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Modern concepts about the pathogenesis of thrombosis of various etiologies

R.R. Khismatullin[1]*, R.I. Litvinov[2]

[1]Institute of Fundamental Medicine and Biology, Kazan (Volga Region) Federal University, Kazan, Russia;
[2]Department of Cell and Developmental Biology, School of Medicine, University of Pennsylvania, Philadelphia, United States of America

Abstract
Thrombosis becomes the cause and complication of many cardiovascular diseases, and their prevalence remains a leader in the structure of morbidity and mortality in Russia and throughout the world. Modern fundamental and clinical research has significantly supplemented traditional ideas about the mechanisms of thrombus formation. First of all, Virchow's triad has been rethought, in which, according to new data, the leading role is assigned to vascular damage, and slowing down blood flow plays a primary role in the formation of only venous, but not arterial, blood clots. In recent years, the mechanisms of endothelial dysfunction underlying thrombosis associated with inflammatory (immunothrombosis) and atherosclerotic (atherothrombosis) damage to the vascular wall have been studied in detail. The cellular and molecular mechanisms of acquired hypercoagulability and hereditary thrombophilia have been deciphered. The traditional concept of dividing blood clots into "red" (venous, consisting of fibrin and red blood cells) and "white" (arterial, platelet) is being revised. It has been shown that red blood cells can occupy most of the volume of not only venous, but also arterial thrombi, and play an important role in thrombus formation reactions. The process of compression (contraction, retraction) of blood clots, caused by contraction of activated platelets, changing the structure of the blood clot and affecting the course and outcome of thrombosis, is being actively studied. A deep understanding of the pathogenesis of thrombosis, taking into account modern concepts, is necessary for effective prevention, early diagnosis and treatment of thrombotic conditions.

Keywords: thrombosis, arterial thrombus, venous thrombus, immunothrombosis, review.

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Background

Thrombosis is the formation of an abnormal blood clot in a medium or large blood vessel and multiple clots that disrupt regional blood flow. It may cause deadly cardiovascular diseases, namely, cerebral ischemia, acute ischemic heart disease, and acute pulmonary embolism. Circulatory diseases, including thrombosis, are one of the leading causes of death worldwide, claiming 17.5 million lives per year [1].

In Russia, approximately one million people die each year of cardiovascular diseases, accounting for approximately half of the mortality rate [2]. Globally, thrombosis is responsible for approximately one-fourth of all deaths [3]. Prevention, early detection, and treatment of thrombotic diseases and complications remain urgent medical, scientific, and social issues [4, 5].

Cellular and molecular mechanisms of thrombosis: Virchow's triad

The pathogenetic triad that underlies thrombosis includes blood composition changes due to hypercoagulability, vascular wall damage, and slowing of blood flow (Fig. 1). This classical concept, known as Virchow's triad [6], has retained its significance to the present day, albeit with significant additions [7]. Contemporary concepts indicate that vascular wall damage plays a critical role in thrombosis. Slowing of blood flow is crucial for venous thrombosis, whereas arterial thrombosis is associated with high blood flow velocity [8].

Mechanical or biological damage to the vascular wall is the primary cause of thrombosis

Normally, intact endothelial cells lining the inner surface of blood vessels have antithrombotic

*For correspondence: rafael.khismatullin@gmail.com

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properties because of several mechanisms. The endothelial monolayer acts as a physical barrier between the thrombogenic subendothelium and platelets and proteins of the blood coagulation system. Intact endothelial cells secrete prostacyclin and nitric oxide, which inhibit platelet aggregation [9, 10]. Furthermore, endothelial cells synthesize physiological anticoagulants such as thrombomodulin and protein S [11] and secrete t-PA, a fibrinolytic enzyme and tissue plasminogen activator, into the blood [12].

Under pathological conditions, the endothelium can be mechanically damaged by trauma [13] or biologically activated in atherosclerosis or by viruses, endotoxins, or inflammatory cytokines (e.g., interleukin-1β, tumor necrosis factor α, and interferon γ) [14] produced following infection and systemic or local inflammation. The damaged or activated endothelium, which is normally neutral or antithrombotic, becomes a prothrombotic surface upon contact with blood.

Under pathological conditions, the endothelium may express tissue factor, which triggers the coagulation cascade via the extrinsic pathway [15, 16]. Moreover, it may secrete PAI-1 protein, which inhibits fibrinolysis [17]. Vascular damage results in the exposure of subendothelial components of the extracellular matrix, namely, collagen and von Willebrand factor, which mediate platelet adhesion and activation. Stimulated or damaged endothelium expresses cell adhesion molecules, such as E-selectin, ICAM-1, and P-selectin, which increase its adhesiveness to leukocytes [18–20]. Additionally, activated endothelial cells express and secrete chemotactic cytokines, such as interleukin-8 and platelet activation factor, which stimulate the accumulation, activation, and adhesion of leukocytes to the vascular wall [21, 22]. These reactions initiate the development of inflammatory thrombosis or immunothrombosis.

Thus, in the resting state, the vascular wall maintains blood in the liquid state. However, in response to damage, whether mechanical or biological, it acquires prothrombotic properties. These properties arose because of phylogenesis, an evolutionary defense mechanism initially aimed at hemostatic blood clot formation to stop bleeding. In numerous pathological conditions that alter the structure and functional state of the endothelium, adaptive hemostatic reactions can become pathogenic and contribute to occlusive thrombi formation.

Endothelial dysfunction and/or disruption of the endothelial cell monolayer underlie numerous cardiovascular diseases and thrombotic complications. This is most commonly associated with inflammatory and/or atherosclerotic vascular wall damage.

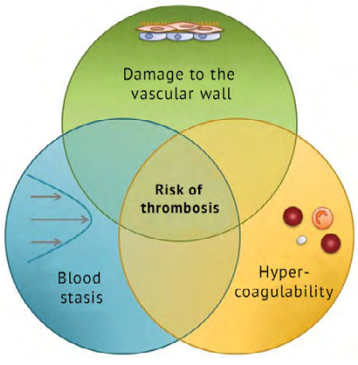

Fig. 1. Virchow's triad and venous thrombosis

Changes in the cellular and protein composition of blood causing hypercoagulability

Blood coagulation can be decreased (hypocoagulability) or increased (hypercoagulability) because of regulatory, pharmacologic, or pathologic influences. Laboratory tests and/or clinical manifestations can detect hypocoagulability and hypercoagulability by hemorrhagic syndrome or thrombosis, respectively. Both hypocoagulability and hypercoagulability can be inherited or acquired. Hypercoagulability is a significant mechanism in thrombosis of various etiologies.

The term "thrombophilia" refers to *hereditary hypercoagulability,* which predisposes individuals to thrombosis due to congenital hypercoagulability. Thrombophilia is often caused by genetic polymorphisms or mutations that decrease the concentration and/or activity of physiological anticoagulants that inhibit thrombin formation at the site of vascular injury [23]. An association between antithrombin III deficiency and an increased risk of venous thrombosis has been established [24]. Antithrombin III deficiency is caused by over 250 mutations in the *SERPINC1* gene on chromosome 1q [25]. Although the prevalence of antithrombin III deficiency is relatively low in the general population (0.02%–0.2%), it has been identified in 1% of patients with venous thromboembolic complications [26].

Activated protein C, in complex with its cofactor protein S, is a physiological anticoagulant that inhibits thrombin formation by cleaving factors Va and VIIIa [27]. Therefore, a deficiency in protein C and/or protein S is associated with a high risk of venous thrombosis due to thrombinemia [28, 29]. Congenital protein C and S deficiency affects 0.1%–0.4% of the general population [30] because of over 200 mutations in the *PROC* and *PROS1* genes on chromosomes 2 and 3, respectively.

In addition to hereditary defects in physiological anticoagulants, genetic abnormalities in blood coagulation factors are crucial in the development

of thrombophilia. For instance, the Leiden mutation of factor V is characterized by a point mutation (G1691A) in exon 10 of the *F5* gene [31]. This genetic alteration results in the absence of a sensitive region (Arg506) in protein factor V, which is responsible for inhibiting thrombin formation when activated protein C is present. This absence significantly weakens the protein's ability to inhibit thrombin formation, leading to a 7-fold increase in venous thrombosis risk in heterozygous individuals and up to an 80-fold increase in homozygous individuals [26, 33].

The G20210A prothrombin gene polymorphism, along with the Leiden mutation, is predominantly found in the European population. This polymorphism increases prothrombin levels in plasma and is associated with an increased venous thrombosis risk [34].

A point mutation in the *F9* gene (R338L) results in a significant elevation of factor IX levels in the blood, which is also associated with a higher risk of thrombotic complications [35].

Acquired hypercoagulability is more prevalent than hereditary thrombophilias and can be due to infections, surgical interventions, medications, poisoning, reptile bites, etc. [36]. Hypercoagulability associated with oral contraceptives or hyperestrogenemia during pregnancy results from increased clotting factor synthesis and decreased antithrombin III formation [37]. Malignant neoplasms contribute to hypercoagulability because of the ability of tumor cells to express tissue factors and secrete other procoagulants [38].

Antiphospholipid syndrome and heparin-induced thrombocytopenia are autoimmune pathological conditions that alter blood composition and can cause both venous and arterial thrombosis [36]. Thrombosis in patients with antiphospholipid syndrome is caused by the binding of circulating autoantibodies to phospholipids on the cell membranes of endothelial cells and platelets, leading to their activation. Furthermore, antiphospholipid antibodies bind to protein epitopes that bind to phospholipids, such as β_2-glycoprotein and annexin V. This binding results in increased platelet activation, endothelial cell stimulation, and subsequent procoagulant production [39].

Up to 5% of patients treated with unfractionated heparin develop immune heparin-induced thrombocytopenia. This condition is characterized by the production of autoantibodies in response to the formation of complexes between negatively charged heparin and positively charged platelet factor 4 (PF4), a protein secreted by activated platelets. Autoantibodies bind to PF4 and glycosaminoglycan complexes on the surface of monocytes, endothelial cells, and platelets, resulting in hypercoagulability. Platelet immune activation can cause their consumption as part of a clot or increased elimination from the bloodstream, leading to thrombocytopenia. Heparin-induced thrombocytopenia results in a (pro)thrombotic state due to multiple immune activations of blood cells and endothelial cells, which persists even after heparin withdrawal [39].

In prothrombotic states, activated platelets secrete bioactive molecules that contribute to thrombus formation (adenosine diphosphate and thromboxane A_2) and inflammation (proinflammatory cytokines) [40]. Platelet activation causes membrane reorganization, phosphatidylserine externalization, and transformation of the membrane into a matrix for assembling blood coagulation enzyme complexes [41].

Simultaneously, αIIbβ3 integrin is expressed and activated, ensuring fibrinogen binding and platelet aggregation, whereas P-selectin is translocated from α-granules to the platelet surface. Increased P-selectin expression during hypercoagulability is crucial in the formation of platelet–leukocyte aggregates, primarily because of the binding of P-selectin to a specific protein ligand (PSGL-1) on the leukocyte surface [43–45]. Platelet–leukocyte aggregates in the bloodstream are a significant indicator of inflammatory prothrombotic conditions of various etiologies [46].

Role of blood flow disorders in the development of thrombosis

The relationship among hemodynamic disorders, blood coagulation, and thrombosis is the least studied aspect of Virchow's triad. However, turbulence and blood stasis are the leading pathogenetic factors in venous thrombosis [7]. Blood flow stasis and turbulence have various pathophysiological consequences, and their combination predisposes patients to intravascular fibrin formation.

First, blood flow disturbances stimulate endothelial cell activation, increasing their procoagulant activity, which subsequently leads to tissue factor expression [47]. The expression of the tissue factor gene is caused by increased transcription in response to the activation of intracellular signaling systems triggered by mechanosensitive structures of the cell membrane [48], such as mechanosensitive cation channels, namely, Piezo1 [49]. Prolonged immobilization can cause slow blood flow in the veins, decreasing the expression of FOXC2 and PROX1 transcription factors by perivalvular endotheliocytes. This then causes increased local concentration of prothrombotic proteins, such as the von Willebrand factor, P-selectin, and ICAM-1,

and decreased levels of anticoagulants such as protein C and tissue factor pathway inhibitor [50].

Second, blood stasis creates conditions for prolonged contact of platelets and leukocytes with the endothelium. This interaction is enhanced by the degree of margination and the concentration of platelets and leukocytes near the vascular wall, which increase significantly when blood flow slows down [51, 52].

Furthermore, in cases of blood stasis, platelets may be further activated by substances released from aggregated and deformed erythrocytes, specifically adenosine triphosphate (ATP) and ADP [52]. Blood stasis, which induces local hypoxia, intensifies inflammatory endothelial damage. This damage prompts endothelial cells to express tissue factors in response to the effects of proinflammatory cytokines, such as interleukin-1 [53].

Additionally, during hypoxia-induced stasis, endothelial cells overexpress P-selectin molecules, which promote leukocyte adhesion via PSGL-1 protein [24]. Leukocyte adhesion to the vascular wall during inflammation and the interaction of leukocytes with erythrocytes in conditions of blood stasis can increase and inhibit resistance to blood flow [54]. These circumstances together predispose patients to thrombus formation and aggravate thrombotic occlusion [55].

Blood stasis affects cells, slows down the removal of activated clotting factors from the site of vascular wall damage, and prevents physiological anticoagulant influx. Reducing blood flow velocity increases the local concentration of fibrin monomers and soluble oligomers, leading to the intensive formation of protofibrils and fibrin fibers [56]. Blood stasis is a significant factor in the development of venous thrombosis; however, it is not applicable to arterial thrombi as part of Virchow's triad [8]. Arteries, particularly those with stenosis, have considerably higher blood flow velocity than veins [55, 57–61].

Thrombogenic mechanisms related to high blood flow velocity are primarily associated with the hydrodynamic activation of platelets through mechanosensitive calcium channels [62] and activation and mechanical unfolding of large von Willebrand factor molecules on the endothelial surface and in the exposed subendothelium [63]. Complex functional relationships exist between hemodynamic disorders and prothrombotic changes in blood cells, endothelial cells, and blood protein composition. These relationships include the local concentration of pro- and anticoagulants, and along with other components of Virchow's triad, they create the pathogenetic basis of arterial and venous thrombosis.

Arterial and venous thrombi: thromboembolism

Arterial thrombosis

The occurrence and severity of clinical manifestations of *arterial thrombosis* depend on the artery's innate predisposition to thrombosis, including its hemodynamic features, diameter, tortuosity, presence of bifurcations, and blood flow turbulence, and the hypoxic resistance of the tissues supplied by the corresponding artery [60]. The coronary, cerebral, and lower extremity arteries are the most vulnerable to acute arterial thrombosis and have clinically significant consequences. Thrombosis in coronary arteries can cause myocardial infarction, whereas thrombosis in cerebral arteries can lead to ischemic cerebral infarction or stroke. Thrombosis in the lower extremity arteries may cause gangrene.

Arterial thrombosis is commonly caused by atherosclerosis, a chronic pathological process that affects large and medium-sized arteries. It is characterized by the infiltration of lipoproteins into the arterial wall and the accumulation of monocytes and macrophages, which together form an atherosclerotic plaque that protrudes into the vessel lumen. When the fibrous capsule of an atherosclerotic plaque is eroded or ruptured, blood comes into contact with procoagulant subendothelial structures (tissue factor-expressing macrophages, collagen, etc.), resulting in fibrin deposition and platelet adhesion, activation, and aggregation.

Platelet adhesion. When the integrity of the endothelial layer is disrupted, platelet adhesion receptors bind to subendothelial proteins such as collagen, von Willebrand factor, laminin, fibronectin, and thrombospondin [64]. Platelets have two protein receptors for collagen: glycoprotein VI and integrin $\alpha_2\beta_1$ [65]. In addition to direct interaction with collagen, platelet adhesion to collagen is mediated by the von Willebrand factor [66], whose active sites bind to both subendothelial collagen and platelet GPIb receptors on the resting platelet surface [41, 67].

Platelet activation is accompanied by the release of biologically active substances from platelet alpha and gamma granules. The secretion of δ-granules plays a role in hemostasis, providing a positive feedback mechanism, as the ADP released from these granules stimulates new platelets via the purinergic receptors P2Y12 and P2Y1 [68].

Platelet aggregation. Platelets adhere to each other (aggregation) after their activation and adhesion under the action of physiological inducers such as ADP, thrombin, adrenaline, and thromboxane A_2. The key mechanism in platelet aggregation is the activation of the integrin receptor αIIbβ3, the conformational change of which leads to its binding to the von Willebrand factor and to soluble

fibrinogen, which acts as a bridge between platelets [69, 70]. Recent data revealed a crucial role of soluble oligomeric fibrin precursors in platelet aggregation [71]. Aggregation is mediated by molecules of the active integrin αIIbβ3 and fibrin (fibrinogen) that adhere platelets together.

The growing fibrin–platelet thrombus aggravates vascular stenosis, leading to increased local blood flow velocity [72], which becomes an additional prothrombotic factor because of hydrodynamic unfolding and activation of von Willebrand factor molecules and additional mechanical activation of platelets and endotheliocytes. These and other mechanisms underlie the atherothrombosis of arteries [7]. Atherothrombosis can be complicated by the dislodgement of thrombotic and atherogenic masses, leading to local or systemic embolism.

Venous thrombosis and thromboembolism

Venous thrombosis commonly occurs in the deep veins of the lower extremities and pelvis [73]. It is dangerous not for its own sake, but because if the thrombus is completely or partially dislodged, thrombotic masses can be carried by the bloodstream and cause occlusion of the pulmonary artery and its branches. Pulmonary embolism is a life-threatening venous thrombosis complication that can lead to sudden death due to acute cardiopulmonary failure and cardiopulmonary reflex. In contrast to arterial thrombosis, the severity of the clinical course of venous thrombosis is not associated with tissue hypoxia/ischemia in the area of vascularization but with thromboembolic complications.

Pathogenetically, venous thrombosis differs from arterial thrombosis in that it is due to hypercoagulability and can develop with physically intact endothelium because of local inflammation and/or blood stasis during prolonged immobilization.

Thrombus composition and dynamic properties

Different conditions and mechanisms of thrombus formation result in significant differences in structure, composition, and properties depending on the localization (artery or vein), age of formation, and blood composition [74, 75]. Arterial thrombi are believed to mainly consist of platelet aggregates ("white" thrombi), whereas venous thrombi ("red" thrombi) are composed of fibrin and erythrocytes [76].

However, in recent years, with the development of techniques for extraction and visualization of vestibular thrombi, this outdated view has been revised [60]. Recent publications [77–79], including our studies [80, 81], showed that erythrocytes can occupy a larger part of the volume of both venous and arterial thrombi (up to ≈80% of the volume fraction), whereas the platelet content can be only ≈10% [82]. In the complex pathogenesis of thrombosis, one of the least studied processes is the remodeling or compression (contraction, retraction) of the thrombus by the contraction of activated platelets, which may influence the course and outcome of thrombosis [83, 84]. Activated platelets contract because of the ATP-dependent interaction of actin and nonmuscle myosin IIA fibers with subsequent cytoskeletal reorganization [85]. Intracellular contractile forces of platelets are transmitted to the fibrin network through focal adhesive contacts (mechanotransduction), resulting in mechanical fibrin compaction and "squeezing" of serum [86]. Contraction (retraction) of the thrombus is accompanied by decreased volume and mass of the clot and reorganization of its structure.

Two structural consequences and features of contraction are most obvious [74, 87]: redistribution of fibrin–platelet aggregates from the interior to the periphery of the thrombus and concentration of densely packed erythrocytes in the "core" of the thrombus and change in the shape of the erythrocytes from biconcave to polyhedral.

Deformed erythrocytes formed inside contracted blood clots and thrombi are called polyhedrocytes or piezocytes [88].

Changes in the structure of blood clots during contraction make them more rigid and resistant to mechanical action. Dense packing of polyhedrocytes and their concentration in the central part reduce clot porosity and permeability, including for fibrinolytic enzymes, which is of great pathophysiological and clinical importance in resistance to proteolysis. According to existing data, the pathogenetic significance of thrombus contraction is determined by the effect on thrombus size and obstruction [89], changes in permeability and sensitivity to fibrinolysis [90], and influence on mechanical properties and the risk of embolization, specifically detachment [91].

Thrombus contraction may be impaired in various clinical pathologies, particularly those associated with the risk of thrombotic complications [92–95]. The most common mechanisms of impaired blood clot contraction are platelet dysfunction and changes in the protein and cellular composition of blood. Thrombocytopenia and hyperfibrinogenemia are among the leading causes [75, 96].

In vivo contraction of blood clots and thrombi may be critical in thrombosis and a potential therapeutic target. *In vitro* contraction kinetics serve as a diagnostic and prognostic laboratory test for threatening and ongoing thrombosis [89, 92, 93, 95].

Conclusions

Thrombosis is a severe hemostasis disorder that underlies deadly diseases, such as ischemic stroke, acute myocardial infarction, and pulmonary embolism. Thrombotic conditions have diverse etiologies and can complicate the course of cardiovascular diseases, inflammatory processes (primarily autoimmune and infectious), dysmetabolic conditions, oncological diseases, trauma, and others. Thrombosis is caused by complex pathogenetic mechanisms, which can be reduced to three main factors: vascular wall damage, changes in the cellular and protein composition of the blood, and systemic, regional, and local hemodynamics disruptions.

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Author details

Rafael R. Khismatullin, M.D., Cand. Sci. (Med.), Senior Lecturer, Depart. of Morphology and General Pathology, Institute of Fundamental Medicine and Biology; Pathologist, Pathoanatomical Depart., Medical and Sanitary Unit, Kazan (Volga Region) Federal University, Kazan, Russia; rafael.khismatullin@gmail.com; ORCID: http://orcid.org/0000-0001-8597-811X

Rustem I. Litvinov, M.D., D. Sci. (Med.), Prof., Senior Research Investigator, Depart. of Cell and Developmental Biology, University of Pennsylvania School of Medicine, Philadelphia, USA; rustempa@gmail.com; ORCID: http://orcid.org/0000-0003-0643-1496