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Venous thrombosis unchained: Pandora's box of noninflammatory mechanisms

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Venous thromboembolism, which includes deep vein thrombosis (DVT) and pulmonary embolism, represents a complex pathological process extending far beyond inflammatory mechanisms. This review comprehensively examines the multifaceted noninflammatory mechanisms underlying thrombosis development, integrating insights from molecular, physiological, and systemic levels. Blood flow dynamics and endothelial function are known to be critical regulators of thrombus development. Platelets and microparticles play important roles beyond conventional inflammatory responses, actively contributing to thrombus formation through intricate molecular interactions. Metabolic syndrome and insulin resistance are associated with thrombotic risk, demonstrating the complex interplay between metabolic disorders and DVT. Certain genetic mutations also predispose individuals to venous thrombosis. Emerging research has discovered the essential role of previously underappreciated factors such as products of gut microbiota or endothelial glycocalyx modifications. Molecular regulators such as microRNAs and hormonal disbalance further illustrate the complex mechanisms of venous thrombosis. Interestingly, circadian rhythms exhibit certain influence on thrombotic potential, introducing chronobiology as an emerging variable affecting the risk of thrombosis. On the basis of these insights, future therapeutic strategies may include various interventions targeting or at least considering metabolic, molecular, and systemic noninflammatory factors. Potential approaches include personalized risk stratification, microbiome modulation, endothelial protection approaches, and chronotherapy-based therapeutic modalities, which would ensure more efficient and safe thrombosis management.

Introduction

Venous thromboembolism (VTE), encompassing deep vein thrombosis (DVT) and pulmonary embolism, remains a significant global health burden affecting millions annually.[1,2] Although the role of inflammatory processes in thrombosis is well-documented,[3] there is growing recognition of crucial noninflammatory mechanisms in VTE pathogenesis.

The hemodynamics of venous valve pockets creates areas of disturbed flow and stasis that concentrate procoagulant factors, platelets, and microparticles (MPs) while subjecting the local endothelium to mechanical stresses. This convergence of factors at valve sites explains why systemic risk factors such as metabolic syndrome (MetS), circadian variations, and altered glycocalyx function manifest as

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localized rather than systemic thrombosis. However, there seems to be no direct experimental (in vivo) or clinical evidence that thrombi originate in valvular pockets, and it cannot therefore be ruled out that thrombus formation starts upstream of the valve and continues downstream reaching the valve, which prevents its further development.

Virchow triad, which includes blood stasis, hypercoagulability, and endothelial injury, remains fundamental to understanding venous thrombosis (VT).[4] However, advanced molecular biology, genetics, and imaging techniques have revealed complex noninflammatory contributors to thrombosis, including altered blood flow dynamics, changes in blood components, and vascular endothelial modifications.

Current research focuses on how hemodynamics, endothelial dysfunction, and genetic factors contribute to thrombotic risk through pathways relatively independent of inflammation. This expanding understanding creates opportunities for developing targeted therapies beyond traditional approaches, which do not address the underlying noninflammatory processes. Current preclinical data have mostly been obtained using the model of inferior vena cava (IVC) ligation in rodents. These data should be interpreted with caution given substantial differences between mice and humans. For example, the murine IVC does not contain valves or have vasa vasorum, and mice operate in a horizontal plane, making the effects of gravity less significant than in humans.

This review examines the current knowledge of noninflammatory mechanisms in VTE, exploring the interactions between hemodynamics, blood components, endothelial function, and genetic factors, and provides an insight into how MetS, the gut microbiota, and circadian rhythms contribute to VTE. Understanding these mechanisms is crucial for advancing both our fundamental knowledge and the development of novel diagnostic tools and therapeutic strategies.

Hemodynamics in VT

Blood flow dynamics significantly influence VT development. Venous stasis allows for the accumulation of procoagulant factors, leukocytes, and platelets, which are essential for thrombus formation.[5] Altered shear stress activates platelets through glycoprotein Ibα (GPIbα) and phosphoinositide 3-kinase/protein kinase B signaling, followed by the initiation of coagulation.[6-8] It was recently demonstrated using fluidic devices that pathological flow conditions in the valvular pockets can induce the formation of cleavage-resistant platelet–von Willebrand factor (VWF) complexes, which mediate neutrophil recruitment and the formation of prothrombotic neutrophil extracellular traps. This provides in vitro evidence that the valvular pockets could serve as launchpads for thrombus initiation.[9] The role of different hemodynamic patterns was demonstrated in mouse models in which venous side branch closure affected thrombosis prevalence.[10] Stasis-induced thrombosis operates through multiple mechanisms, for example, increasing tissue factor (TF) expression in monocytes and smooth muscle cells.[11]

The endothelium plays a crucial regulatory role in hemostasis through the production of antithrombotic factors such as nitric oxide (NO) and prostacyclin (Figure 1A).[12] Venous stasis alters endothelial function (Figure 1B), downregulating protective factors, and increasing the expression of adhesion molecules such as VWF and P-selectin.[13,14]

Endothelial cells (ECs) use various mechanosensors (receptors, caveolae, ion channels, and EC cilia) to convert mechanical signals into biochemical responses. For example, the mechanosensor PECAM-1 (platelet EC adhesion molecule 1) was shown to affect thrombus size in mouse models.[15] The mechanosensitive ion channel PIEZO1 affects endothelial response to altered blood flow.[16] Under pathological flow conditions, altered PIEZO1 activity may predispose to thrombosis, in particular via reduced NO production.

The relationship between stasis and VT warrants careful consideration because of the differences between experimental models and human pathophysiology. Although complete stasis is commonly used in murine models, human VT typically involves disturbed flow, particularly in the context of valve dysfunction. This distinction has implications for understanding disease mechanisms and therapeutic approaches. The role of P-selectin expression and VWF release in thrombosis also requires specific context. Although these molecules play important roles in DVT, their presence alone is insufficient to trigger thrombosis. This concept is illustrated by the clinical use of desmopressin (DDAVP), which induces acute VWF release and P-selectin expression but rarely causes thrombosis. Therefore, additional factors or specific conditions must converge with these molecules to promote thrombosis, highlighting the complexity of thrombotic processes in different clinical contexts.

Prevention strategies focusing on improving venous flow and reducing stasis, such as mechanical compression devices and early postsurgery mobilization, have proven effective in reducing thrombosis.[17] Ongoing research into blood flow dynamics and endothelial function will continue to inform new therapeutic approaches.

Platelets and MPs

Platelets underlie the development of arterial thrombi on the surface of ruptured atherosclerotic plaques and activate the coagulation cascade through expressing phosphatidylserine (PS). Although the endothelial layer is still present in DVT, aspirin is considered a promising therapeutic in patients with thrombosis.[18,19]

Platelet depletion strongly reduces DVT in the IVC ligation model in mice.[14] Targeting one of the major platelet receptors, integrin $\alpha_{IIb}\beta_3$, by m-tirofiban, inhibits experimental DVT without increasing bleeding.[20] Platelet signaling pathways (eg, RAS-like guanosine triphosphatases) implicated in DVT have recently been identified.[21] Interestingly, chronic overproduction of erythropoietin, leading to elevated red blood cell counts, exacerbates experimental DVT despite reduced platelet count in these mice.[22] A high hematocrit level also results in the development of thrombi enriched with red blood cells, but with a reduced platelet and fibrin content.

Recently, elevated amounts of procoagulant platelets, a subpopulation of platelets expressing high levels of PS, were reported in patients with VTE and mice after IVC stenosis.[23] Interestingly, methazolamide, which reduces platelet procoagulant activity, also suppresses experimental DVT without affecting normal hemostasis. Therefore, procoagulant platelets represent a promising antithrombotic target.

MPs are released from outer membranes of many cell types such as platelets, leukocytes, and ECs.[24,25] Regardless of the origin,

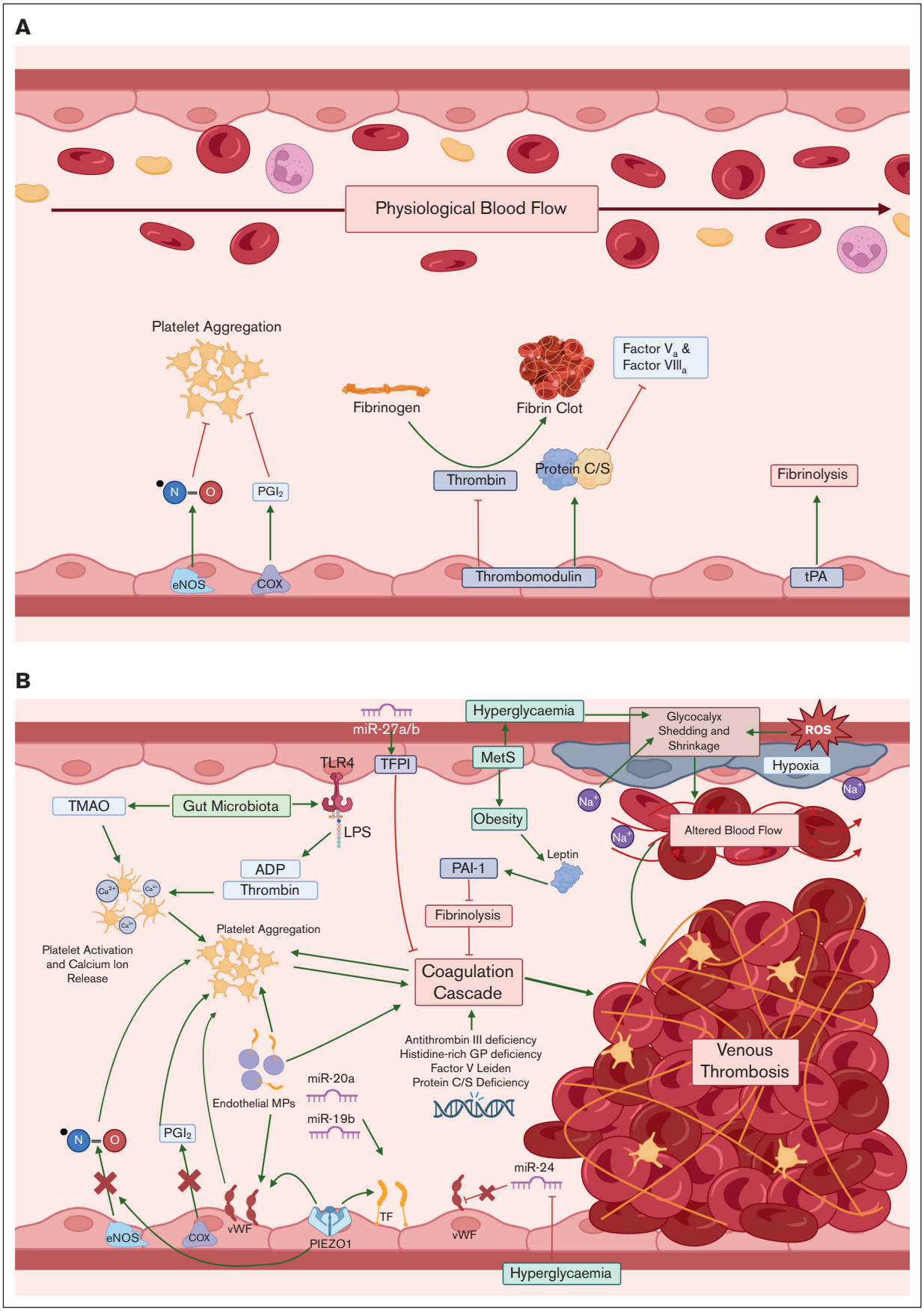

Figure 1.

MPs have procoagulant properties and contribute to thrombosis through providing the surface for activating coagulation factors.[26]

Endothelial MPs are released in response to injury or apoptosis, and promote thrombus formation by exposing PS and TF, expressed on the MP surface.[27,28] TF activates the extrinsic pathway of the cascade, leading to the activation of factor X,[29] which then complexes with factor Va, leading to thrombin generation. Thrombin amplifies its own production via positive feedback and contributes to the final fibrin clot through hydrolyzing fibrinogen into fibrin, which is crosslinked by factor XIIIa, forming the insoluble clot. In the venous valves, where blood flow is naturally slower, MPs can accumulate and contribute to coagulation. The prothrombotic potential of endothelial MPs is significantly enhanced under conditions where TF is present. However, TF expression typically requires vascular injury or monocyte preactivation. In the absence of TF, the coagulative capacity of endothelial MPs is less pronounced, raising questions about their stand-alone impact on thrombus formation. Thus, the contribution of EC-derived MPs to DVT likely depends on local conditions that may facilitate their interaction with coagulation pathways. Further research is needed to understand the specific circumstances under which these MPs might exert a prothrombotic effect in veins.

Endothelial MPs can further contribute to thrombosis through releasing VWF.[30] VWF mediates platelet aggregation and supports clotting through protecting factor VIII from degradation. Mice deficient in VWF or subjected to blockade of VWF-GPIbα interaction are protected from flow stagnation–induced thrombosis, suggesting that VWF initiates thrombus formation and mediates its progression and stability.[13] Recently, it was shown that endothelial MPs enriched in protein disulfide isomerase promote DVT in mice via interaction with platelet αIIbβ3 and glucose-regulated protein 94.[31]

Platelet and megakaryocyte MPs are the most abundant MP type in the circulation.[32] Platelet MPs are released from platelets after activation or apoptosis and may originate from either plasma membranes or α-granules.[33,34] Platelet MPs have a greater procoagulant activity than activated platelets themselves, largely credited to the abundant presence of PS on their surfaces.

The number of circulating MPs of different origins increases in patients with DVT.[35] The presence of MPs can be viewed as both a causative agent and consequential product of thrombosis. Although the usefulness of MPs as independent and specific markers of DVT is still unclear, measuring their levels in blood may aid in evaluating the severity or risk of thrombosis.[36]

The role of microbiota in VTE

The human microbiota, consisting of bacteria, viruses, fungi, and archaea, is most densely populated in the gut but also exists in other areas such as the skin, mouth, and lungs. Dysbiosis, an imbalance in the microbiome, is implicated in multiple diseases, from inflammatory bowel disease to MetS and cardiovascular disorders.[37,38] The link between microbiota and coagulation suggests a potential involvement in thrombotic events.

Several studies have suggested a potential association between gut microbiota and VTE, implying that changes in the gut microbiome composition could impact VTE risk. A causal relationship between gut microbiota, metabolites, and VTE has been suggested.[39,40]

The gut microbiota produces a variety of metabolites such as trimethylamine N-oxide (TMAO) through the metabolism of dietary components including choline, phosphatidylcholine, and L-carnitine. TMAO levels can vary across different populations. For instance, a study comparing young, middle-aged, and older adults found mean TMAO levels of ~2.5, 4.8, and 10 μM, respectively, suggesting that TMAO levels increase with age.[41]

TMAO has been shown to enhance platelet hyperactivity through increasing cytoplasmic calcium release in platelets, thus predisposing to thrombosis.[42-44] This effect is compounded by the role of TMAO in promoting macrophage foam cell formation and enhancing lipid accumulation, which contributes to atherosclerosis, a condition that can exacerbate DVT.[45,46] The gut microbiota can produce lipopolysaccharides (LPSs), which can activate ECs and platelets, thereby initiating the coagulation cascade.[47]

Dysbiosis can lead to increased gut permeability, facilitating the translocation of LPSs into the bloodstream.[48] Dysbiosis can underlie a twofold increase in the levels of circulating LPSs (from ~45 to ~75 ng/mL) in patients with diabetes with chronic kidney disease.[49] The role of gut microbiota LPS was recently demonstrated in experimental DVT.[50] LPSs stimulate platelet activation and secretion via Toll-like receptor 4–dependent pathways, and enhances platelet aggregation induced by classical agonists such as thrombin.[51]

TMAO and other microbial metabolites may work synergistically to promote a hypercoagulable state, increasing DVT risk. However, some studies do not report a direct association between TMAO levels and thrombotic risk in certain populations, suggesting a complex interplay between TMAO, gut microbiota, and individual patient factors.[52,53] Moreover, TMAO is associated with conditions such as heart failure and chronic kidney disease, which are known

Figure 1. Noninflammatory mechanisms of VT. (A) In healthy veins and physiological blood flow, VWF and TF are not expressed, tPA promotes fibrinolysis, and thrombomodulin blocks the coagulation cascade; NO and PGI_2 block platelet aggregation. (B) A schematic overview of noninflammatory mechanisms that contribute to VT. Alterations in blood flow, resulting in local hypoxia of the venous wall, are detected by the mechanosensors such as PIEZO-1, which increases the expression of TF and VWF on EC surfaces, leading to cell recruitment and activation of the coagulation cascade. miR-20a and miR-19b regulate the TF expression, and MPs contribute to thrombus development through VWF release. miR-27a/b regulates the expression of TFPI, which inhibits the initiation of coagulation. PIEZO-1 can further enhance platelet aggregation through blocking NO production. Hyperglycemia represses miR-24, leading to increased VWF expression, and induces shedding and shrinkage of the EG, resulting in endothelial activation. Leptin, a hormone whose levels increase in obesity, increases levels of PAI-1 to inhibit fibrinolysis. The gut microbiota produces TMAO, which enhances the coagulation cascade and platelet aggregation. The gut microbiota also produces LPS that interacts with TLR4 on the endothelial surface and further promotes a hypercoagulable state. The resultant thrombus is erythrocyte and fibrin rich with the presence of certain amounts of platelets. ADP, adenosine diphosphate; eNOS, endothelial nitric oxide synthase; PAI-1, plasminogen activator inhibitor-1; PGI_2, prostacyclin; ROS, reactive oxygen species; TFPI, tissue factor pathway inhibitor; TLR4, Toll-like receptor 4; tPA, tissue plasminogen activator.

risk factors for thromboembolic events.[54,55] The mechanisms by which TMAO influences these outcomes include oxidative stress,[56] which can further impair endothelial function and contribute to thrombosis. Thus, gut microbiota plays a multifaceted role in regulating DVT through mechanisms involving platelet activation, exacerbation of comorbidities, and interactions with other microbial metabolites.

MetS and insulin resistance in VTE

MetS is a cluster of interrelated metabolic abnormalities that significantly increase the risk of cardiovascular diseases, including VTE. For example, a 2007 case-control study reported that individuals with MetS had a twofold increased risk of VTE compared with those without the syndrome (odds ratio, 2.1; 95% confidence interval, 1.2-3.7).[57] A patient-level meta-analysis of 6 case-control studies found that MetS was independently associated with unprovoked VTE (odds ratio, 1.91; 95% confidence interval, 1.57-2.33).[58]

Central to the pathogenesis of MetS is insulin resistance, which is characterized by a reduced response of peripheral tissues to insulin.[59] The interplay between these factors not only exacerbates the risk of developing type 2 diabetes but also contributes to a prothrombotic state. Insulin resistance is often associated with increased body mass index, which is a risk factor for VTE. As body fat content increases, the likelihood of developing insulin resistance also rises, thereby heightening the risk of VTE.[60] The compensatory mechanisms in insulin-resistant individuals may lead to altered coagulation profiles, which can lead to thrombotic events.[61] The role of adipokines, such as leptin and adiponectin, in the context of MetS and insulin resistance is crucial. Leptin, which is elevated in obesity, enhances thrombotic risk by supporting platelet aggregation and increasing the levels of plasminogen activator inhibitor-1 in ECs.[62,63] However, some studies do not confirm the causal role of leptin in the pathogenesis of VTE.[64] In contrast to leptin, adiponectin, which is typically reduced in insulin-resistant states, exhibits antithrombotic properties, suggesting that its lower levels may contribute to VTE.[65] Increased levels of leptin and decreased levels of adiponectin can also serve as predictors of postthrombotic syndrome, a frequent complication of VTE, in patients with and without obesity.[66] The association between insulin resistance and VTE has been supported by various epidemiological studies. For instance, a population-based cohort study found that individuals with higher insulin resistance exhibited an increased risk of VTE independently of traditional risk factors.[67] This suggests that insulin resistance may be a risk factor for VTE, potentially through mechanisms involving endothelial dysfunction and altered hemostatic balance.[61] The relationship between MetS and VTE is also supported by mechanistic insights. Insulin resistance was shown to induce a state of chronic low-grade inflammation, which is a recognized contributor to both MetS and thrombotic events.[68] This milieu associated with insulin resistance can lead to endothelial malfunction, a critical factor in VTE pathogenesis.[67] Nonalcoholic fatty liver disease, a frequent consequence of insulin resistance, has been linked to increased thrombotic risk, further illustrating the link between these conditions.[69,70] In summary, the interplay between MetS, insulin resistance, and the pathogenesis of VTE is multifaceted. Insulin resistance not only serves as a hallmark of MetS but also contributes to a prothrombotic state through dysregulation of adipokines and endothelial dysfunction. Understanding these relationships is crucial for developing targeted interventions to reduce the risk of VTE in individuals with MetS.

Endothelium and EG

The vascular endothelial glycocalyx (EG) is a negatively charged, carbohydrate-rich complex of proteoglycans, glycosaminoglycans, and GPs on the apical surface of ECs.[71,72] The EG supports the maintenance of vascular integrity and tone, protects the vessel from chemical injury and microbes,[71] and regulates the blood vessel microenvironment.[73] The EG contributes to maintaining endothelial integrity and protecting against thrombosis by mediating flow-induced NO release. Therefore, the loss of EG integrity can enhance thrombosis through endothelial malfunction.

The EG shedding can be mediated by certain enzymes or reactive oxygen species. Dietary salt intake can be implicated in stiffness and shrinkage of the EG. Sodium influx reduces EG buffering capacity and causes oxidative stress. EG and erythrocytes should normally repel each other, to prevent friction and to ensure smooth, uninterrupted, blood flow. However, damage to the EG caused by the excess sodium combined with the reduction of its negative charge permit erythrocytes to interact with the endothelium, causing its activation and thrombosis.[74]

Diabetes mellitus is a known risk factor for VT.[75] Hyperglycemia is destructive to the EG and causes dysfunction by attenuating the synthesis and promoting the shedding of hyaluronan from the EG,[76] which is vital for endothelial quiescence.[77] In hyperglycemia, amplified reactive oxygen species production, which activates hyaluronidase-1, degrades and directly depolymerizes hyaluronan.[77,78] Hyperglycemia also induces robust EG shrinking. All these effects can create a milieu predisposing to VT in patients with diabetes mellitus.

Another important mechanism regulating thrombosis is the endothelial cyclooxygenase (COX)–prostacyclin axis. COX enzymes in ECs convert arachidonic acid into prostacyclin, a potent vasodilator and platelet inhibitor. A disruption in this axis can promote a prothrombotic state, favoring clot formation in the venous system. This is confirmed by increased DVT in COX2-deficient mice[79] and elevated risk of DVT after pharmacological COX inhibition in humans.[80]

Genetic risk factors and inherited thrombophilia in VTE

Thrombophilia comprises a group of disorders wherein acquired or inherited mutations increase VT risk through defective coagulation components.[81] Primary genetic causes include factor V Leiden mutation, antithrombin III deficiency, protein C/S deficiency, histidine-rich GP deficiency, and prothrombin-related thrombophilia.

Factor V Leiden thrombophilia, the most common among these conditions, results from a point mutation in the *F5* gene (c.1691 G>A; p.R506Q), first identified in the Dutch population with 2% allelic frequency.[82] Homozygosity for this *F5* variant alludes to severe thrombosis risk, whereas heterozygous carriers present with a milder phenotype.[83] Antithrombin III deficiency, caused by *SERPINC1* gene mutations, presents as 2 types: type 1 shows reduced antigen and heparin levels, whereas type 2 maintains

normal antigen levels but reduced heparin cofactor activity.[84] Most cases are dominantly inherited, although recessive forms exist. Protein C deficiency, caused by *PROC* gene mutations, reduces substrate availability, thereby lowering circulating levels and impairing the degradation of coagulation factors V and VIII.[85] Protein S deficiency, caused by *PROS1* mutations, presents as severe thrombosis in homozygous/compound heterozygous cases,[86] whereas heterozygous mutations cause milder symptoms.[87]

Rarer forms of thrombophilia include histidine-rich GP deficiency,[88] with some variants showing abnormal heparin binding.[89] The prothrombin G20210A mutation increases the circulating levels of prothrombin by 133%, predisposing the individual to an increased risk of VTE development.[90] The prothrombin G20210A mutation has a higher prevalence in southern Europe (3.0%) and northern Europe (1.7%) and has a lower incidence in Asian and African populations.[91] The difference in genetic predisposition and environmental factors such as obesity could at least partially explain the lower occurrence of VTE among Asians compared with African Americans and Europeans.[92] Finally, dysfibrinogenemia can be associated with VTE and is usually dominantly inherited, with mutations in one of the fibrinogen genes (*FGA*, *FGB*, or *FGG*) causing structural changes in fibrinogen that may modify its function.[93]

Because thrombophilia can be inherited, acquired, or a combination of both, currently the preferred specific test for patients is genetic sequencing to find deficiencies in thrombophilia-related genes. In the United Kingdom, this genetic testing is currently offered for thrombophilia via a panel of genes or more targeted gene sequencing for known familial variants.[94]

However, these genetic causes account for only 50% of thrombophilia cases, suggesting significant genome-environment interactions and potential epigenetic factors.[95] Understanding these inherited thrombophilias is crucial for understanding DVT pathogenesis because they represent key noninflammatory mechanisms that predispose individuals to thrombosis, often requiring lifelong clinical vigilance and prophylactic intervention.

miRNAs in thrombosis regulation

MicroRNAs (miRNAs) are small, single-stranded, noncoding RNAs, which downregulate gene expression. There is growing evidence from population studies and animal models that miRNAs affect, and/or are biomarkers of, VTE.[96] The mechanisms of this are complex, with the role of miRNAs in coagulation and endothelial function being increasingly recognized. Multiple studies have shown that miR-27a/b regulates the expression of TF pathway inhibitor in ECs.[97,98] Some miRNAs (eg, miR-24) suppress the expression, maturation, and secretion of VWF.[99] This miRNA is downregulated by hyperglycemia, leading to increase in VWF levels, which is crucial for DVT initiation.[13] Another miRNA, miR-221/miR-222, also referred to as the miR-221/miR-222 cluster, regulates the proliferation and migration of ECs.[100]

MiRNAs can also exert their effect on procoagulant and anticoagulant gene and protein expression. For example, miR-19b functions as an antithrombotic protector by targeting TF in patients with unstable angina.[101] A whole host of miRNAs may target proteins involved in the coagulation cascade, thereby leading to VTE.[102]

Autoimmune conditions can lead to hypercoagulable states and also predispose to VTE.[103] A comprehensive study, using in silico and in vitro approaches, reported downregulation of miR-19b and miR-20a observed in patients with systemic lupus erythematosus and antiphospholipid syndrome, which could contribute to increased TF expression and provoke thrombosis.[104]

In addition to their mechanistic role in thrombosis, plasma levels of miRNAs, being relatively stable and easy to measure, are thought to be promising biomarkers for the diagnosis and prediction of VTE.[105] Thus, miRNAs represent an emerging field of biomarkers and therapeutic targets in DVT, and future research involving larger patient cohorts and mechanistic studies are clearly needed.

Circadian rhythms and VTE

Circadian rhythms coordinate daily physiological, biochemical, and behavioral processes with the external environment, enabling individuals to maintain homeostasis while responding to changes. Disruption of these rhythms can significantly impact human health.[106] In mammals, the suprachiasmatic nuclei in the hypothalamus serve as the primary biological clock, with melatonin regulating the sleep-wake cycle.[107,108] Melatonin production occurs in the pineal gland in response to darkness, triggering glutamate release from retinohypothalamic tract terminals to the suprachiasmatic nuclei.[108] In addition to this regulation in response to the Earth's natural light/dark cycle, food intake can also significantly influence circadian rhythms. Extended periods of fasting, inconsistent meal times, and frequent eating between meals can dysregulate these rhythms as much as abnormal exposure to the light/dark can. The relationship between circadian rhythms and hemostasis is an emerging research focus.[107]

Studies have revealed an association of circadian rhythms with DVT.[109] Circadian patterns in the levels of coagulation factors have also been demonstrated. Budkowska et al[110] found peak fibrinogen levels at 8 AM, although some studies report midday peaks.[111-113] Despite repeated findings, estimated variability of fibrinogen levels (~3%) does not hold significant clinical value in healthy individuals, and these changes should only be assessed in conjunction with circadian fluctuations in other hemostatic factors. Platelet counts peak at around 2 PM and are lowest at 8 AM, differing by ~6%.[110,114] Blood clotting capacity varies throughout the day, with activated partial thromboplastin time being shortest at 2 PM and prothrombin time showing opposite patterns. One study reported larger thrombi and lower survival rate in mice that underwent IVC ligation at noon vs 7 AM, implying that VT initiation time could affect its outcome in experimental settings.[115]

Lifestyle factors can disrupt circadian rhythms and affect clotting. Night shift work can disturb coagulation balance,[116,117] potentially increasing VTE risk. Similarly, jet lag disrupts natural rhythms, with even 1 night of poor sleep increasing clotting risk.[118,119] Notably, although melatonin supplements are commonly used to treat jet lag, excessive amounts may damage platelets.[120]

Despite these findings, dysregulation of circadian rhythms is believed to be a chronic event, and short-time alterations of these zeitgebers do not significantly elevate thrombosis risk. These parameters are more influential when repeated over long periods, and also pose as events whereby the chances of VTE initiation are higher.

Chronotherapy involves aligning medical treatments with circadian rhythms, and shows promise in optimizing therapeutic outcomes of VTE.[121] However, research in anticoagulation chronotherapy remains limited. For example, one study found no effect of administration time on warfarin efficacy.[122] Thus, circadian cycles represent an important aspect in DVT pathophysiology, and further research could help optimize anticoagulant treatment timing.

Effects of hormonal therapy on VTE

VTE could be a complication of hormonal therapy, with estrogen carrying the most significant risk. Estrogen is often combined with progesterone as a contraceptive pill or for use in hormone replacement therapy, with the former often prescribed to females.[123] However, men have a greater risk of VTE and recurrent thrombotic events.[124] Furthermore, thrombosis risk associated with other, particularly male hormones, has been largely underappreciated.

Testosterone replacement therapy or prescribed supplementation has surged in recent years because of increased clinical recognition and diagnosis of hypogonadism in men.[125] Studies addressing relationships between testosterone therapies and VT risk are scarce and have reported contradictory results. Ayele et al concluded that adult men on testosterone treatments are not at greater risk of VTE.[126] However, a case-crossover study later claimed the opposite, concluding that men with or without hypogonadism are at increased risk of VTE if they are treated with testosterone, with the risk being higher within the first 6 months of therapy.[127] Another study has mirrored these findings, also concluding that there is a correlation between testosterone use and VTE risk, with the greatest risk observed within the first 6 months of treatment.[128] Clarification of this potential causation is necessary to optimize treatment plans, particularly in cases of hypogonadism.[129,130]

Thyroxine (T4) is a major thyroid hormone that circulates in an inactive state until conversion into triiodothyronine (T3). Thyroid hormones are implicated in most body systems, but their role in coagulation specifically is less validated.[131,132] Hyperthyroidism can lead to a hypercoagulable state through elevated levels of VWF, coagulation factors, fibrinogen, and D-dimers, and thyroid dysfunction/hyperthyroidism likely confers higher VTE risk.[132-135]

Future directions and emerging therapies

Future research in VT is expanding beyond traditional inflammatory models, leveraging advanced technologies such as vessel-on-a-chip microfluidics and computational modeling to understand the roles of mechanical and biochemical factors in clot formation.[136-138] These tools provide detailed insights into how blood flow dynamics and shear stress patterns influence thrombosis independently of inflammation.

Endothelial mechanobiology has become a key focus, examining how blood stagnation affects endothelial function through mechanotransduction pathways and glycocalyx modifications. Simultaneously, researchers are investigating how chronic stasis changes the extracellular matrix composition and activates matrix metalloproteinases, leading to altered vessel wall mechanics and increased thrombogenic potential.

New therapeutic approaches target these noninflammatory aspects, including mechanosensitive drug delivery systems and molecules that stabilize the EG. Materials that respond to blood flow changes are emerging as a promising approach for preventing VT.

The field is also exploring the relationship between circadian rhythms and coagulation, studying how daily variations in blood flow and coagulation factors affect thrombosis risk independently of inflammation. This may lead to time-optimized, more personalized treatment strategies. Additionally, vascular aging research examines how senescence-related changes in vessel elasticity and blood flow patterns contribute to thrombosis through mechanical pathways.

Innovative biophysical approaches include devices modifying local blood flow, materials mimicking healthy vessel properties, and therapies restoring normal mechanotransduction. Long-term studies examine how noninflammatory risk factors evolve over time and are helping to develop more comprehensive risk assessment tools.

The distinction between inflammatory and noninflammatory processes is inherently complex and somewhat artificial. For example, platelets participate both in blood clotting and inflammation through cytokine release and immune cell recruitment. Similarly, endothelial activation can be triggered by both mechanical forces and inflammatory mediators, leading to comparable outcomes in terms of thrombosis risk. The relative contribution of inflammatory vs noninflammatory mechanisms likely varies by thrombosis type and location. For example, cerebral venous sinus thrombosis occurs without valves and under different pressure dynamics, wherein endothelial dysfunction may be more significant than flow-related mechanisms. Hemorrhoidal thrombosis develops in a vascular bed subject to variable pressure and mechanical stress, whereas portal vein thrombosis occurs in a specialized system with high exposure to gut-derived factors, suggesting that microbiota-related mechanisms might be more prominent. The challenge lies in studying these mechanisms independently, as they form an interconnected network where mechanical triggers can initiate inflammatory responses and vice versa. This suggests that therapeutic approaches might need to target both pathways simultaneously for optimal efficacy in preventing or treating VT. Furthermore, understanding anatomical differences is crucial for developing targeted therapies.

In conclusion, a broader understanding of thrombosis, incorporating both inflammatory and noninflammatory mechanisms, is driving the development of more personalized and effective treatments, potentially complementing traditional anticoagulation approaches.

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