV, IX, X and II, as well as proteins C and S. Vitamin K is necessary at the last stage of the formation of factors. Factors formed without Vitamin K cannot provide complete hemostasis. These are called PIVKA factors and can be determined by immunological methods.

The reasons for the deficiency of high-grade vitamin K-dependent factors can be:

- violation of the synthesis of vitamin K in premature newborns,
- insufficient intake of vitamin K from the intestine with dysbacteriosis, prolonged use of broad-spectrum antibiotics, with resection of the small intestine,
- impaired absorption of vitamin K in cholestasis,
- taking antagonists of vitamin K - oral anticoagulants (warfarin, etc.).

Hypocoagulation with hemorrhage can manifest itself in a number of general and systemic diseases and conditions. Liver diseases with impaired synthetic function lead to a decrease in all factors, except for XIII (see Table 2). In amyloidosis, there is a deficiency of factor X associated with the absorption of this factor by amyloid. In nephrotic syndrome, the level of factor VII decreases due to the excessive excretion of this factor in the urine. In some types of pathology, antibodies to various factors are observed, which leads to inhibition of factors

the appearance of hemorrhagic syndrome and lengthening of the clotting time in those tests where this factor is involved. The presence of antibodies to factors was recorded in leukemia, myelodysplasia, solid tumors, immune-allergic syndromes, with wasp bites, at the end of pregnancy and in the early postpartum period, etc. Most often antibodies are formed to factors VIII and von Willebrand, as well as to factors IX , V, VII and II.

The primary detection of hemorrhagic coagulopathy is possible after conducting tests of a standard coagulogram to prolong the plasma clotting time in the test in which the deficient factor is involved. So, with a deficiency of factor VII, PT is lengthened, and with a deficiency of factors VIII, IX and XI - APTT, with a deficiency of prothrombin (factor II) both indicators will be lengthened, etc. In specialized medical institutions, differential diagnosis is carried out by conducting clarifying tests.

Paraproteins and cryoglobulins, which are most often formed in immunocomplex diseases and neoplastic processes, disrupt not only platelet function, as mentioned earlier, but also affect the results of some tests of plasma hemostasis. Prolongation of plasma clotting time in the thrombin test with normal fibrinogen content and the absence of thrombinemia (PDP and RFMK are not increased), as a rule, is associated with paraproteins, which disrupt the interaction of thrombin with fibrinogen and prevent fibrin polymerization. The simultaneous lengthening of APTT and PT in some cases may be due to the presence of cryoglobulins.

One of the causes of hemorrhagic coagulopathy is hyperfibrinolysis (active fibrinolysis, fibrinolytic state). Under physiological conditions, plasmin in the bloodstream is neutralized by natural inhibitors, and fibrinolysis is limited to the zone of fibrin formation, that is, a hemostatic plug. The background activity of fibrinolysis in blood plasma is rather low. In women, during menstruation, local activation of fibrinolysis in the vessels of the uterus is observed, which ensures the absence of clots and the unhindered flow of blood. In pathological conditions, fibrinolysis can be generalized, covering both clot fibrin and blood plasma fibrinogen. Clinically, hyperfibrinolysis is manifested by bleeding, and with depletion of plasminogen, by thrombosis.

There are primary and secondary hyperfibrinolysis. Primary hyperfibrinolysis is caused by hyperplasminemia when large amounts of plasminogen activators enter the bloodstream. It can be caused by physical exertion, stress or venous stasis, tumors that secrete activators of fibrinolysis (tumors of the ovaries, pancreas, prostate, intestines, promyelocytic leukemia), surgical interventions on organs rich in activators of fibrinolysis (lungs, prostate, pancreas and thyroid). Very rarely, hyperfibrinolysis is observed with a hereditary excess of tissue plasminogen activator (t-PA) or with a hereditary deficiency of the main inhibitor of plasmin α2-antiplasmin.

Secondary fibrinolysis develops in response to intravascular blood coagulation, thromboembolic processes of various localization, or the introduction of fibrinolytics (streptokinase, actilyse, etc.). Activation of fibrinolysis is also observed with massive transfusions of canned blood, multiple injuries, and sepsis.

Laboratory hyperfibrinolysis can be detected in a test with lysis of an euglobulin clot (fibrinolytic activity - FAC) and by thromboelastography or thromboelastometry. Plasminogen level determination is possible.

Hypercoagulation.

Conditions associated with hypercoagulability are common and are usually diagnosed and treated in general hospitals.

For patients of this group, the concept of thrombophilia is characteristic - a tendency to thrombus formation, an increase in blood viscosity, platelet aggregation, a decrease in anticoagulant potential. Thrombophilia can lead to the development of arterial and venous thrombosis and thromboembolism.

Arterial and intracardiac thrombosis is usually caused by changes in the vascular wall, for example, in atherosclerosis, and platelet activation. Arterial thrombosis can also be associated with the presence of a lupus anticoagulant or with a decrease in anticoagulant levels. Usually these are parietal white blood clots, prone to separation. Clinically, arterial thrombosis is manifested by the cessation of function of the organ affected by thrombosis, for example, myocardial infarction or stroke.

The appearance of venous thrombosis is associated with hypercoagulability and blood stasis. These are more often red blood clots that completely cover the lumen of the vessel. Clinically, venous thrombosis manifests itself in the form of pain syndrome with local redness and fever.

In both arterial and venous thrombosis, especially in deep vein thrombosis of the thigh, there may be a separation of a thrombus with subsequent thromboembolism of the pulmonary artery (PE), cerebral vessels (ischemic stroke) and other small or large vessels. Thromboembolism in arterial thrombosis can be caused by an irregular heartbeat, for example, atrial fibrillation.

The occurrence of thrombotic complications is associated with both hereditary and acquired factors.

Acquired factors can be conditionally divided into 2 groups: factors associated with the characteristics of physiology and lifestyle (age, sex, diet, smoking, physical inactivity, increased body weight, pregnancy), and secondary disorders caused by another disease.

Classification of the main types of thrombophilia.

1. Hemorheological forms in myeloproliferative diseases, polyglobulia, violations of the volume and shape of erythrocytes (hemoglobinopathy,

fermentopathy, etc.), with increased blood viscosity (paraproteinemia, hyperfibrinogenemia, gammopathy).
2. Forms associated with vascular platelet hemostasis: thrombocytosis, increased platelet aggregation, increased von Willebrand factor production, sticky platelets, etc.
3. Forms caused by deficiency or abnormalities of physiological anticoagulants: deficiency and abnormalities of antithrombin, protein C and S, etc.
4. Forms associated with deficiency, overproduction or abnormalities of plasma coagulation factors.
5. Forms associated with fibrinolysis disorders.
6. Metabolic forms (atherosclerosis, diabetes mellitus, hyperlipidemia, hyperlipoproteinemia (a), hypergomocysteinemia).
7. Autoimmune and infectious-immune forms (antiphospholipid syndrome, allergies and other immune diseases, immune and viral thrombovasculitis, thrombohemorrhagic fevers, bacterial endocarditis and other types of chronosepsis).
8. Paraneoplastic thromboembolic syndromes (thrombotic complications in all types of cancer, surgical and chemotherapeutic interventions).
9. Iatrogenic forms (catheterization, vascular prostheses, caval filters, stem cell transplantation, dosage forms - treatment with concentrates of activated factors, heparin, plasminogen activators, taking hormonal contraceptives, etc.).
10. Combined forms.
Thus, the majority of hospital patients and a wide contingent of polyclinic patients need to determine the parameters of blood coagulation and further monitoring of coagulation. Thrombophilic conditions can be detected using laboratory tests that reflect a tendency to hypercoagulability.
To examine patients with suspected thrombophilia, two groups of tests are used. The first group includes markers of blood coagulation activation. These tests are usually available in a conventional clinical diagnostic laboratory. They allow to identify hypercoagulability, but do not provide information about its causes. This group of tests includes PT (shortening), APTT (shortening), fibrinogen (growth), TB (change), markers of thrombinemia (increase in RFMK, PDP, D-dimers).
Tests to identify the causes of thrombophilia are carried out in most cases after the first group tests are performed and thrombosis has been stopped. For example, determining the concentration of homocysteine or detecting

hereditary factor abnormalities. Unfortunately, the cause of thrombophilia is not established in all cases.

Recently, much attention has been paid to hyperhomocysteinemia and antiphospholipid syndrome.

Hyperhomocysteinemia is a proven risk factor for the development of both arterial and venous thrombosis. The molecular mechanisms of the thrombogenic action of homocysteine have not been identified.

Homocysteine is a sulfur-containing amino acid, an intermediate metabolic product of the essential amino acid methionine.

Hyperhomocysteinemia can be

• congenital, associated with a deficiency of one of the enzymes for converting homocysteine,

• acquired, due to a lack of vitamins B12, B6, and / or folic acid, as well as the use of drugs that disrupt the function of enzymes or the exchange of vitamins.

Severe congenital hyperhomocysteinemia leads to recurrent arterial and venous thrombosis, which manifests itself from childhood. The individual risk of thrombosis in hyperhomocysteinemia increases 3-10 times.

The reference limits for the content of homocysteine in blood plasma depend on the analysis method, the available method of determination is ELISA.

Antiphospholipid syndrome (APS) is a special syndrome based on the development of an autoimmune reaction to negatively charged phospholipid determinants on the membranes of platelets, endothelium, nerve cells and glycoproteins associated with the phospholipids of these membranes. The syndrome refers to autoimmune thrombogenic hemophilia as one of the hypocoagulable forms. APS is a common cause of acquired immune thrombophilia. The syndrome is characterized by a combination of thrombotic and ischemic manifestations with moderate thrombocytopenia. APS is more common in women. The pathogenesis of APS has not been definitively established.

With APS, repeated venous thrombosis and arterial thrombosis are observed. Obstetric pathology is manifested by habitual miscarriage (44%), placental vascular thrombosis, intrauterine fetal death, preeclampsia. Patients with APS may have lesions of the nervous system, the head of the femur, skin, changes in the heart and blood vessels.

With APS, autoimmune antibodies appear that inhibit the activation of prothrombin and cause hypocoagulation in phospholipid-dependent coagulation tests without specifically inhibiting any coagulation factor.

In patients with APS, the following appear in the blood:

1. Antibodies to membrane phospholipids - cardiolipin, phosphatidylserine, etc., therefore, the syndrome is sometimes called anticardiolipin. Patients

with APS may have a positive Wasserman reaction because cardiolipin is a reagent.
2. Antibodies to glycoproteins associated with phospholipids (annexin V, β2-glycoprotein-I, prothrombin).
3. Lupus anticoagulants (VA).
Lupus anticoagulants are a group of antiphospholipid antibodies of all classes of immunoglobulins (IgA, IgM, IgG) that prolong plasma clotting time in phospholipid-dependent tests such as the prothrombin test and APTT. Anticoagulants are called lupus because they were first discovered by Conley and Hartman, 1952, in the study of the blood of patients with systemic lupus erythematosus (SLE). In addition to APS and SLE, lupus anticoagulants can be found in other autoimmune diseases.
Thus, APS is characterized by a state of hypercoagulation in the body with hypocoagulation in phospholipid-dependent tests against a background of moderate thrombocytopenia.
There are 3 types of antiphospholipid syndrome (Table 5).
Classification of antiphospholipid syndromes

The name	The specifications
Primary API	There are no manifestations of "background" immune disease. Sometimes they detect a / t to DNA, the growth of circulating immune complexes (CIC). Subsequently, SLE or other collagenoses may appear.
Вторичный АФС	Complication of the "background" immune systemic disease (SLE, RA, scleroderma, etc.), viral infections, tumors, medication (procainamide, quinidine, chlorpromazine)
Catastrophic APS	Rapidly progressive polythrombotic syndrome with circulation of BA and / or antiphospholipid antibodies in the blood. Cannot be treated and is fatal

The main clinical signs for a targeted examination of a patient for the presence of APS are thrombosis, organ ischemia, and obstetric pathology. Additional signs include the presence of immune pathology, thrombocytopenia, positive RW.
For the correct diagnosis of APS, it is necessary to conduct a laboratory examination, including:
• Detection of antibodies to cardiolipin and phosphatidylserine in a high titer.
• Detection of antibodies to glycoproteins (prothrombin, etc.).
• Identification of lupus-type anticoagulants.
Antibodies to phospholipids and glycoproteins are detected by enzyme immunoassay. The effect of lupus anticoagulant is detected by clotting methods in phospholipid-dependent tests. The detection of VA is carried out

in 2 stages: screening tests that detect the effects associated with VA, and confirmatory tests, which establish that hypocoagulation in screening tests is corrected by the addition of platelet phospholipid membranes or erythrophosphatide.
Examination of a patient for APS should be comprehensive. A single immunological test does not provide reliable detection of APS. The error is 35 to 60%.
For the treatment of APS, anticoagulants of direct and indirect action, antiplatelet agents, immunosuppressants of cytostatic action, and discrete plasmapheresis are used.

Disseminated intravascular coagulation syndrome.
Disseminated intravascular coagulation syndrome (DIC) is a universal process that manifests itself in critical and terminal conditions. The process was first described by the classic of medieval oriental medicine Abu-Ibrahim Djurdjani in 1110, and deciphered in the 60s of the last century by the research of M.S. Machabeli and other authors.
DIC-syndrome reflects a breakdown in the mechanisms of adaptation of hemostasis and generalization of the blood coagulation process. The mechanism of development of DIC syndrome is due to the activation of the blood coagulation system and platelet hemostasis, followed by their depletion (coagulopathy and consumption thrombocytopenia). Activation and depletion concerns all other plasma proteolytic systems - fibrinolytic, kallikrein-kinin, complement, renin-angiotensin.
The main agent causing blood clotting and platelet activation is thrombin. In most cases of disseminated intravascular coagulation (DIC), prothrombin is activated by tissue factor secreted from damaged tissues and bacteria (external pathway of prothrombinase activation). Less commonly, prothrombin activation occurs by cancerous procoagulants or snake coagulases. Excess thrombin in a short time activates such an amount of fibrinogen that the resulting fibrin monomer circulates in the blood both in unchanged form and in the form of soluble fibrin (RF). Activation of blood coagulation and excess RF leads to the formation of microclots, which, blocking the lumen of the vessels, lead to the cessation of organ function - multiple organ failure. Initial hypercoagulation, as coagulation factors and platelets are depleted, goes into hypocoagulation with the development of hemorrhagic syndrome, and an excess of RF and RFMK activates fibrinolysis. Thus, disseminated intravascular coagulation is characterized by hypercoagulation, which turns into a hemorrhagic syndrome with secondary systemic hyperfibrinolysis.

It has been proven that the depletion of reserves of physiological anticoagulants (primarily protein C, antithrombin) occurs much faster than plasma coagulation factors. The discovery of this phenomenon made it possible to revise the tactics of treating patients with disseminated intravascular coagulation and save the lives of thousands of patients.

DIC is diagnosed clinically. One of the main manifestations of the syndrome is the development of multiple organ failure as a result of blockade of microcirculation in organs by blood clots. The main target organs are:

1. Lungs: progressive respiratory failure (shortness of breath, cyanosis, dyspnea, hypoxemia) up to the development of distress syndrome.
2. Kidney: oliguria reaching anuria, proteinuria, azotemia, renal failure and uremic intoxication.
3. Gastrointestinal tract: the formation of bleeding erosions and ulcers, diapedetic bleeding, a decrease in the barrier function of the intestinal mucosa for the intestinal flora, the penetration of which into the blood causes the development of secondary endogenous sepsis.
4. Liver: hyperbilirubinemia, increased enzyme activity. In some cases, hepato-renal syndrome develops.
5. Nervous system: deep depression.

The main reasons for the development of disseminated intravascular coagulation.

1. Entering the bloodstream of a large amount of tissue thromboplastin during tissue destruction.

• Severe trauma, including compression syndrome, traumatic surgery.
• Malignant neoplasms, including hematological ones. Acute promyelocytic leukemia is always accompanied by disseminated intravascular coagulation.
• Acute massive destruction of organs and tissues (pancreatitis, burns, etc.).
• Obstetric pathology (placental abruption, fetal death, amniotic fluid embolism, eclampsia, etc.).

2. Violation of microcitrulation. All terminal states and shock.
3. All acute infectious and septic conditions. The leading role is played by endotoxins of gram-negative microorganisms.
4. Foreign surfaces when using extracorporeal treatments.
5. Violation of the phagocytic function of EMMC cells in liver cirrhosis.
6. Acute intravascular hemolysis and cytolysis.
7. Poisoning with hemocoagulating snake poisons.

The nature of the course and phase of the disseminated intravascular coagulation syndrome.

The nature of the course of the DIC is determined by the rate at which substances activating hemostasis enter the bloodstream. There are the following types of flow:

• Acute, including catastrophic form.
• Subacute with a long period of hypercoagulability.
• Chronic with a low rate of thrombin generation.

The presence of chronic ICE is not recognized by all authors. Sometimes it is called "laboratory" DIC syndrome, since the revealed hemostasis disorders are not accompanied by characteristic clinical manifestations.

The internal combustion engine in its development goes through the following main phases:

1. Hypercoagulation and hyperaggregation. In acute disseminated intravascular coagulation, the phase is very short, it is not always possible to identify it in the laboratory. When drawn, blood may clot in the needle.
2. Hypocoagulation with consumption coagulopathy.
3. Generalization of fibrinolysis (defibrination). The manifestation of hypocoagulation and defibrination is the absence of blood clots flowing from the uterus, intestines, nose, wounds.
4. Recovery.

All these phases can overlap with each other, and in the later stages of development occur simultaneously. The mortality rate of acute forms against the background of modern treatment is 10-15%, without treatment - 50%.

Changes in laboratory parameters with disseminated intravascular coagulation.

The diagnosis of DIC is made clinically, therefore, laboratory tests are used to confirm the diagnosis, objectively assess the effectiveness of the therapy and the state of various links of hemostasis in the corresponding phase of the development of the syndrome (Table 6).

Table 6.Laboratory indicators in different phases of disseminated intravascular coagulation

The Phase	Laboratory tests
Hypercoagulability (thrombinemia)	↑ activation markers (fibrin monomer, RFMK, FPA) ↓ APTV, TV, ↓ AT, protein C Thrombocytopenia (<150,000),
Consumption coagulopathy	High activation markers ↓ fibrinogen and other factors ↑ AChTV, TV
Hyperfibrinolysis	↓ fibrinogen before defibrination ↑ PDF and D-dimers ↑ APTT, TV, prothrombin time

Note: arrows indicate ↑ - increase, ↓ - decrease the parameter.

The hypercoagulable phase is characterized by severe thrombinemia. Since it is not possible to measure the content of thrombin in plasma due to the short life span, the level of thrombinemia is determined indirectly by an increase in the content of coagulation activation markers. In the blood, the content of

fibrin monomers, soluble fibrin monomer complexes (RFMK), fibrinopeptide A (FPA) and some other compounds. In connection with the activation of coagulation, APTT and TB are shortened and the activity of antithrombin and protein C decreases. Determination of the content of anticoagulants in dynamics is useful for individualizing the prognosis, since their increase in the course of treatment suggests a favorable outcome. Characterized by progressive, but not reaching a critical level, thrombocytopenia with increased platelet aggregation.

In the hypocoagulation phase, against the background of high rates of coagulation activation markers, the content of fibrinogen and other factors decreases. APTV and TV are lengthening.

In the phase of hyperfibrinolysis, a decrease in fibrinogen up to defibrination and an increase in fibrinolytic activity (FAA) are pronounced. In connection with the activation of fibrinolysis, the content of PDP and D-dimers formed only from fibrin increases. Other coagulation tests (PT, APTT, TV) are usually lengthened.

In laboratory monitoring of DIC syndrome, the main attention should be paid to identifying tests that reflect stably progressive changes, and, on the basis of these tests, to carry out laboratory control of the therapy. These tests may differ from patient to patient. They can include an assessment of the content of platelets, fibrinogen, fibrinolytic activity, antithrombin, protein C, RF and other indicators. Coagulation tests such as PT, APTT, TB have multidirectional values depending on the phase of the disease and may be of little informative for follow-up.

With DIC syndrome, an increased number of fragmented erythrocytes appears in the blood. They can be detected in a blood smear and according to hemanalyzer indicators: a histogram of erythrocytes, a decrease in MCV and an increase in RDW.

Principles of treatment for disseminated intravascular coagulation.

Hypercoagulant phase: usual heparin IV, fresh frozen plasma with a low level of anticoagulants, antibiotics, replacement of the function of vital organs.

Hypocoagulation phase: fresh frozen plasma, sometimes factor concentrates, thrombosis, antibiotics, replacement of the function of vital organs.

Before treating patients who are in the hypercoagulable phase, it is advisable to measure the antithrombin content, since when its level decreases, heparin will not have the desired effect. In treatment, as a rule, ordinary heparin is used, since its main effect is antithrombin. Antibiotics are administered to all patients to relieve possible secondary sepsis caused by intestinal flora that

has entered the blood as a result of a decrease in the intestinal barrier function.

Essential drugs for the treatment of hemostasis disorders.

In a healthy person, all the components of hemostasis are in dynamic equilibrium. With various kinds of violations, hemostasis shifts either to the area of hypercoagulation, or to the area of hypocoagulation. To restore balance and prevent severe and sometimes life-threatening complications, various groups of pharmacological drugs are used.

Principles of bleeding treatment.

For hemorrhagic manifestations, depending on the cause of hemorrhage, fresh frozen plasma is used as a source of plasma coagulation factors, coagulation factor concentrates, activated coagulation factors, platelet concentrates.

Drug therapy includes antifibrinolytics, immunosuppressants, immunoglobulins, vasopressin analogs (desmopressin), vitamin K (vicasol), topical preparations.

Antifibrinolytics (fibrinolysis inhibitors).

With generalized or local fibrinolysis, drugs are used that inhibit fibrinolysis. These include aminocaproic acid, tranexamic acid, aminobenzoic acid (Pamba) and aprotinin (trade names trasilol, counterkal). Aminocaproic acid blocks the action of plasminogen activators, inhibits the action of plasmin, and partially blocks kinins. Tranexamic and aminobenzoic acids block the lysine binding sites in the plasminogen molecule, preventing the latter from being activated. The protein aprotinin, isolated from the lungs of cattle, inhibits the enzymatic action of plasmin by forming complexes with it. In addition to plasmin, aprotinin inhibits trypsin, chymotropsin and kallikrein.

Drug treatment of hypercoagulability.

Of the acquired disorders, hypercoagulation is most common, which is expressed in thrombophilia, thrombosis or thromboembolism. The administration of almost all drugs requires laboratory control. The choice of tests depends on the mechanism of action of the drug.

Inhibitors of platelet function (antiplatelet agents).

To reduce the level of thrombophilia prophylactically, antiplatelet agents are used. These drugs inhibit the adhesion and aggregation of platelets, prevent the development of the atherosclerotic process. They are used in the treatment of patients with stenosis and thrombosis of the arteries, ischemia, organ infarctions, in the presence of thrombophilia.

Currently, various groups of drugs are used: cyclooxygenase-1 (COX-1) inhibitors, phosphodiesterase inhibitors, platelet ADP receptor inhibitors, IIb / IIIa receptor inhibitors

platelets. In our country, COX-1 inhibitors and platelet ADP receptor inhibitors are most commonly used.

Cyclooxygenase-1 inhibitors include **aspirin** preparations, which are used in small doses (30 - 300 mg / day). The mechanism of action of aspirin is associated with the irreversible inhibition of COX-1 platelets, thereby preventing the formation of a procoagulant thromboxane A2 from arachidonic acid, which increases the adhesion of platelets. Platelets cannot restore the enzyme level, since they do not contain a nucleus and are not capable of protein synthesis. The level of the antiplatelet prostacyclin, which is formed from arachidonic acid with the participation of COX-1 in endothelial cells (EC), is practically unaffected by aspirin: the EC containing the nucleus replace COX-1 by a new synthesis.

The antiplatelet effect of aspirin is rarely laboratory controlled, despite the fact that one-fifth of patients have genetically determined or acquired resistance to aspirin. To control antiplatelet therapy, it is optimal to investigate the aggregatogram of a patient with induction of platelet aggregation with adrenaline before taking aspirin and on the 2-3rd day after starting treatment. A 3-4-fold decrease in aggregation confirms the effectiveness of aspirin.

Sometimes a urinary test is used to assess the individual response to aspirin intake: with sufficient sensitivity to aspirin in the urine, the level of the arachidonic acid metabolite thromboxane B2 decreases.

Side effects of aspirin include bleeding and neutropenia (0.17%).

Of the platelet ADP receptor inhibitors (tinopyridines), **Plavix** (clopidogrel) is the most widely used. It irreversibly inhibits one of the three known platelet ADP receptors, blocking their ADP-dependent aggregation. Genetically determined resistance to Plavix has not been identified. Shown is laboratory control by evaluating the aggregatogram during the induction of aggregation by adding ADP (decrease in the aggregatogram indices from the original by 3-4 times), but it is possible to use Plavix without laboratory control.

Side effects of Plavix can be bleeding and neutropenia (0.1%).

Direct anticoagulants.

Direct anticoagulants include heparins, heparinoids, and hirudin. Their name is due to the direct effect on plasma clotting: neutralization of thrombin or inactivation of the Xa factor.

Heparins are polymers of a sulfur-containing disaccharide and are acidic polysaccharides. They differ in terms of GMM and priority mechanism of action.

Conventional unfractionated heparin (OH) is a mixture of heparins with a HMW of 1700 to 24000 D. It acts in two main ways: through catalytic enhancement of the action of antithrombin (antithrombin action), and through inactivation of factor Xa (antithrombotic action). The most pronounced antithrombin effect of OG. Conventional heparin is widely used both for the prevention of venous thrombosis and thromboembolic complications, and for the treatment of thrombosis, thromboembolism, and disseminated intravascular coagulation. In the treatment of disseminated intravascular coagulation, the use of OH is preferable, since it neutralizes excess thrombin via AT. EG is used in heart-lung machines (AIC), in hemodialysis, in hemosorption, etc. Its advantages are availability, ease of monitoring and the possibility of complete neutralization of the anticoagulant effect by introducing protamine sulfate, a protein from fish sperm. Conventional heparin is given either subcutaneously or intravenously. Heparin resistance is rare. In this case, it is replaced with hirudin.

Laboratory monitoring of OH consists in determining the APTT 4 hours after heparin administration. When using high doses, it is considered curative that the plasma clotting time is 1.5-2.5 times longer than the average APTT value for a given laboratory, and not with the patient's initial APTT value. With the introduction of low doses of OH for the purpose of prophylaxis, the lengthening of APTT may be insignificant and laboratory control is not necessary. In the conditions of AIC, control over heparin therapy can be carried out according to the time of clotting of whole blood. Since the main anticoagulant effect of OH is carried out through antithrombin, it is recommended that the patient measure the level of antithrombin before treatment with high doses of heparin. At a level less than 70%, the introduction of fresh frozen plasma or antithrombin concentrate compensates for the lack of anticoagulant. Exhaust gas doses are measured in antithrombin units.

Side effects of high doses of OH include bleeding and heparin-induced thrombocytopenia (HIT). A decrease in platelet count is observed in 10% of patients receiving heparin, which is due to the ability of heparin to activate platelets. However, the platelet count is never lower than 80.0009 / L. A more pronounced drop in the level of platelets is associated with the formation of antibodies against platelets and heparin. With such a complication, the risk of thromboembolism increases, since platelets activated by antibodies are capable of aggregating. A decrease in the level of platelets is observed no earlier than 5-14 days from the start of treatment. In this regard, with the introduction of high doses of heparin, it is recommended to control the platelet count before treatment, on the 7th and 12th days, and

then with the continuation of treatment 1-2 times a week. In rare cases, patients may develop heparin-dependent osteoporosis as a complication.
Low molecular weight heparins (LMWH) are produced by chemical or enzymatic treatment of common heparin. They have a lower GMM and differ in their mechanism of action. The main thing for LMWH is the antithrombotic effect through the inactivation of factor Xa. LMWH are used subcutaneously, mainly for the prevention of thrombosis and in the treatment of venous thromboembolic complications.
Laboratory control is carried out either by the residual activity of factor Xa (anti-Xa) before the administration of the next dose, or not. Complications can be the same as with the introduction of OH, but they are extremely rare.
The dose of LMWH is measured in anti-Xa units, which do not correspond to antithrombin units of OG.
Hirudin is a polypeptide from the saliva of a medicinal leech, which has a pronounced direct antithrombin effect without the participation of any blood coagulation factors. Recombinant hirudin preparations are used for medical purposes. Hirudin is administered intravenously for anticoagulant therapy in patients with heparin-induced thrombocytopenia, and subcutaneously for the prevention of thrombosis in high-risk settings. The use of hirudin is limited due to the risk of bleeding, since hirudin is not metabolized. It is excreted through the kidneys unchanged and, in excess, accumulates in the tissues. For hirudin, unlike heparin, there is no antidote. For kidney disease, the dose is adjusted.
Laboratory control of hirudin therapy is carried out by APTT. The criteria are the same as for heparin. However, in some cases, with an increase in the dose, the plasma clotting time in the APTT test reaches a plateau and no longer lengthens, which indicates a relative or absolute drug overdose.
Indirect anticoagulants (vitamin K antagonists).
Indirect anticoagulants (AEDs) are widely used in medical practice, especially when long-term anticoagulation therapy is required. They are used for primary prevention of atrial fibrillation, prevention of recurrence of thromboembolism, thrombotic complications in the presence of artificial prostheses or a filter in the vena cava.
The advantages of these drugs are that they can be taken orally, and their effect does not depend on the presence of other blood clotting factors, for example, antithrombin. The group of AEDs (oral anticoagulants) includes coumarin anticoagulants (warfarin, etc.) and indandione series (phenylin). Recently, warfarin has been widely used as the least toxic and most "manageable".
Warfarin is a coumarin derivative. Its effect, like other oral anticoagulants, is to disrupt the reaction of γ-carboxylation of vitamin K-dependent coagulation

factors: II, VII, IX, X, proteins C and S. Without vitamin K, PIVKA factors that are defective during plasma hemostasis are formed, in which the ability to bind with calcium ions, membrane phospholipids and other factors of blood coagulation is impaired, which leads to an anticoagulant effect.

When taking AEDs, in particular, warfarin, both the external and internal pathways of prothrombinase activation change. Factor VII is involved in the external pathway, IX in the internal, and II and X in both pathways of prothrombinase activation. Nevertheless, the action of AEDs has a greater effect on the external pathway, therefore, the administration of warfarin is controlled by prothrombin time (test - PT, PT).

Proper use of warfarin requires constant laboratory monitoring. The dosage of the drug does not lend itself to any preliminary calculations. The effect of the drug is influenced by the synthetic ability of the liver, food regimen, other drugs, or concomitant diseases. The desired level of factor activity is set individually. Low-dose AED therapy is also currently being used. In the initial phase of saturation, an induction dose of warfarin is prescribed, and laboratory control is carried out by a prothrombin test in percentage according to Quick. Further, when selecting a dose, control is carried out by the Quick index every 3-4 days. As an additional test before starting treatment, it is useful to assess the protein C system. If the activity of protein C is low, taking warfarin can lead to thrombosis and necrosis of the skin.

In the phase of stable hypocoagulation, the effect of the drug is assessed according to a more objective indicator, the International Normalized Ratio (INR).

When using warfarin, the following rules should be followed:

1. Use warfarin according to the expiration date.
2. When taking warfarin, if possible, limit the intake of vitamin K. Vitamin K is found in vegetables, fruits, liver, yeast.
3. Move aside the intake of warfarin from food intake, since the drug is absorbed by food.
4. Use the lowest dosage regimen that provides a therapeutic effect (by INR control).

The INR interval for warfarin treatment depends on the nature of the pathology and is determined by the attending physician. In most cases, it ranges from 2 to 4.

With long-term treatment with warfarin, it is useful to determine the APTT as a test for the intrinsic pathway of prothrombinase activation. The patient's plasma clotting time is usually 2-2.5 times longer than normal laboratory APTT values.

The most dangerous complications in the form of bleeding are relatively rare. With prolonged use, increased hair loss is possible. The drug is contraindicated in women in the first trimester of pregnancy.

PRACTICAL PART

Basic concepts.

Laboratory tests are inseparable from the diagnosis and monitoring of hemostatic disorders, especially when monitoring anticoagulant therapy. To assess the hemostatic system, the following groups of tests are used:

1. Plasma coagulation (coagulation link).
• Screening tests.
• Study of factors (activity and concentration).
• Markers of activation of coagulation.
• Clotting inhibitors.
2. Fibrinolysis.
• Screening tests.
• Factors of fibrinolysis (plasminogen, t-PA).
• Markers of fibrinolysis.
• Inhibitors of fibrinolysis.
3. Study of platelets (number, function, factors of platelets).

In general hospitals, as a rule, screening tests are carried out, which assess the function of individual phases of coagulation processes, that is, the joint activity of several factors. Other tests are usually performed in specialized laboratories or specialized medical institutions, although, in principle, they can be performed in any CDL with the appropriate equipment.

When prescribing tests for hemostasis, the doctor is guided by the presence of clinical signs, anamnesis, the likelihood of surgery, as well as the laboratory's capabilities. Depending on the diagnostic need, patients are assigned a coagulogram or hemostasiogram study.

A coagulogram involves the study of the plasma link of hemostasis. The hemostasiogram includes the simultaneous assessment of plasma and vascular-platelet hemostasis. In routine studies, the minimum set of tests for the vascular-platelet link consists of determining the bleeding time and the number of platelets. A more subtle study of platelet function is carried out using special methods and devices.

Patients without visible clinical symptoms and before surgery are assigned a baseline coagulogram. The minimum number of basic coagulogram parameters is four: prothrombin time (PT), activated partial thromboplastin time (APTT), thrombin time (TB), and fibrinogen. These tests are screening tests and can detect violations at all stages of coagulation. This set of tests can be performed both manually and on a coagulometer. However, the minimum set of tests is not enough for patients with impaired hemostasis.

Considering the laboriousness and considerable cost of the tests, the CDL should have "backup" tests, which are performed only as directed by the attending physician in addition to the basic coagulogram. These tests, as a rule, are screening tests and allow assessing the functional activity of other links of hemostasis. These include: fibrinolytic activity (FAC, screening test for fibrinolysis), assessment of the activity of physiological anticoagulants (antithrombin and the total activity of the protein C system), assessment of the level of thrombinemia (RFMK, D-Dimers, etc.), identification of the effects of lupus anticoagulant and some others ... All these tests are available for execution, and for them, in addition to D-dimers, there are domestic reagents. D-dimers are determined by immunological methods and require special equipment. The test suite should be tailored to the needs of the hospital. It is more rational to carry out rare tests in a specialized laboratory because of their unprofitability.

For a general assessment of the hemostatic system, thromboelastography (TEG) or thromboelastometry (TEM) is performed. The methods are expensive, but in some cases they are extremely necessary.

Thus, each CDL identifies a range of studies, which is determined by the needs of the hospital and the capabilities of the laboratory.

The main stages of the study of hemostasis.

When studying the hemostatic system, as in other laboratory studies, the analysis consists of 3 stages:

- preanalytical, including the purpose of the study, registration of an order form for analysis, collection, storage and transportation of biological material, sometimes primary processing of biological material (centrifugation and separation of plasma from erythrocytes);
- analytical, that is, direct analysis;
- post-analytical, including the issuance of an analysis to the department, the attending physician or patient, and the interpretation of the results of laboratory tests.

At each of these stages, mistakes can be made. According to statistical data, 70-80% of all errors occur at the preanalytical stage, therefore all components of this stage must be standardized and strictly followed.

Preanalytical stage. The prescription of tests is carried out by the attending physicians and includes both a standard coagulogram and additional tests. The purpose of the tests must be justified. The rationale is the history data, clinical examination data (the presence of hemorrhages, signs of venous stasis or arterial insufficiency). It also takes into account information on taking medications that directly or indirectly affect hemostasis.

When prescribing tests, it is necessary to correctly fill out the referral, which will help both the clinician and the doctor of clinical laboratory diagnostics in

interpreting the results obtained. Close cooperation of clinicians with a laboratory doctor on hemostasis helps both in the correct assignment of tests and in their decoding, especially since the basic information is provided not so much by a specific test as by a combination of tests.
The registration of the form includes the following points:
• Full name, gender, age
• Department or name of the attending physician
• Complete diagnosis (diagnosis of a hemorrhagic or thrombotic state, control of therapy with anticoagulants, thrombolytics, pregnancy, etc.).
• Indication of pharmacological drugs that indirectly affect hemostasis (antibiotics, oral contraceptives, etc.).
Date Doctor's signature

Coagulation processes are influenced by many drugs, the effects of which can be both therapeutic and side effects. Pharmacotherapy for hemostasis disorders has been discussed previously. Side effects of drugs can manifest themselves in the form of an effect on platelets or plasma factors. Analgesics tend to reduce platelet aggregation. Taking oral contraceptives and hormone replacement therapy drugs activates platelet aggregation and clotting factors, reduces antithrombin levels, which can contribute to thrombophilia. Broad-spectrum antibiotics suppress the intestinal flora and associated endogenous vitamin K synthesis, and can also cause thrombocytopenia. The presence of a tendency to hypocoagulation was established in patients after the introduction of blood substitutes and during treatment with asparaginase and valproic acid. The effects of drugs must be considered when interpreting laboratory results.
Physical and emotional stress activates the coagulation and fibrinolysis system.
The biological material for the study of hemostasis can be blood, plasma and blood serum.
Blood is required when registering a thromboelastogram. Serum is rarely used to measure the content of a number of factors or PDP. Serum is a liquid that remains after blood clotting and separation of cellular elements. It does not contain fibrinogen and some other factors. The main material for the study of the hemostasis system is blood plasma. To obtain plasma during blood sampling, a stabilizer (anticoagulant) is added to the test tube, which prevents blood from clotting. Plasma is a liquid obtained after the separation of cellular elements without preliminary blood clotting. Plasma contains fibrinogen and all factors.
Blood sampling for hemostasis is carried out from a finger, earlobe or from a vein. Capillary blood is drawn for some specially adapted

methods such as platelet counts, bleeding time, APTT, PT, and in children. When capillary blood is taken, a sufficiently deep puncture with a sterile scarifier and a quick (10 sec) collection of the first drops of blood obtained by gravity without pressure or compression are required. When transferring blood to a tube containing an anticoagulant (sodium citrate), it should be lowered into the center of the tube and not flowed around the edges. Blood with an anticoagulant must be thoroughly and carefully mixed.

The standard is the study of blood taken from a vein. Blood is taken on an empty stomach or after a light breakfast without fatty foods from the cubital vein with a silicon needle with a wide lumen into tubes with sodium citrate. When taking blood, the application time of the tourniquet should be minimal (no more than 2 minutes), since blood stasis activates coagulation. If blood is taken simultaneously in several tubes, then the tube for hemostasis should be the second. When taking blood from a catheter, to exclude the effect of heparin, up to 20 ml of blood is preliminarily drained. Blood with sodium citrate is immediately mixed 2-3 times without shaking and foaming, and left at room temperature. Silicon glass tubes, plastic tubes, or blue-capped vacuum tubes containing sodium citrate can be used as containers for blood collection. Blood sampling into ordinary glass tubes is unacceptable, since glass activates some blood coagulation factors (contact activation).

For tests where it is important to avoid the release of platelet factors in the period between blood collection and analysis, use STAD tubes. These tubes contain sodium citrate, theophylline, adenosine, and dipyridamole. The last three components prevent platelet aggregation and the release of biologically active substances from platelets.

To prevent blood clotting after collection for coagulation studies, sodium citrate (hereinafter citrate) is used as a blood stabilizer. Citrate reversibly binds calcium ions, thereby preventing blood clotting. Citrate also stabilizes factors VIII and V and makes it possible to obtain clear plasma after centrifugation. The ratio of citrate to blood is strictly defined - 1: 9. According to the WHO recommendations, the concentration of citrate solution is 109 mM, which corresponds to 3.8% citrate prepared from trisubstituted 5.5 aqueous crystalline hydrate (Na3C6H6O7x5.5H2O), or 3.2% citrate prepared from trisubstituted 2-aqueous crystalline hydrate (Na3C6H6O7x2H2O). Using conventional graduated tubes in the laboratory, pour the required amount of citrate and mark the blood level with a felt-tip pen. Blue-capped vacuum tubes contain a standard amount of citrate and the blood level is already marked.

Table 7.

Dependence of the ratio of anticoagulant and blood on hematocrit

Hematocrit%	*Anticoagulant ml (3.8% sodium citrate)*	Total volume ml
20	1,4	10
22-26	1,3	10
28-32	1,2	10
34-38	1,1	10
40-44	1,0	10
46-50	0,9	10
52-56	0,8	10
58-60	0,7	10
>65	0,5	10
The newborns	0,25	5,0

Note: Bold hematocrit values assume standard citrate amounts.

High and low hematocrit require correction of the citrate-blood ratio (Table 7). In this case, the blood is taken into plastic tubes with the corrected amount of citrate. In practice, with a hematocrit of 25 to 55%, the amount of citrate can be left unchanged.

The largest number of errors is associated with an incorrect ratio of citrate to blood and poor mixing of samples. Very often, procedural nurses draw blood above or below the mark. In this case, non-standard dilution of factors occurs, which can lead to the formation of clots or impaired coagulation in the test tube if the patient does not have it. Clotted blood is not analyzed!

The time for conducting studies to assess platelet function and some other tests (PAI-1) is 30-40 minutes. Such samples should be taken at the hospital where they are analyzed. When assessing plasma hemostasis, samples should be analyzed within 4 hours. The use of vacuum tubes extends the analysis time, blood samples can be transported to a specialized laboratory. Samples are stored at room temperature prior to testing.

The preliminary stage of sample processing includes centrifugation. By changing the centrifugation regimen, plasma is obtained containing different numbers of platelets. Platelet-rich plasma (PRP) is obtained by centrifuging the blood for 5 min at 1000 rpm. PRP is used to count and assess platelet function. Platelet-poor plasma (PRP) is obtained by centrifuging citrated blood at 3000 rpm for 15 minutes. It is used to study the parameters of plasma hemostasis. Platelet-free plasma is obtained by centrifuging citrated blood for 30 minutes at 4000 rpm. Centrifugation in the study of hemostasis is best carried out in centrifuges with a bucket rotor. When removing tubes from such a centrifuge, the phases are not mixed. Slow stopping of the centrifuge also prevents mixing of the phases.

If necessary, plasma can be frozen once (-200 - -700C). It is recommended to use platelet-free plasma for freezing. At a high freezing temperature (-200C), plasma should be analyzed within 2 weeks. Plasma defrosting is carried out in a water bath at 370C. Thawed plasma is analyzed immediately. In plasma containing heparin and PDP, hemostasis parameters change after freezing.
Hemolyzed plasma is not analyzed except in cases of intravascular hemolysis in the patient.
The analytical stage includes the direct analysis. The result of the analysis depends on the research method, the quality of the reagents, the equipment used and the human factor. Reducing the influence of the human factor ensures the automation of the analytical process.
The following groups of methods are used to study hemostasis:
1. Clotting, based on recording the time of clot formation (clot - clot, thrombus). Used to perform screening tests to determine the activity of certain coagulation factors and inhibitors.
2. Chromogenic, based on photometric determination of the activity of factors for the cleavage of chromogenic or fluorogenic substrates. Used to perform screening tests to determine the activity of certain coagulation factors and inhibitors. The methods are sensitive and specific, easily adaptable to biochemical analyzers. With their help, it is possible to reliably measure the content of a particular factor, but it is not always possible to assess its biological activity.
3. Immunological, based on the antigen-antibody reaction (ELISA, latex agglutination, nephelometry, turbidimetry, etc.). Used to determine the concentration of factors.
4. Other methods for special research (for example, clot dissolution, molecular methods, etc.).
When choosing methods, it should be remembered that the concepts of activity and concentration of factors are not always identical. Thus, during treatment with warfarin, the concentration of vitamin K-dependent factors, measured by ELISA, may be within the normal range, while their activity in clotting tests will be reduced (lengthening of PT and APTT) due to the synthesis of functionally defective PIVKA factors.
A pool of citrated plasma is used to calibrate the study of activity and most factor concentrations.
Normal plasma is a pool of platelet-free citrate plasma obtained from at least 20 clinically healthy donors. In this plasma
the activity of all factors is considered by definition as 100% or 1 unit / ml. Normal plasma and its serial dilutions are used for multipoint calibration.
Reference plasma (100% value) - centrally prepared according to the standard normal plasma.

Calibration plasma is a pool of citrated plasmas in which the activity or concentration of hemostasis components is precisely determined, but may differ from 100%. Calibration plasma and its serial dilutions are used for multi-point calibration based on the activity or concentration specified by the manufacturer.

Coagulological research methods do not lend themselves well to standardization. Test performance depends on the quality and composition of the reagents and the equipment used. Some advances have been made in the standardization of PT using the MIC and calculating the INR in the control of AED treatment. To avoid mistakes, it is advisable to use ready-made reagent kits supplied by domestic and foreign companies. Among the domestic manufacturers of test systems, the most popular are the reagents of the companies Renam (Moscow), Tekhnologiya-Standard (Barnaul), MedioLab, (Moscow). It is recommended to use kits from the same manufacturer and to work out the reference values of indicators in your own laboratory. To be confident in the correctness of the research and obtaining reliable results, each laboratory must carry out daily internal quality control and participate in external quality control, domestic or international. Due to insufficient standardization of tests for external quality assessment, for example, FSVOK, for most tests, not the absolute values of the plasma clotting time are taken into account, but the degree of lengthening or shortening of the clotting time of the control plasma in comparison with donor plasma, measured under the conditions of this laboratory.

All clotting tests are carried out in silicone or plastic containers. Tests are carried out manually in a water bath with transparent walls or on semi-automatic and automatic hemostasis analyzers - coagulometers. Manual methods are acceptable, but they have the lowest reproducibility and accuracy. This is due to the impossibility of standardizing the analysis process carried out by different operators, as well as the complexity of visual registration of the time of clot appearance. Clotting tests are performed in duplicates. The discrepancy between clot formation time in parallel studies should be less than 0.5 sec.

More modern and widespread is the measurement of hemostasis parameters using coagulometers. In semi-automatic coagulometers, only the registration system is automated. According to the system for recording clot formation, there are optical, optical-mechanical, mechanical and turbidimetric coagulometers. Optical and optical-mechanical devices can only analyze plasma. These devices have a so-called automatic start: the addition of reagent to the cuvette to be measured automatically starts recording the time of clot formation. When using multiple reagents, only the last (starting) reagent should be added to the cuvette in the measuring cell. Mechanical

coagulometers can analyze both plasma and whole blood. These devices do not have an automatic start, the timing is started by mechanical pressing of the button. Mechanical coagulometers are especially useful for polyclinics when monitoring patients taking warfarin for capillary blood prothrombin. Automatic coagulometers carry out the entire analysis procedure in accordance with a given program. Some coagulometers calculate fibrinogen by prothrombin. This technique can be used for mass screening examinations, but is of little use for examining patients with hemostatic pathology.

The post-examination stage includes filling out the analysis form, issuing analyzes from the laboratory and helping the attending physician in interpreting the results. Some laboratories write a conclusion on the coagulogram. Errors of the post-analytical stage are most often associated with incorrect entry of the analysis results into the form. The use of modern technologies (automation, computerization, installation of laboratory information systems - LIS) dramatically reduces the percentage of errors.

METHODS FOR STUDYING HEMOSTASIS

Methods for assessing total coagulation.

To assess the overall complex state of hemostasis, there are methods of graphic registration of coagulation processes: thromboelastography (TEG) and thromboelastometry (TEM). TEM differs from TEG using computer analysis and blood coagulation activators.

Table 8.

Basic standard parameters of TEG and TEM

parameters	Abbreviation	content
Coagulation start time sec	CT (r)	Time from start of analysis to swing (amplitude) 2 mm Reflects the time of thrombin formation
Clot formation time sec	CFT (k)	Time from amplitude 2mm to 20mm Kinetics of persistent clot formation from platelets and fibrin
Angle α (0)	Alpha	Tangent to curve at point 2 mm Clot formation rate
Maximum amplitude mm	MCF (MA)	Mechanical properties of the clot at maximum clotting

The principle of the method is based on the involvement in the vibrational movement of movable cuvettes or a pin by polymerized fibrin. Depending on the amount of fibrin, the degree of its polymerization, the activity of platelets, the presence of hyperfibrinolysis, a characteristic curve is displayed on thermal paper or in a computer in the amplitude - time coordinates. Manual or computer processing of the curve allows you to calculate a number of necessary parameters. The main standard parameters are shown in Table 8. A graphic representation of a TEM of a healthy person is shown in Figure 5.

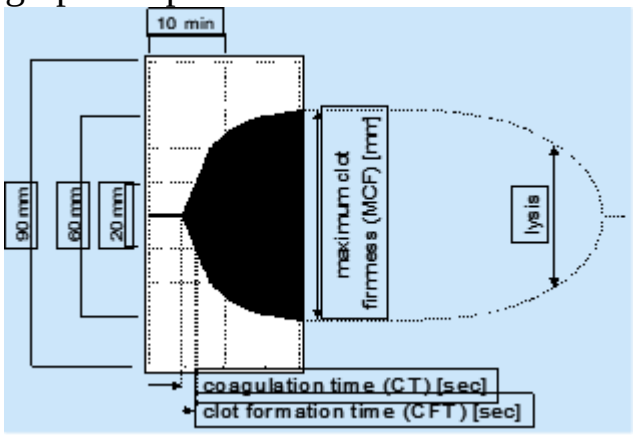
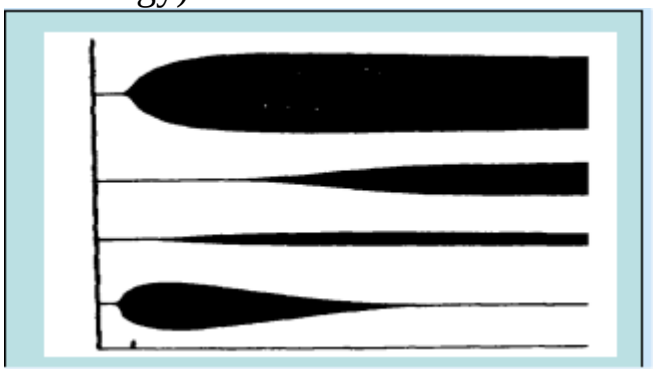


Fig 5. Image and parameters of TEM.

The graphic representation of the process clearly reflects the state of hemostasis (Fig. 6). It contains information about the current state of all major links. The method makes it possible to qualitatively and semi-quantitatively characterize the process of clot formation, its mechanical characteristics, density, stability and the process of fibrinolysis. The analysis is most useful in cases where the doctor needs to quickly make the right decision regarding the functional state of the patient's hemostasis system (hematology, obstetrics and gynecology, oncology, traumatology, burn departments, newborn examination, transplantology, cardiovascular surgery, cardiology).



Rice. 6. Various TEM options. From top to bottom: norm, hemophilia, thrombocytopenia, hyperfibrinolysis.

Assessment of the vascular-platelet link.

1. Samples for the duration and amount of capillary bleeding from superficial microvessels after violation of their integrity. Includes Duke's test and other methods, for example, a sensitive test (Ivy) against the background of artificially created venostasis at a pressure of 40 mm Hg. Reference values up to 4 minutes (newborns 1-3 minutes).

Bleeding time characterizes the functional activity of platelets and capillaries and does not depend on the processes of plasma coagulation. The clotting time is lengthened in case of impaired adhesion and aggregation of platelets, in some types of von Willebrand disease, severe thrombocytopenia, toxic damage to the capillaries. However, normal test results do not exclude platelet defects (Duke's test in 2/3 of patients with thrombocytopathies shows normal results). In patients with hemophilia, the bleeding time is within acceptable values. The test is important in preoperative screening and in children.

2. Calculation of the number of platelets is carried out in patients during the initial examination, before surgery, in hemorrhagic and thrombophilic conditions, while monitoring thrombocytopenic state or thrombocytosis.

The reference values are 150-380 thousand / ml. With functionally high-grade platelets, bleeding phenomena are observed when the platelet level drops to 50 thousand / ml or less. Thrombocytosis is considered to be an increase in the platelet count of more than 600 thousand / ml.

Thrombocytopenia in most cases is caused by autoimmune or heteroimmune pathology, as well as taking medications, in particular heparin. Thrombocytopenia can also be associated with increased platelet deposition in the spleen in portal hypertension, chronic myeloid leukemia and other types of splenomegaly, in viral and bacterial infections, in myelophthisis, certain types of anemias, increased platelet consumption in DIC and some other conditions.

Distinguish between primary thrombocytosis associated with a stem cell defect and reactive thrombocytosis. It is observed in myeloproliferative disorders, malignant diseases, some anemias, inflammatory processes, after splenectomy, etc.

Platelet counting is performed using several methods:

one). Direct blood count.

- Using a counting chamber in a phase-contrast microscope from platelet-rich plasma (reference method). The method error is 10% or more.
- Using an automatic counter. The method error is minimal in the absence of platelet aggregates. The most accurate calculation is in the range from 60 to 600 thousand / ml.

2) Counting in blood smears according to Fonio. The method error is up to 25%.

Thus, in most cases, the most accurate platelet count is provided by a hematology analyzer, which determines the following platelet parameters: platelet count (Platelets, PL, PLT), mean platelet volume (MPV), platelet volume distribution variance (PDW) and draws platelet histograms. that is, graphs of the distribution of platelets by volume. In case of deviation of platelet counts from the reference interval or in the presence of abnormal histograms of platelets or erythrocytes, it is necessary to examine a peripheral blood smear and count platelets in a blood smear according to Fonio.

The most common cause of platelet count errors in a hematology analyzer is platelet aggregation, which manifests itself as false thrombocytopenia - pseudothrombocytopenia. Pseudothrombocytopenia may be associated with EDTA-induced agglutination (EDTA-induced pseudothrombocytopenia) or adhesion of platelets to neutrophils under the influence of platelet agglutinins (Figure 7).

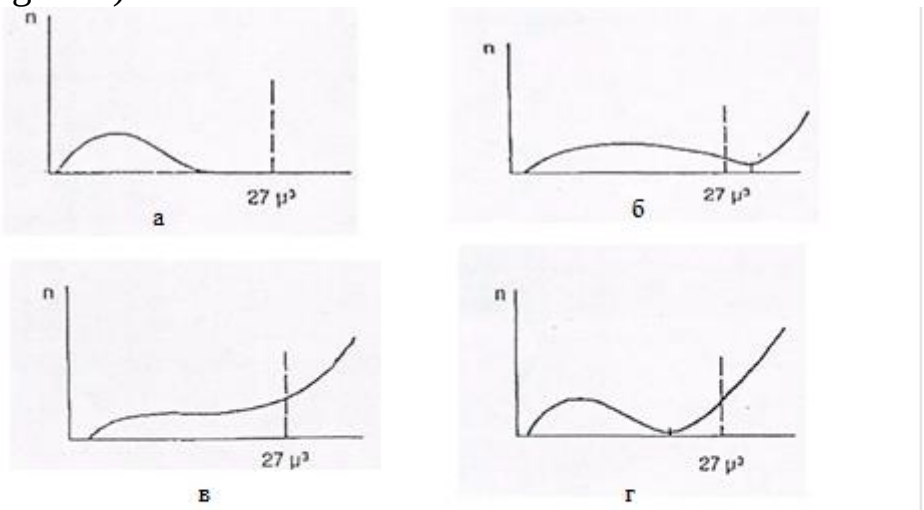


Рис.7. Гистограммы тромбоцитов: а – норма; б, в – гигантские тромбоциты или их агрегаты (на гистограмме отсутствует нормальный пик распределения, график смещается вправо и меняет свою форму); г – гистограмма тромбоцитов при наличии микроцитов

EDTA-induced pseudothrombocytopenia occurs in 0.1-1% of patients when EDTA is used as an anticoagulant. It is caused by the presence of antibodies that, in the presence of EDTA, bind to platelet membranes, cause platelet aggregation in vitro and a decrease in the number of platelets during counting. For EDTA-induced thrombocytopenia, it is recommended to count platelets in a blood sample with citrate or heparin immediately after blood collection.

Pseudothrombocytosis may be due to additional red blood cell counts with microcytosis or the presence of fragmented red blood cells.
When analyzing a blood smear, it is necessary to pay attention to platelet morphology. Visually under a microscope, one can see the size of platelets, the presence of aggregates, and some morphological changes. In thrombocytopathy associated with a violation of the formation of protein granules, platelets do not stain, they look gray ("gray" platelet syndrome).

Assessment of the functional state of platelets.
Evaluation of platelet function is carried out in the study of their aggregation using various inducers of aggregation. The previously proposed practically qualitative methods with the assessment of aggregation on a glass slide, in a test tube, or using FEC are now obsolete. Platelet function is assessed using special devices - aggregometers.
The principle of operation of most aggregometers is based on a decrease in the light absorption of platelet-rich plasma during the formation of platelet aggregates. Aggregation is triggered by the addition of various aggregation inducers or assessed without their addition (spontaneous aggregation).
The use of aggregometers is necessary in the following situations:
1. When detecting hereditary or acquired thrombocytopathies and thrombocytopenias.
2. When diagnosing von Willebrand disease.
3. In a comprehensive study of the hemostasis system in patients with blood coagulation disorders.
4. When treating with antiplatelet agents, especially aspirin, since 10-38% of patients either have congenital or acquired tolerance to the action of the drug.
ADP, adrenaline, thrombin, collagen, ristocetin, arachidonic acid and some other agents are used as inducers of aggregation.
Differential diagnosis of platelet pathology is usually carried out in specialized medical institutions. In polyclinics and multidisciplinary hospitals, the aggregatogram is relevant for a comprehensive study of the hemostasis system and monitoring of treatment with antiplatelet agents.
When treated with aspirin, the initial aggregatogram of spontaneous (without an inducer) or adrenaline-induced platelet aggregation is compared with the aggregatogram obtained after 3-4 days of aspirin treatment. A 3-4-fold decrease in aggregation indicates the presence of an aspirin effect and the adequacy of the dose. The lack of a decrease in aggregation is associated with aspirin tolerance and suggests replacement of the antiplatelet agent.
When using Plavix, the assessment of ADP-induced platelet aggregation is useful for dose adjustment. A 3-4-fold decrease in aggregation with ADP on day 3-4 indicates the presence of an effect and the adequacy of the dose.

Fresh platelet-rich plasma (PRP) is used to analyze platelet aggregation. The storage of blood samples should not exceed 45 minutes.

Screening coagulogram tests.

Screening coagulogram tests include prothrombin time (PT), activated partial thromboplastin time (APTT), thrombin time (TB), and fibrinogen. PT reveals violations of the external coagulation pathway, APTT - the internal coagulation pathway, TB - the final stage of coagulation, that is, the formation of fibrin from fibrinogen. When conducting tests, the clotting time of the patient's plasma is compared with the clotting time of plasma from healthy donors. Shortening of clotting time indicates hypercoagulability, lengthening - hypocoagulation. The prolongation of the clotting time in all three tests simultaneously occurs with heparin therapy, the presence of fibrin / fibrinogen degradation products (FDF) or pathological inhibitors in the sample. These tests are basic because they allow you to decide which qualifying test to perform.

Prothrombin time (PT).

PT (PT) is a test for the external (fast) mechanism of hemocoagulation. Tissue factor (TF), also known as tissue thromboplastin, which triggers the plasma coagulation reaction, vitamin K- and at the same time Ca ++ - dependent factors VII, X and II (prothrombin), factor V and factor I (fibrinogen). Under physiological conditions, TF enters the bloodstream from damaged or destroyed cells, including leukocytes, macrophages, tumor cells and tissues, and activates the blood coagulation process.

The main areas of application: as a screening test for the study of the blood coagulation system, control of hemostasis in the treatment of indirect anticoagulants (AED), assessment of the synthesis of prothrombin complex factors in the liver.

The principle of the method is to determine the clotting time of platelet-poor citrated plasma after adding tissue factor and Ca ++. Tissue thromboplastin is used as a tissue factor. Tissue thromboplastins are aqueous extracts of mammalian tissue lipoproteins rich in tissue factor. Phospholipids are part of tissue thromboplastin. Reagents differ in activity, sensitivity and solubility. To obtain reproducible results, it is preferable to use ready-made reagent kits. PT is a phospholipid-dependent test.

PV can be expressed in several ways:

1. Clotting time in sec.
2. Prothrombin index (PI, PTI)

$$PTI = \frac{\text{Clotting time of normal plasma}}{\text{Patient plasma clotting time}} \times 100\%$$

3. Prothrombin ratio (PO)

Patient plasma clotting time
PTI = ---------- ----------
Clotting time of normal plasma
4. Prothrombin according to Quick in%. Determined by the calibration graph.
5. INR (International Normalized Ratio), which represents the software raised to the degree of the International Sensitivity Index (IIR).
INR = POMICH

Clotting time in seconds, PTI and PO are qualitative indicators. The clotting time in seconds is not determined due to the low reproducibility of results when changing reagents, software is used as an intermediate indicator when determining INR, and PTI has low information content and is determined only in Russia and neighboring countries. Limitations in the use of IPT are associated with the dependence of the parameter on the initial activity and sensitivity of tissue thromboplastin. The values of the indicator of the same patient, measured in different laboratories, can differ significantly. The reference limits are 80-110%.

All attempts to completely replace the definition of IPT with prothrombin in percentage according to Quick in Russia were unsuccessful. This is due to objective circumstances. First, a number of laboratories are still acquiring unapproved thromboplastin. Secondly, many laboratories do not have programmable coagulometers. Water bath testing increases the likelihood of subjective errors.

Currently, it is customary to express PV in% according to Quick. Although the clotting time depends on the thromboplastin used, the type of addition to the reagent, the detection method (manual, automatic), the type of samples and the calibration material, it is a quantitative method, the final results of which are obtained according to the calibration graph. To plot a graph, several concentrations of prothrombin of donor plasma are prepared: without dilution (100%) and with saline dilution several times, for example, 2 times (50%) and 4 times (25%). Other dilutions are allowed, for example 75%. If the percentage of prothrombin indicated in the passport of the plasma calibrator differs from 100, then the percentage is calculated based on real numbers. For example, if the initial content of prothrombin according to the passport of the control plasma is 96%, then the following dilutions will be 48% and 24%, respectively. After analyzing the calibration samples, a graph is plotted in the reverse coordinate system, and the percentage of prothrombin is determined from the graph. With manual calibration, the results are limited to 100% activity and can only be determined from the calibration graph, which is difficult for nurses. Thus, laboratories without proper equipment are doomed to IPT.

Coagulometers, as a rule, have a program that, after calibration, converts the results in seconds into% according to Quick, so manual plotting is not required. Reference limits 70-130%.

PTI% does not correspond to the Quick percentage.
Clinical interpretation.
Decrease in activity (hypocoagulation) - elongation of PT, decrease in PTI and prothrombin according to Quick in%, is observed with a lack of blood coagulation factors due to a natural or drug-induced decrease in the synthesis or inhibition of factors.
1. Hereditary or acquired deficiency of factors involved in the external pathway of prothrombinase activation (VII, X, V, II, I).
2. Deficiency of vitamin K (factors II, VII and X are formed in hepatocytes in the presence of vitamin K).
3. Taking medications - antagonists of vitamin K, AEDs (warfarin, phenylin) and drugs that enhance their effect: anabolic steroids, clofibrate, glucagon, thyroxine, indomethacin, neomycin, oxyphenbutazone, salicylates; heparin, urokinase.
4. DIC syndrome.
5. The presence in the sample of primary and secondary anticoagulants (heparin, PDF).
6. The presence of pathological coagulation inhibitors.
7. Diseases of the liver with a decrease in synthetic function.
Increased activity (hypercoagulation) - shortening PT, an increase in PTI and prothrombin according to Quick in%, reflects a massive influx of tissue factor into the bloodstream (trauma, necrosis), activation of coagulation during pregnancy or after childbirth, a lack of physiological anticoagulants.
AEDs, being vitamin K antagonists, affect both the external and internal pathways of prothrombinase activation. However, the effect is more pronounced in the external cascade, and PT changes more than APTT. In the treatment of AED at the beginning of therapy, due to the large number of fluctuations, PT is used, and when the treatment is stabilized, INR is used.
INR is a calculated indicator adopted by WHO in 1983. to control treatment with indirect anticoagulants. The International Normal Ratio (INR) Index of Sensitivity is necessary to bring the PO data determined with various thromboplastins to a value that would have been determined by the primary thromboplastin standard (reference). Manufacturers of reagents determine the sensitivity of each series of thromboplastins by comparing it with a standard and indicate the MIC value in the passport for the kit. It is optimal to work with thromboplastins with a MIC close to 1 (0.96-1.5).
In the presence of hypocoagulation, the INR increases. The use of INR makes it possible to assess the degree of hypocoagulation regardless of the thromboplastin used. As a result, it becomes possible to compare data obtained in different laboratories. Determination of INR is possible only with the use of thromboplastin certified by MIC. Manual analysis is allowed. The

optimal INR limits to be achieved during treatment with indirect anticoagulants depend on the therapeutic goals and are determined by the attending physician. Usually, for the prevention of hypercoagulability, the INR is maintained in the range of 2-2.5. A high INR (4.5) is sometimes used medicinally, but is fraught with the possibility of bleeding.

INR is not used:

1. For screening studies.
2. At the beginning of AED therapy.
3. When assessing liver function.

Activated partial thromboplastin time (APTT).

The term partial (partial) is due to the use of phospholipids in the composition of reagents, and not tissue factor. APTT refers to phospholipid-dependent tests. It is designed to assess the internal mechanism of prothrombinase formation. The internal cascade involves IUD, PK, XII, XI, IX, VIII, X, V, II and I factors, of which IX, X and II are vitamin K- and Ca ++-dependent.

Main areas of application: as a screening test, monitoring heparin therapy, primary detection of lupus anticoagulant (VA).

The principle of the method is to determine the clotting time of PPP when it is triggered by an internal mechanism after adding calcium under conditions of standardization of the contact and phospholipid phases of coagulation. The contact phase can be activated by substances of inorganic (kaolin, sulfatides) and organic (ellagic acid) origin.

The results of the study are significantly influenced by the composition of phospholipids, which are a substitute for platelet factor 3 (P3). They can be represented by phospholipids of platelet membranes, phospholipids of synthetic origin or isolated from tissues of animals and humans, phospholipids of plant origin (from soybeans), a mixture of animals and plant phospholipids, phospholipids from the membranes of human erythrocytes (erylide, erythrophosphatide). Depending on the priority of identifying any violations for a given laboratory, appropriate reagent kits are selected. Thus, the combination of ellagic acid and soybean phospholipids is sensitive to factor deficiency and the presence of heparin, and kaolin and erylide is sensitive to both factor deficiency, heparin, and lupus anticoagulant.

The method is poorly standardized, the results depend on the instrument and the reagents used, therefore the properties of the reagents must be known. It is advisable to use reagents from the same manufacturer. In some cases, it is useful to calculate the patient's APTT / donor APTT ratio (normal control plasma).

In the APTT test, the reproducibility of the results depends on the observance of the plasma preincubation time with phospholipids and the contact phase

activator before adding CaCl2. The pre-incubation time specified in the analytical procedure must be strictly observed.

Clinical interpretation.

Prolongation of APTT (hypocoagulation):

1. Deficiency of internal path factors.
2. Presence of hemophilia (congenital deficiency of factors VIII and IX causes hemophilia A and B, respectively).
3. Vitamin K deficiency or treatment of AEDs (vitamin K-dependent factors II, IX and X are involved in the intrinsic pathway).
4. Treatment with heparin and other direct anticoagulants.
5. DIC-syndrome in the phase of hypocoagulation.
6. Diseases of the liver with a decrease in synthetic function.
7. The presence of secondary anticoagulants in the sample (PDF, etc.).
8. Presence of pathological coagulation inhibitors.
9. Presence of lupus anticoagulant (VA).

Heparin therapy and the introduction of heparin into the tested plasma leads to a dose-dependent lengthening of the APTT. With the introduction of therapeutic doses of OH, the coagulation time in the APTT test should be extended by 1.5-2.5 times compared with the average for the series, and not with the patient's APTT. With prolonged treatment with heparin, as well as after fibrinolytic therapy, resistance (resistance) to heparin may develop. It manifests itself in a shortening of the APTT time against the background of a constant dose of heparin. This fact indicates the ineffectiveness of treatment and an increased risk of thrombosis. Resistance to heparin is most often associated with a lack of antithrombin, therefore, patients on heparin therapy are advised to determine antithrombin. Other reasons may be the release of heparin-neutralizing substances by platelets or an increase in their level in the acute phase of inflammation. The introduction of LMWH is currently accepted to control the residual activity of factor Xa, although the APTT is lengthened similarly to the introduction of OG.

When treating AEDs, APTT is recommended to be measured at the beginning of treatment, as well as with prolonged use of drugs. Clotting time, as a rule, lengthens 2-2.5 times in relation to the norm. When taking oral anticoagulants, there was no correlation between the APTT and PT numbers.

The effects of pathological inhibitors and lupus anticoagulants (LAs) will be discussed in more detail in the VA test.

Shortening of APTT (hypercoagulability) indicates thrombophilia. The reason may be the resistance of factor V to protein C, an increase in the level of factor VIII, activation of factors, a physiological increase in blood clotting during pregnancy.

Often the shortening of APTT is associated with impaired work with blood at the preanalytical stage.

Thrombin time

Thrombin time (TB) characterizes the final stage of the plasma coagulation process: the conversion of fibrinogen to fibrin under the action of thrombin. The test is a screening test for fibrinogen / fibrin polymerization and plasma anticoagulant activity.

The principle of the method is to determine the clotting time of PPP by adding a standard concentration of thrombin to it. Since thrombin directly activates fibrinogen, the test does not require the addition of calcium or any additional reagents. The test uses low to moderate thrombin concentration. Low concentration thrombin causes clotting within 15-20 seconds. (reference values are indicated in the analysis method). It is used for screening studies. When working with plasma containing heparin, a higher concentration of thrombin is recommended, since the antithrombin-heparin complex, which is formed with the introduction of heparin, quickly neutralizes the added thrombin. The test is very sensitive to heparin. Immediately after intravenous administration of heparin and in the terminal phase of disseminated intravascular coagulation, blood does not clot in the thrombin test. TB is lengthened when blood is drawn through a heparin-treated catheter.

For correct interpretation of test results, protamine sulfate can be added to a blood sample containing heparin to neutralize heparin or the reptilase time can be measured. Reptilase or batroxobin is a coagulase from the venom of the snake snake. It acts similarly to thrombin, but is not affected by heparin.

TV lengthening (hypocoagulation):

1. Decrease in the concentration of fibrinogen to 1-0.5 g / l.
2. A qualitative change in the fibrinogen molecule (dysfibrinogenemia).
3. Introduction of direct anticoagulants (heparin).
4. High content of PDP with increased fibrinolysis, treatment with fibrinolytics, disseminated intravascular coagulation syndrome.
5. The presence of pathological inhibitors.

TB-lengthening inhibitors are divided into polymerization inhibitors, which are the majority, and thrombin inhibitors. Polymerization inhibitors are abnormal immunoglobulins that interfere with the polymerization of fibrinogen. All inhibitors are associated with an underlying disease such as multiple myeloma and are represented by paraproteins or cryoglobulins. In the presence of thrombin inhibitors, the clotting time in the reptilase test remains normal.

In some cases, TB lengthening is observed with uremia and in the presence of VA.

The definition of TB is useful for fibrinolytic and heparin therapy.

Shortening of TB is indicative of hypercoagulability and risk of thrombosis.

Fibrinogen.

Fibrinogen is a blood plasma protein that is synthesized in the liver. The fibrinogen content is practically independent of gender and age. Although it does not activate hemostasis by itself, epidemiological studies have shown that fibrinogen is an independent risk factor for myocardial infarction and ischemic stroke. An increase in the content of fibrinogen increases the tendency to thrombus formation, increases platelet aggregation, impairs the rheological properties of blood, promotes atherosclerosis and increases the risk of thrombosis. However, the limits for therapeutic intervention have not yet been determined.

Fibrinogen is a protein of the acute phase of inflammation. In severe bacterial infections, trauma, thrombosis, it can increase to 10 g / l or more. An increase in the content of fibrinogen correlates with an increase in ESR.

The reference range is 2-4 g / l.

Basic methods of determination.

1. Clotting method according to Claus is the most adequate and widespread method and belongs to functional tests. It is based on the determination of the clot formation time when thrombin in high concentration is added to the plasma diluted 10-20 times. A logarithmic relationship was found between the time of clot formation and the concentration of fibrinogen. The method requires preliminary construction of a calibration graph in accordance with the instructions for the kit of reagents. In the absence of programmable coagulometers, it is undesirable to use this method: a small error in determining the coagulation time leads to significant errors in the content of fibrinogen. The limitations of the method include the sensitivity of the results to hyper-, dis-, hyperfibrinogenemia and an excess of PDP, which reduce the activity of fibrinogen polymerization.

2. Determination of fibrinogen by R.A. Rutberg. The method is based on weighing the dried fibrin clot after adding a thromboplastin-calcium mixture or thrombin to 1 ml of plasma. The method is manual, not very sensitive and reproducible, and is considered obsolete. However, with hypo- and dysfibrinogenemia, its results are more reliable than with the Claus method.

3. Other methods (turbidimetric, photometric using chromogenic substrates, immunochemical) relate to concentration methods, since they directly determine the concentration of fibrinogen.

Increase in content (hypercoagulation): inflammation, necrosis, smoking, kidney disease, collagenosis, neoplasms, atherosclerosis, taking oral contraceptives, pregnancy, diabetes mellitus, obesity, stress.

Decrease in content (hypocoagulation): congenital deficiency, DIC, hepatocellular failure, acute fibrinolysis, leukemia, infectious mononucleosis, the effect of drugs (reptilase, fibrates, phenobarbital, streptokinase, urokinase, actilize, etc.), exercise.

Dysfibrinogenemia is characterized by the presence of a functionally defective fibrinogen molecule in the blood plasma. Hereditary dysfibrinogenemia is rare. They are more often asymptomatic, sometimes with a tendency to bleeding or thrombosis. Acquired dysfibrinogenemia occurs in patients with severe liver disease. Dysfibrinogenemia is characterized by the following laboratory parameters:

• an increase in thrombin and reptilase time,

• discrepancy in the results when measuring the level of fibrinogen functional test (Claus, low) and concentration (higher or normal).

Additional tests.

Evaluation of the fibrinolysis system. Fibrinolytic activity.

To assess the fibrinolysis system, tests can be carried out that measure the concentration of certain factors (plasminogen, α2-antiplasmin, the concentration of the plasmin-antiplasmin complex, etc.). However, the most general idea of the fibrinolysis system can be obtained by carrying out a traditional functional test - the assessment of the fibrinolytic activity of the blood (FAC). The test is distinguished by its simplicity of execution and the absence of special equipment. The disadvantage is the impossibility of automation. The result of the FAK test depends on the level of fibrinogen, the quality of fibrin polymerization and the presence of heparin.

There are currently two test modifications in use.

1. Spontaneous euglobulin fibrinolysis. When plasma proteins are precipitated by a weak, usually acetic acid, the so-called euglobulin fraction is precipitated, which contains fibrinogen, coagulation factors, plasminogen and its activators, but is practically free of fibrinolysis inhibitors. With such a gentle sedimentation, the proteins do not lose their properties. After dissolving the precipitated proteins in a buffer solution, adding CaCl2 and the formation of a fibrin clot thereafter, the clot lysis time reflects the FAA. Reference limits 2-4 hours.

2. XIIa-dependent fibrinolysis (or Hageman-dependent fibrinolysis, f. XII - Hageman factor) differs from spontaneous in that kaolin is added to the reaction mixture during the precipitation of proteins. Kaolin is an activator of f. XII and the internal pathway of fibrinolysis. The analysis time is sharply reduced, since the lysis of the clot is 4-12 minutes.

When choosing a method for assessing FAA, it is necessary to take into account the peculiarities of the patient's pathology. So, in screening studies,

in examinations of pregnant women and other patients with thrombophilia, the definition of XIIa-dependent fibrinolysis will be adequate. For patients with disseminated intravascular coagulation syndrome, after surgery on the organs of internal secretion or with metastatic tumors, as well as in other conditions with the possibility of hyperfibrinolysis, the definition of spontaneous euglobulin fibrinolysis is more appropriate: when fibrinolysis is activated, the clot lysis time is reduced, and reliably visually track clot dissolution over a period time less than 4 minutes is almost impossible.
Activation of fibrinolysis (shortening of clot lysis time, hypocoagulation) is observed with a decrease in the concentration of fibrinogen, hypo- and dysfibrinogenemia, pancreatitis and pancreatic necrosis, operations on the organs of internal secretion and lungs, obstetric complications, adrenaline administration, pyrogenic tumors, metastasis syndromes the level of plasminogen inhibitors, treatment with fibrinolytics and plasminogen activators.
Inhibition of fibrinolysis (prolongation of clot lysis time, hypercoagulation) is observed with hyperfibrinogenemia, congenital deficiency of plasminogen or its abnormalities, a decrease in plasminogen and its activators with increased consumption or decreased synthesis (thrombosis, vasculitis, sepsis, liver disease, disseminated intravascular coagulation), pregnancy.
The test can be used to monitor the effectiveness of fibrinolytic treatment.

Assessment of the anticoagulation link in hemostasis.
The anticoagulant system, which limits blood clotting, is represented by several proteins. The main physiological anticoagulants include antithrombin (AT, formerly antithrombin III), and the protein C system ("si"). These systems have a different mechanism of action: AT inhibits coagulation enzymes, and the protein C system inactivates cofactors of enzymes, therefore, studies should include an analysis of both systems. Deficiency or defect of both one and the other anticoagulant are confirmed risk factors for the development of thrombosis.
Antithrombin.
Antithrombin is a glycoprotein with OMM 58000D, which is synthesized in the liver. Due to the low BMM (<70,000 D), it is easily excreted through the kidneys. AT inhibits serine proteases, primarily thrombin (f. IIa) and f. Ha. In the presence of heparin, the activity of AT increases thousands of times. The anticoagulant effect of heparin it is realized through AT, without which heparin does not have an anticoagulant effect. According to the literature data, AT accounts for 75-90% of the total anticoagulant potential.
Determination methods.

1. Clotting methods. These are screening functional methods that assess the activity of antibodies by their inhibitory effect on thrombin. The test is calibrated against normal donor plasma and is graded as a percentage. The reference values range from 75 to 125%.
2. Methods with a chromogenic substrate make it possible to assess the amidolytic activity of AT, which may not correspond to its biological activity. However, the simplicity and availability of the method are widely used in CDLs.
3. Immunochemical methods determine the concentration of antithrombin.
Decreased activity (hypercoagulability).
Congenital: deficiency or abnormalities of AT.
Acquired:
Liver disease (decreased synthesis).
Severe proteinuria (increased excretion of AT through the kidneys).
Malignant neoplasms (activation of coagulation, increased consumption).
Taking oral contraceptives, corticosteroids, L-asparaginase treatment.
Pregnancy and late toxicosis of pregnancy (gestosis).
Massive thrombin formation (DIC, sepsis, terminal conditions).
Treatment with large doses of heparin.
Most cases of AT deficiency are acquired. It should be borne in mind that with a decrease in AT <60%, the risk of thrombosis increases sharply, therefore, all patients with thrombophilia and the above conditions should be determined for AT activity. Before treatment with large doses of heparin, including disseminated intravascular coagulation, control of AT is extremely useful, since heparin has no effect when the AT level falls. It was found that the restoration of the level of anticoagulants in acute and subacute disseminated intravascular coagulation syndrome indicates a favorable prognosis of the disease.
Increased activity (hypocoagulation): acute viral hepatitis, cholestasis, taking anabolic steroids, treatment of AED.

Protein C.
The main proteins of the protein C system are protein C and its cofactor protein S. These are vitamin K-dependent serine proteases that are synthesized in the liver. The action of the protein C system is aimed at inactivating factors VIIIa and Va - accelerators of the plasma clotting process. When the protein C system is activated, PAI-1 is also inhibited, which leads to the initiation of fibrinolysis. Proteins of the protein C system under physiological conditions are activated by thrombin. Activation requires the presence of phospholipids and Ca ++, which is typical for all vitamin K-

dependent factors. Under in vitro conditions, the system is triggered by a protac - an activator made from the venom of the snake snake.
Protein C system tests include an assessment of the overall activity of the protein C system, determination of the activity of protein C, protein S, factor Va.

Determination methods.
Protein C system assessment can be performed using clotting and immunochemical methods, as well as using chromogenic substrates.

A screening method for assessing the overall activity of the protein C system. The method was developed based on the modified APTT test. In the presence of activated protein C (APC) in the plasma, the clotting time of the plasma of a healthy person is lengthened due to the inactivation of factors VIIIa and Va. Activation is carried out by adding protak. The test measures the clotting time of the patient's plasma with and without APS. In some modifications of the method, the clotting time of the patient's plasma is compared with the clotting time of normal donor plasma. The analytical procedure provides a formula for calculating the normalized ratio and criteria for evaluating the test in numerical terms, which depend on the reagents used.
In a number of patients, there is practically no lengthening of APTT with the addition of APT. In this regard, the term "activated protein C resistance" was introduced.
The decrease in the activity of the protein C system is due to one of three reasons: protein C deficiency, protein S deficiency, or factor Va resistance to APP. Structural change of the molecule f. V leads to the impossibility of its complete inactivation under the action of APS. For the differential diagnosis of disorders, the tests indicated above are carried out in full.
Despite the fact that the protein C system accounts for only 10-15% of the total anticoagulant activity of the blood, a decrease in the level of protein C and its cofactors to 50% of the norm leads to the appearance of recurrent venous thrombosis, juvenile thrombosis, which appear at a young age, and thromboembolism. Homozygous protein C deficiency causes the development of fulminant purpura in children, which is practically incompatible with life. Heterozygous deficiency is manifested by early thrombosis.
Decreased activity (hypercoagulability). There are hereditary and acquired factors that reduce the activity of the protein C system.
Hereditary factors: mutations in the genes of protein C, protein S, factor V. Factor V Leiden mutation, the most common among the European population, causes resistance to APP.

Acquired factors: presence of lupus anticoagulants, pregnancy, excess factor VIII, age over 50, disseminated intravascular coagulation, liver disease, sepsis, L-asparaginase treatment, vitamin K deficiency, treatment with warfarin and other AEDs.

Indirect anticoagulants reduce the synthesis of all vitamin-K-dependent factors, including protein C and protein S. Warfarin, prescribed to patients with low protein C activity, provokes thrombosis and skin necrosis. Before prescribing warfarin, especially the elderly and persons with early thrombosis, it is necessary to test for the general activity of the protein C system. At low activity of the system, warfarin is recommended to be introduced gradually, starting with small doses, or replaced with fractionated heparin.

Assessment of the level of thrombinemia.

The processes of blood coagulation in humans are ongoing, but they are local in nature. When coagulation processes are activated, an increased generation of thrombin occurs, which, under physiological conditions, triggers the process of converting soluble fibrinogen into insoluble fibrin. An obvious estimate of the level of coagulation by the amount of thrombin is attractive, but not available, since thrombin has a short lifespan, and it is almost impossible to determine its amount in plasma. The level of thrombinemia, and, accordingly, the activity of the coagulation processes, is assessed by the products that are formed during the activation of prothrombin and the conversion of fibrinogen by thrombin into fibrin.

When prothrombin is activated, various products are formed, but the formation of thrombin and the F1 + 2 fragment is mandatory, between which there is a stoichiometric ratio. At the F1 + 2 level, both hypercoagulation (growth) and hypocoagulation (decrease) can be monitored. The F1 + 2 fragment has a lifetime of 30 min. When thrombin interacts with antithrombin, thrombin-antithrombin complexes (TATs) are formed, which can also be used as a marker of thrombinemia. However, the most accessible way to assess the level of thrombinemia is to determine the substances that are formed during the activation and destruction of fibrinogen and fibrin.

When fibrinogen is activated by thrombin, two fibrinopeptides A (FPA), two fibrinopeptides B (FPV) are cleaved from the latter, and a fibrin monomer is formed. Determination of FPA has been used for a long time as a marker of hemostasis activation, but it is rapidly excreted by the kidneys. Fibrin monomer forms fibrin oligomers and soluble fibrin polymers, which are converted into insoluble fibrin under the action of fibrin-stabilizing factor (XIII) (Fig. 3). Further, the fibrin clot is lysed by plasmin with the formation of D, E, D-E and D-D fibrin fragments - D-dimers. With a high activity of plasmin, a decrease in the level of antiplasmins and a high level of

fibrinogen, plasmin can cleave fibrinogen with the formation of D, E, and D-E fragments. All of these products are called fibrin / fibrinogen degradation products (FDP). Their amount can be measured by immunological methods using polyclonal antibodies.
In a healthy person, the level of PDP is very low and is not always determined by routine laboratory tests. In addition, PDP is utilized by phagocytes. When blood coagulation is activated, the level of PDP increases, the fibrinogen pool expands, and fibrinolysis is simultaneously activated. The products begin to interact with each other, forming soluble fibrin-monomeric complexes (RFMC), which prevent fibrinogen polymerization and thereby act as secondary anticoagulants. Of all PDPs, the only fragment that is formed only from thrombus fibrin is D-dimers.

Available methods for determining the components of the PDF.
Paracoagulation tests.
Paracoagulation tests include ortho-phenentroline, ethanol, and protamine sulfate. They are based on the formation of a gel or flakes when ortho-phenanthroline, 50% ethanol or protamine sulfate is added to the blood plasma of a patient with thrombinemia. The o-phenanthroline test is currently used as a semi-quantitative test. The rest of the methods are considered poorly sensitive, poorly reproducible, and outdated.
Determination of RFMC by the ortho-phenanthroline method.
The method is based on the appearance of paracoagulum flakes when o-phenanthroline is added to the platelet-poor plasma of patients. The time of flake appearance reflects the degree of thrombinemia: the higher the RFMK level, the earlier the flakes appear. Manual semi-quantitative test. Domestic reagents.
An increase in RFMK (hypercoagulation) is observed in all cases of thrombinemia: with thrombophilia, thrombosis and thromboembolism, pregnancy, high levels of fibrinogen, disseminated intravascular coagulation. The test can be used to assess the effectiveness and sufficiency of anticoagulant therapy based on the final result - the elimination of thrombinemia. The method cannot be used to confirm or exclude the presence of blood clots.
Soluble fibrin (RF).
Immunological test systems for determining RF are produced by foreign manufacturers. Since the reaction is based on the interaction of fibrin monomers with antibodies to them, the test actually determines fibrin monomers, which are formed only from fibrinogen. Their level rises sharply with the generalization of coagulation processes, that is, with disseminated intravascular coagulation. In all other situations, fibrin monomers either

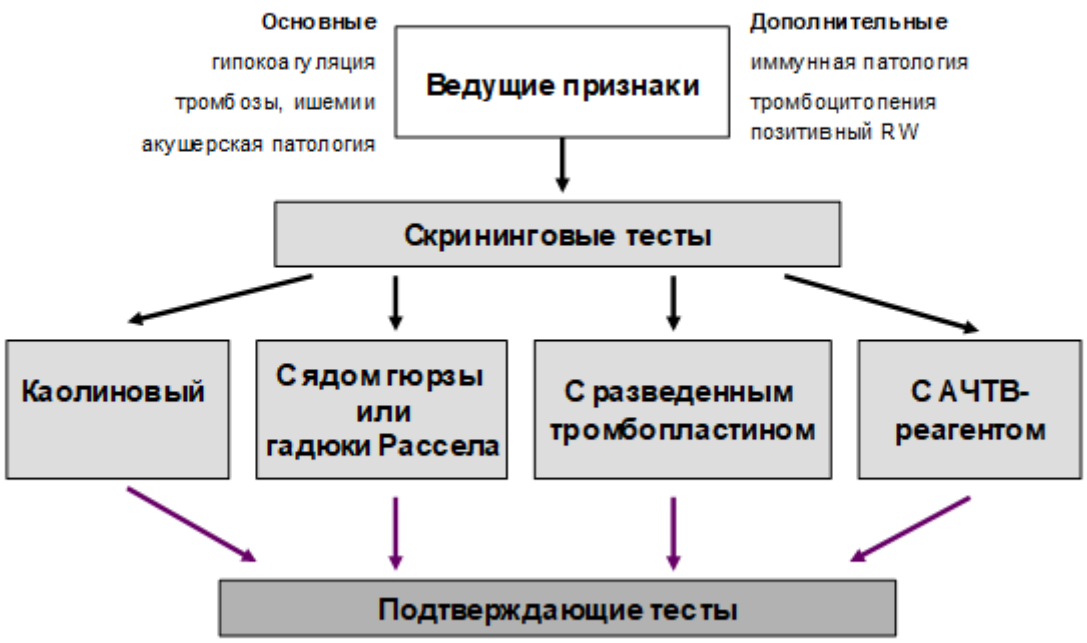
polymerize or bind to other PDPs and are found in free form at extremely low concentrations. Therefore, this method is suitable exclusively for the diagnosis and monitoring of disseminated intravascular coagulation.
D-dimers.
These are specific products of degradation of fibrin, which is part of the thrombus. The concentration of D-dimers is proportional to the activity of fibrinolysis and the amount of lysed fibrin, that is, the size of the thrombus.
To determine the concentration of D-dimers, immunological methods using monoclonal antibodies are used. Kits of reagents for ELISA diagnostics, latex agglutination, immunodiffusion, immunoturbidimetry are produced.
An increase in the level of D-dimers is observed in deep vein thrombosis, PE, DIC, thrombophilia, pregnancy (increase in 3-4 r.), Treatment with thrombolytics, extensive hematomas, as well as in cancer, infections, inflammation, liver diseases, at the age of older 80 years old, with rheumatoid factor, with wound healing.
A decrease in the level of D-dimers is observed during treatment with direct and indirect anticoagulants.
For D-dimers, negative diagnostic significance is most characteristic (about 100%). The method can be used to exclude the diagnosis of thrombosis, since a negative result is highly likely to exclude this diagnosis.

Identifying the effects of lupus anticoagulant (VA).
Lupus anticoagulants (Lupus Anticoagulants, LA) are class A, M, and G immunoglobulins that act against phospholipid-protein complexes. Since phospholipids are inseparable from the clotting processes, antibodies to them slow down the plasma clotting processes in all phospholipid-dependent tests without reducing the activity of individual factors. The concept is relatively virtual, since VAs are not highlighted or identified. Their presence is assessed by the effect rendered - the prolongation of the clotting time of PTP in phospholipid-dependent tests (APTT, PT). VA are found in APS, SLE and other collagenoses, viral infections, treatment with certain pharmacological drugs. The emergence of VA is based on autoimmune pathology.
An analysis for VA is prescribed in the presence of clinical symptoms (arterial or venous thrombosis, especially in childhood and young age, relative thrombocytopenia, etc.). Currently, pregnant women are examined for VA, since almost half of spontaneous abortions in early pregnancy are associated with the presence of APS. The algorithm for detecting VA is shown in Fig. eight.
In a conventional CDL, to detect the effect of VA, as a rule, screening tests are carried out, which reveal the effects of VA, that is, an extension of the clotting time in phospholipid-dependent tests:

kaolin clotting time of PPP, test with diluted thromboplastin, test with venom of Gyurza or Russell's viper, test using thromboplastin with high sensitivity to VA. The last two tests are most commonly used.

Рис. 8. Алгоритм выявления волчаночного антикоагулянта

(З.С. Баркаган, А.П. Момот)



Correctly obtained platelet-poor plasma (PPP) is used for VA studies. The use of heparinized and frozen plasma is not allowed. All tests are carried out by the clotting method. Manual testing is possible, but the accuracy of the analyzes increases when working on a coagulometer. The evaluation of the tests is based on a comparison of the coagulation indices of the patient and the healthy donor, therefore, the analysis of the patient's plasma is carried out in parallel with the analysis of normal plasma.

Basic screening tests.

Tests with diluted venom of Gyurza or Russell's viper. The coagulases of these snakes are phospholipid-dependent activators of factor X. The prolongation of the patient's plasma clotting time compared to the control (donor) plasma may be associated with VA. For the test, the so-called normalized ratio (NR, NR) is calculated, which takes into account the degree of slowing down of plasma coagulation. The calculation of the indicator and the interpretation of the results depend on the reagents used and are indicated in the analysis method.

Test with aPTT reagent sensitive to VA. The sensitivity of APTT reagents to VA depends on the phospholipids included in the set. The use of APTT

reagents insensitive to VA practically does not lengthen the clotting time of plasma in patients with VA. The use of VA-sensitive APTT reagents significantly lengthens the clotting time of plasma in patients with VA compared to donor plasma. The calculation of the degree of slowing down of plasma coagulation and the interpretation of the results obtained depend on the reagents used and are indicated in the analysis method.

The prolongation of the PPP clotting time may be associated not only with the effect of VA, but also with the deficiency or pathological inhibition of factors. Therefore, it is recommended to conduct confirmatory tests that establish that hypocoagulation in screening tests is due to the presence of VA. For this, it is necessary to have a pool of donor plasma and a solution of the membranes of destroyed platelets or erythrophosphatide.

1. In case of deficiency of factors, the addition of 10% of donor plasma shortens the APTT time, and the addition of 50% of the volume of donor plasma normalizes the time.
2. In the presence of VA, the normalization of the clotting time of PPP occurs after the addition of membranes of destroyed platelets or erythrophosphatide.
3. With pathological inhibition of factors, there is no correction of hypocoagulation in both cases (with the addition of membranes and plasma) (Table 9). Inhibition of factors in most cases is associated with the formation of antibodies of the IgG class. Antibodies usually appear in autoimmune diseases, during treatment with antibacterial drugs, after pregnancy, in the elderly, and during blood replacement therapy.

Table 9.

Tests to confirm the presence of VA.

Pathology	VA test	Correction with platelets or erythrophosphatide	Plasma correction
Availability of VA	+	+	-
Deficiency of factors	+	-	+
Inhibition of factors	+	-	-

The presence of VA is not synonymous with antiphospholipid syndrome or systemic lupus erythematosus (SLE). To diagnose APS, it is necessary to measure the level of antibodies to membrane phospholipids and to glycoproteins associated with phospholipids. Only with an increase in the content of both groups of antibodies, detection of VA and the presence of clinical symptoms

APS diagnosis is possible. SLE is diagnosed by other methods.

Laboratory diagnostics and monitoring of DIC syndrome.

To confirm the diagnosis and objectively assess the effectiveness of the therapy in the laboratory, the following groups of tests are used (Table 10).

Table 10

Available laboratory tests for disseminated intravascular coagulation

test	Pathology
Platelet count	Thrombocytopenia (<150,000), not reaching critical
Fibrinogen concentration	Relative or absolute decrease (<1.5 g / l)
Thrombinemia level (RFMK) soluble fibrin, D-dimer, ethanol test)	Enhancement (DIC develops against the background of thrombinemia)
Anticoagulant level	decline
Fibrinolytic activity (euglobulin fibrinolysis)	Activation against the background of a decrease in plasmin
AChTV, PV, TV	Phase changes (usually lengthening)

1. Calculation of the number and assessment of platelet function, since the syndrome is characterized by progressive, thrombocytopenia with increased platelet aggregation. When counting platelets in a hematology analyzer, it is necessary to view histograms of platelets and erythrocytes. This need is associated with the appearance of a large number of erythrocyte fragments in DIC syndrome.

2. Assessment of the level of thrombinemia, the choice of tests for which is determined by the capabilities of the laboratory. Tests for soluble fibrin (RF), RFMK, fibrinopeptides A, D-dimers and others can be used. It should be noted that an increase in RF is observed only with disseminated intravascular coagulation syndrome, while the level of RFMC is increased with a large list of pathologies, and D-dimers are formed upon activation of fibrinolysis and cleavage of fibrin clot.

3. Assessment of the level of anticoagulants: antithrombin and protein C. When treating a patient in the hypercoagulable phase, it is most important to determine the activity of antithrombin before and during treatment, since heparin does not work without antithrombin, and an increase in the content of AT during treatment indicates a favorable course of the disease.

4. Measurement of the concentration of fibrinogen, the content of which is progressively decreasing. With a low level of fibrinogen, its concentration is

best determined not by clotting methods, but by other methods, including the Rutberg method.

5. Assessment of fibrinolytic activity (FAA). FAA should be determined by the method of euglobulin fibrinolysis, since the test for XIIa-dependent fibrinolysis with activation of the contact phase will be of little information due to the short time of clot dissolution (4-12 min) and the inability to track the degree of activation of the process.

6. Other coagulation tests (PT, APTT, TV) can have different directions depending on the phase of the disease. They are used for the general assessment of coagulation. In heparin treatment, the test time can be lengthened due to the presence of heparin. Heparin can be inactivated with protamine sulfate, and the thrombin time test can be replaced with a reptilase time test, which is not prolonged by heparin.

Total mean thrombogenicity index (SITI).

The total average thrombogenicity (thrombophilia) index of the SIT is the total average indicator calculated from the values of any number of indicators of the functioning of the hemostasis system by links. For the calculation, the average values of the norm and the value obtained from the patient are taken. The index of each indicator (ITj) is calculated, reduced to one, so that values below one indicate hypocoagulation, and above one, indicate hypercoagulation. For this, the indicator of the average norm (N) is divided by the indicator of the patient (A), or vice versa. All indices are summed up and divided by the number of indicators in a given link of hemostasis (nj) - the thrombogenicity index for a specific link (ITi) is obtained. Then the link indices are summed up, and the sum is divided by the number of links examined (ni). As a result, a total average thrombogenicity index is obtained.

$$ИТj = \frac{N}{A} \qquad ИТi = \frac{\sum ИТj}{nj} \qquad ССИТ = \frac{\sum ИТi}{ni}$$

SSIT is an integral indicator of the balanced functioning of the hemostasis system.

The following is an example of calculating the SIT of the patient's coagulogram (Table 11).

The table shows 7 measured parameters, each of which reflects a separate link in hemostasis. On the basis of the reference values, the average indicators were calculated according to the norm, for example, for APTT: (35 + 45): 2 = 40 (sec). The next step was to calculate the index of each indicator. To do this, it is necessary to decide in which case to divide the patient's indicator by the average value of the norm, and in which case the average value of the norm should be divided by the patient's indicator. In accordance

with the calculation conditions, all parameters that indicate hypercoagulation should be> 1, and for hypocoagulation - <1. The direction of the shifts for the corresponding coagulogram index (hypocoagulation - hypercoagulation) is indicated for each test (see the description of the tests).

APTT. With hypercoagulation, the clotting time decreases, and with hypocoagulation, it increases. Therefore, in order to obtain a value of> 1 during hypercoagulation, the average time of the norm must be divided by the time of clotting of the patient's plasma. 40: 29 = 1.38

Prothrombin according to Quick. The higher the coagulation, the greater the percentage of prothrombin. Therefore, to obtain a figure of> 1 during hypercoagulation, it is necessary to divide the patient's prothrombin percentage by the average value of the norm. 98: 100 = 0.98

Thrombin time. With hypercoagulation, the plasma clotting time decreases, and with hypocoagulation it increases. Therefore, in order to obtain a value> 1 during hypercoagulation, the average value of the norm must be divided by the patient's plasma clotting time: 30: 32 = 0.94

Fibrinogen. The higher the concentration of fibrinogen, the higher the coagulation. Therefore, to obtain a figure of> 1 during hypercoagulation, it is necessary to divide the patient's fibrinogen concentration by the average value of the norm: 4.1: 3 = 1.37.

Antithrombin. With hypercoagulation, the percentage of antithrombin activity decreases, and with hypocoagulation it increases. Therefore, in order to obtain a value> 1 during hypercoagulation, the average value of the norm must be divided by the percentage of the patient's antithrombin activity: 100: 80 = 1.25

Fibrinolysis. With hypercoagulation, the fibrinolysis time increases, and with hypocoagulation, it decreases. Therefore, in order to obtain a value> 1 during hypercoagulation, the patient's fibrinolysis time must be divided by the average value of the norm: 14: 8 = 1.75.

RFMK. For RFMK, values within the normal range are denoted as 1, and increased as 2.

In this example, there is 1 test for each coagulation link, so all indices are summed up and divided by the number of tests (7): (1.38 + 0.98 + 0.94 + 1.37 + 1.25 + 1.75 + 2): 7 = 1.32.

With a balanced hemostasis system, the fluctuations of the SIT are in the range of 0.9-1.1.

The calculation of the SIT helps in the interpretation of the results of the coagulogram. In this example, SIT 1.38 indicates an imbalance and a predominance of hypercoagulable processes in the patient. When evaluating specific tests, it becomes clear that hypercoagulation is associated with activation of the internal plasma coagulation pathway (APTT index 1.38), a

relative decrease in antithrombin content and a decrease in fibrinolysis activity (FAK index 1.75). The growth of RFMK is due to the activation of hemostasis.

SSIT is extremely useful for the interpretation of coagulogram analyzes, as it allows you to determine the resultant shifts in the hemostatic system. The presence of an appropriate computer program that allows you to calculate the SIT automatically when the results of coagulation studies are entered, greatly simplifies the task. Calculating the index without a computer program using a calculator is very time consuming and practically impossible. Apparently, this is why such a useful indicator is rarely used in the practice of CDL.

CONCLUSION

In a short textbook it is impossible to describe in detail the mechanisms of hemostasis and the diagnosis of all types of pathology of the blood coagulation system. Nevertheless, the paper summarizes the mechanisms of hemocoagulation, identifies the main disorders associated with the blood coagulation system, and also indicates the drugs that are most widely used to treat the pathology of hemostasis.

In the section of research methods, the main attention was paid to those tests that can be determined in the conditions of a conventional clinical diagnostic laboratory. For each test, the principle of determination is indicated, as well as the main reasons for the decrease or increase in the indicator. For a number of tests, diseases and conditions are indicated in which the study of the indicator has the greatest diagnostic value. The tutorial is aimed, first of all, at helping in practical work. The literature indicated in the bibliography will help to improve qualifications in the field of hemostasis.

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