

ILLUSTRATED REVIEW

Illustrated State-of-the-Art Capsules of the ISTH 2019 Congress in Melbourne, Australia

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*["Correction Statement added on December 11, 2019: ISTH State of the Art Speakers added in the author byline"]

Abstract

The 27th Congress of the International Society of Thrombosis and Haemostasis (ISTH) is an international conference held July 6-10, 2019, in Melbourne, the capital of the state of Victoria, Australia. The ISTH congress has previously been held every other year, with the Scientific and Standardization Committee (SSC) meeting held annually, until 2019 when it became one combined annual meeting of the ISTH and SSC. The conference covers clinical and basic aspects of hemostasis and thrombosis, and this year includes 5 Plenary lectures and >50 State of Art (SOA) lectures, presented by internationally recognized speakers, as well as numerous oral session and poster presentations selected from submitted abstracts, including many early career and reach the world support recipients. This SOA review article in RPTH contains concise Illustrated Review Articles or 'Capsules' consisting of short text, three references and a figure, with topics including stroke, cancer-associated thrombosis, hemophilia, coagulation, the interface between infection and inflammation, and in the experimental and discovery areas, megakaryocyte biology and platelet production, structure-function of key receptors and coagulation factors, and emerging new roles for thrombotic/hemostatic factors. Together, these articles highlight novel findings which will advance knowledge and with the potential to change clinical practice and improve outcomes. It is hoped that conference attendees and followers will enjoy utilizing the images for ongoing education and during the conference for live tweeting during sessions, to assist in the broadcasting and promotion of the science to those unable to attend, or who have chosen to attend a concurrent session. Use #IllustratedReview and #ISTH2019 on social media.

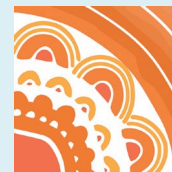
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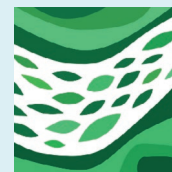
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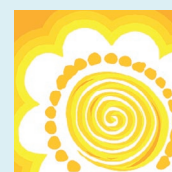
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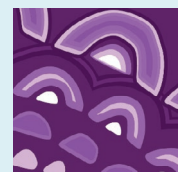
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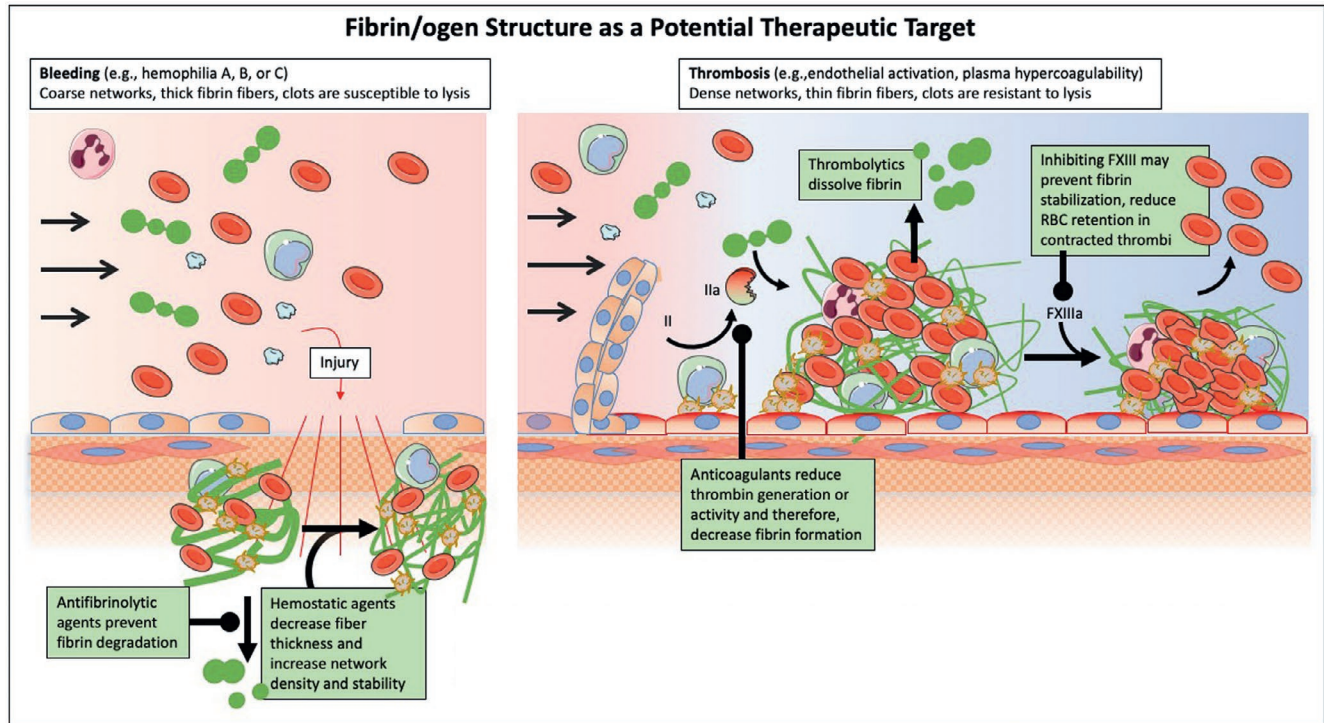


Fibrin/ogen structure as a potential therapeutic target



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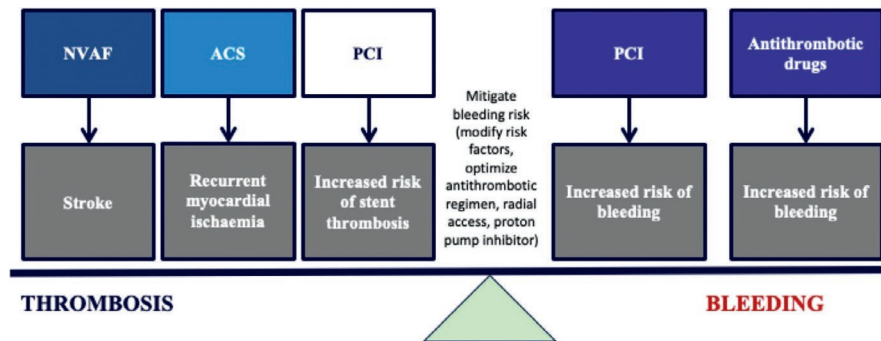
For references, see¹⁻³.

Triple therapy in patients with atrial fibrillation and acute coronary syndrome or PCI/stenting



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Several considerations are necessary when managing patients with atrial fibrillation and acute coronary syndrome or percutaneous cardiovascular intervention/stenting:

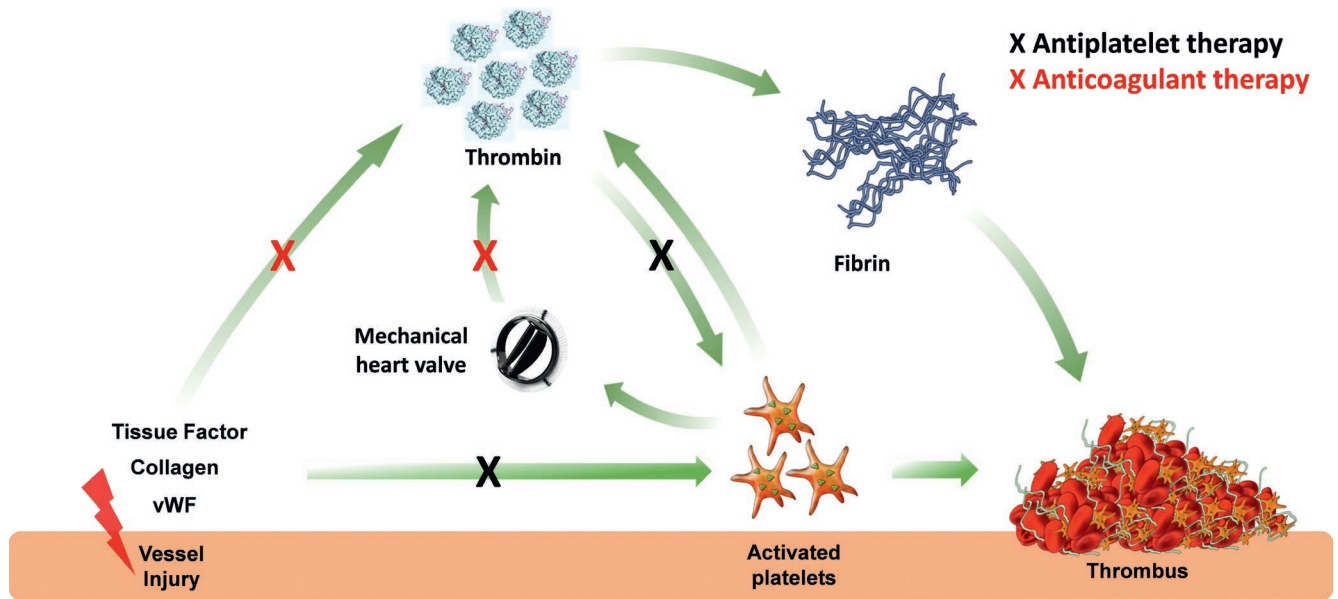
- Stroke prevention, which requires oral anticoagulation (OAC) with well-managed vitamin K antagonists (VKAs) or optimal doses of direct OACs.⁴
- Prevention of recurrent cardiac events or ischemia with antiplatelet drugs.⁵
- Reducing stent thrombosis with antiplatelet drugs.⁵
- Managing risk of serious bleeding related to combinations of OACs and antiplatelets.

Mitigating bleeding in this setting requires attention to modifiable bleeding risk factors (eg. uncontrolled hypertension, excess alcohol, concomitant nonsteroidal anti-inflammatory drugs, labile international normalized ratios if on a vitamin K antagonist, etc), then flagging up the “high-risk” patients (eg. HAS-BLED score ≥ 3) for early review and follow-up.⁶ Percutaneous cardiovascular intervention with radial access reduces periprocedural bleeding, and proton pump inhibitors may be considered. In combination with a P2Y₁₂ inhibitor (not aspirin), bleeding is lower with direct OACs than VKAs, without compromising thrombotic outcomes.⁶

Combining antiplatelet and anticoagulant therapy

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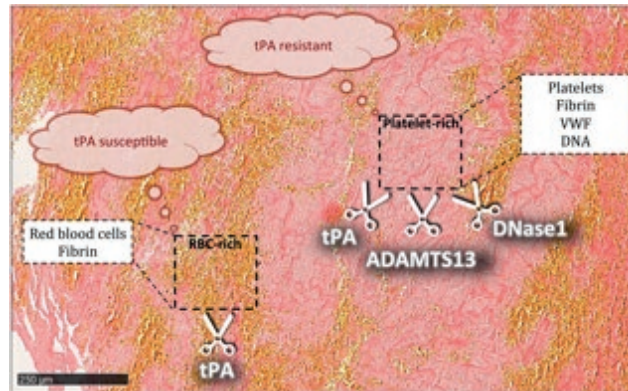
In the arterial circulation, thrombus formation is most commonly triggered by atherosclerotic plaque disruption, which leads to activation of the tissue factor pathway to generate thrombin, and activation of platelets. Thrombin in turn cleaves fibrinogen to form fibrin and is also a potent platelet activator. Less commonly, thrombus formation is triggered by exposure of blood to artificial surfaces (eg, mechanical heart valves), which leads to activation of the contact pathway to generate thrombin. Activated platelets provide a phospholipid surface for thrombin generation and release inorganic polyphosphates that trigger activation of the contact pathway.

When used in combination, antiplatelet and anticoagulant therapy can work synergistically to reduce fibrin formation and platelet activation and thereby prevent thrombus formation. Emerging clinical evidence demonstrates that dual pathway inhibition is beneficial if safe drug combinations are used.

Novel therapeutic targets in stroke

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Structural analysis of occluding thrombi retrieved from ischemic stroke patients reveals histologic indications for therapy resistance. This picture shows a Martius Scarlet Blue staining, showing the typical RBC-rich areas stained yellow and platelet-rich areas stained pink/red in a stroke thrombus. RBC-rich areas have limited complexity as they consist of red blood cells that are densely packed in a meshwork of thin fibrin. In contrast, platelet-rich areas are characterized by dense fibrin structures aligned with von Willebrand factor (VWF) and abundant white blood cells and extracellular DNA, possibly part of neutrophil extracellular traps (NETs). Hence, in addition to targeting fibrin via tissue-type plasminogen activator (t-PA), targeting DNA with DNase1⁹ and targeting VWF with ADAMTS-13¹⁰ are new approaches to improve thrombolysis. Such novel targets could become particularly relevant for treatment of t-PA-resistant (platelet-rich) occlusions in stroke patients.¹¹

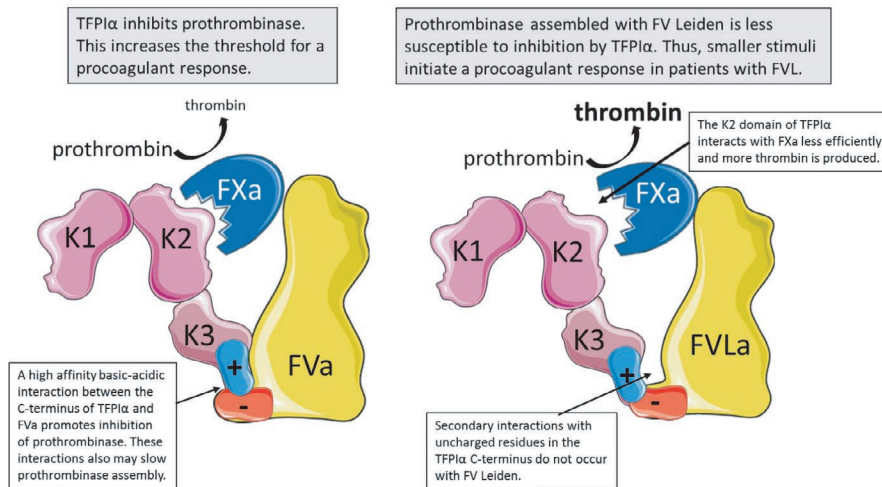
TFPI: structure, function, and therapeutic potential

Alan Mast MD, PhD¹

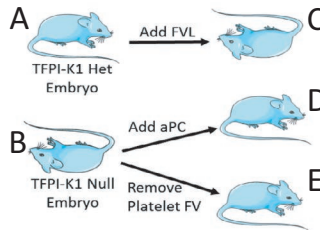
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Biochemical Interaction of TFPIα with Factor V



Physiological Interaction of TFPIα with Factor V



- Mice heterozygous for deletion of the first Kunitz domain of TFPI (TFPI-K1) survive to adulthood.
- TFPI-K1 null mice die during embryogenesis.
- Addition of FV Leiden to TFPI-K1 heterozygous mice results in perinatal lethality.
- A transgene overexpressing activated protein C rescues ~30% of TFPI-K1 null mice to adulthood.
- Removal of factor V from platelets rescues ~80% of TFPI-K1 null mice to adulthood.

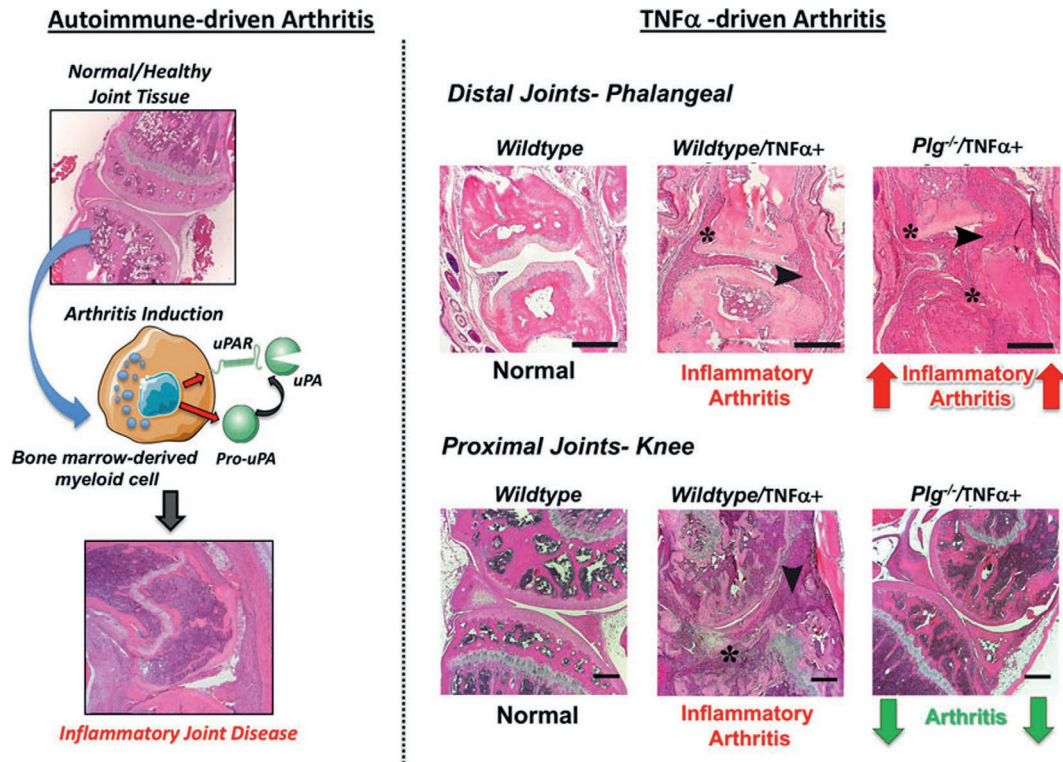
For references, see¹²⁻¹⁴.

Plasminogen activation in inflammatory joint and bone disease

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Rheumatoid arthritis (RA) is a common, yet heterogeneous, chronic inflammatory disease that affects approximately 1% of the population worldwide, with considerable variation among patients in disease progression and severity. A contribution for hemostatic system components is implied by the fact that (1) fibrin deposits along cartilage surfaces and within the inflamed synovium of affected joints is a commonly observed feature of RA patients and experimental animals with inflammatory arthritis, and (2) fibrin degradation products (eg, D-dimer) accumulate in the synovial fluid of RA patients. Studies of mice with plasminogen activation (PA) system deficiencies have yielded mixed results, suggesting that the contribution of PA is context dependent. Nevertheless, multiple studies suggest 2 potent contributions of PA to inflammatory joint disease pathogenesis. Findings indicate a fundamental role for urokinase plasminogen activator (uPA) and uPA receptor-expressing hematopoietic cells in driving arthritis incidence and progression in autoimmune-driven arthritis (left panel).^{15,16} Additionally, plasminogen appears to be a key molecular determinant of tumor necrosis factor α -driven inflammatory joint disease capable of driving or ameliorating arthritis pathogenesis in distinct anatomic locations in the same subject (right panel).¹⁷

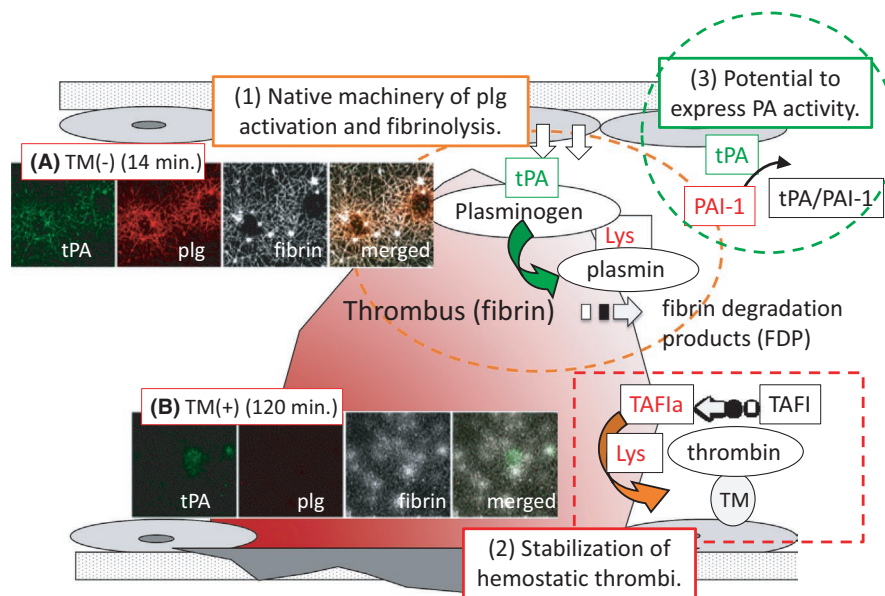
Spatiotemporal regulation of plasminogen activation and its disruption



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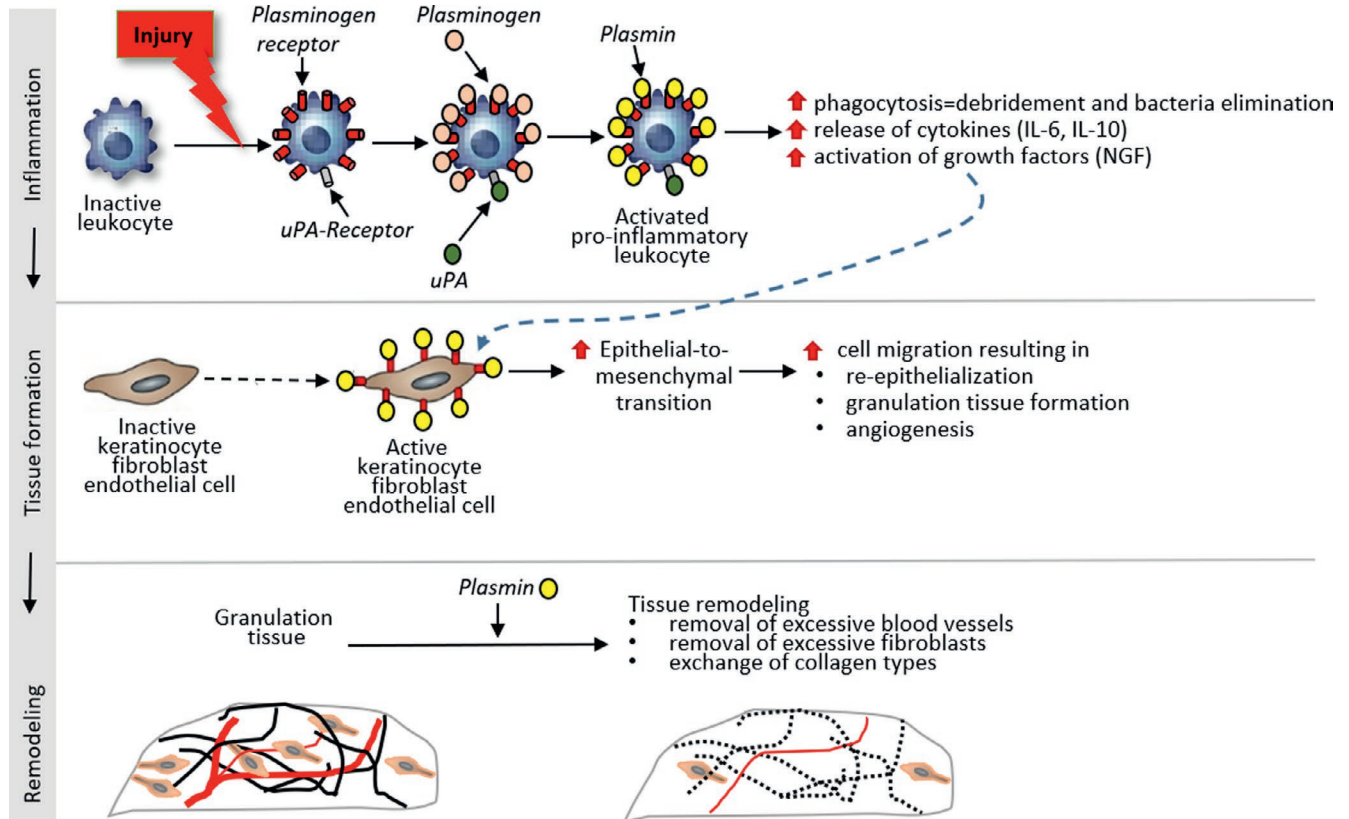


C-terminal lysine in partially digested fibrin is a key factor in spatiotemporal regulation of fibrinolysis. A quick dissolution of generated thrombi is achieved by (1) "native machinery of plasminogen activation and fibrinolysis," in which plasminogen binding to fibrin surface through its lysine-binding sites together with tissue-type plasminogen activator (t-PA) is a key event. Activated platelet surfaces on which t-PA and plasminogen assembled appeared to initiate fibrinolysis after forming fibrin (A). In (2) "stabilization of hemostatic thrombi," a removal of C-terminal lysine by thrombin-activatable fibrinolysis inhibitor (TAFI) after activation by thrombin/thrombomodulin is a key event. Soluble thrombomodulin attenuated both plasminogen accumulation and fibrinolysis through TAFI activation (B). (3) "Potential to express PA activity on fibrin" is determined by the balance between t-PA and plasminogen activator inhibitor-1. These spatiotemporal regulatory mechanisms of fibrinolysis were revealed by recently advanced real-time imaging techniques.^{18,19}

Plasminogen in wound healing

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Wound healing consists of partially overlapping inflammatory, proliferation, and tissue-remodeling phases. A successful wound healing depends on a proper activation and subsequent termination of the inflammatory phase. Plasminogen is a critical regulator of the wound healing processes that mediate its effects by activating both immune and nonimmune cells.²⁰ Early after wound formation, plasminogen is transported to acute wounds by inflammatory cells, where it leads to intracellular signaling events that result in induction of cytokines that potentiate the early inflammatory response and increased levels of growth factors. Plasminogen also plays an important role in later phases of wound healing, where it indirectly or directly through plasmin activates wound debridement and resolution of inflammation. The multiple and vital functions of plasminogen during wound healing makes plasminogen a very attractive novel drug candidate to treat various wounds, including acute wounds, burns, and various chronic wounds. IL-6, interleukin-6; IL-10, interleukin-10; NGF, nerve growth factor; uPA; urokinase-type plasminogen activator.

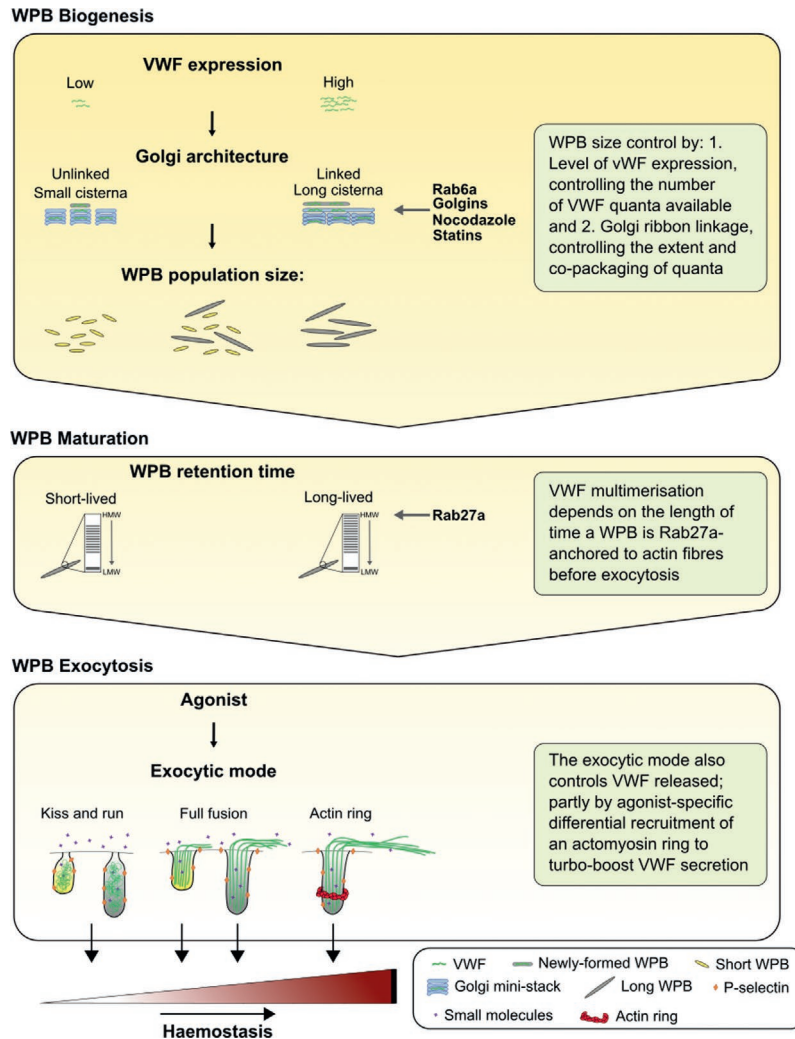
Cellular controls on Weibel-Palade bodies affecting von Willebrand factor function



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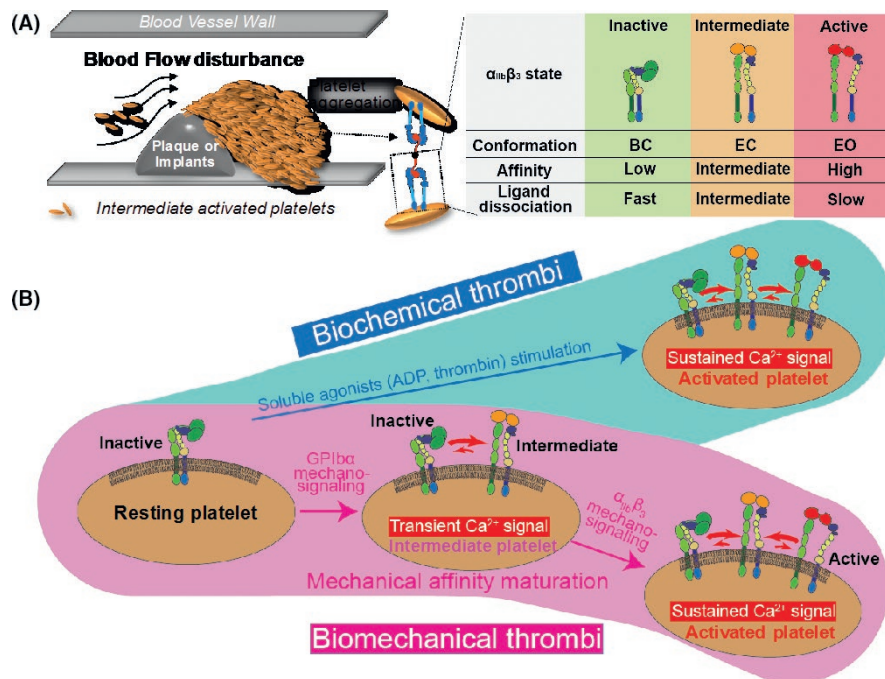
Many cellular controls can affect the Weibel-Palade body population available for release and ultimately the hemostatic response initiated. The left side of the figure shows factors reducing VWF functioning (hemostatic response). The right side shows those producing increased haemostatic response (extremes illustrated).²¹⁻²³

Intermediate state of integrin $\alpha\text{IIb}\beta 3$ for platelet mechanosensing in disturbed blood flow



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Platelets often aggregate near vessel stenosis caused by atherosclerotic plaques or medical device implants, attesting to their extreme mechanosensitivity to blood flow disturbance. Such platelet aggregation is driven by biomechanical rather than biochemical mechanisms, as such prothrombotic effect cannot be eliminated by aspirin and clopidogrel.²⁴ Using a stenosed microfluidic model, we demonstrated that the biomechanical platelet aggregation is mainly supported by integrin $\alpha\text{IIb}\beta 3$ with an extended-closed (EC) conformation (A).²⁵ We also developed a dual biomembrane force probe to visualize integrin biomechanical activation on a living platelet.²⁶ We found that, upon mechano-signaling of GPIIb, $\alpha\text{IIb}\beta 3$ adopts the EC conformation and possesses affinity and bond lifetime that are intermediate between the well-characterized active and resting states. This inside-out mechano-signaling is distinct from the biochemical signaling by agonists and potentiates the outside-in mechano-signaling of $\alpha\text{IIb}\beta 3$ for affinity maturation (B).²⁴ Our work highlighted the existence, genesis, and regulation of a semistable intermediate state of $\alpha\text{IIb}\beta 3$ that is fundamental in mechanically driven thrombosis.²⁶

VWF regulation of angiogenesis and angiodysplasia

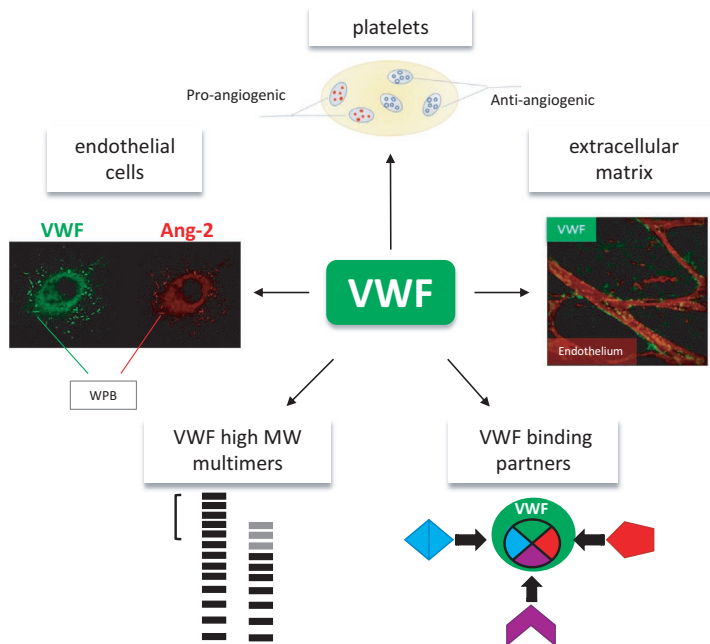


Anna M. Randi MD, PhD¹

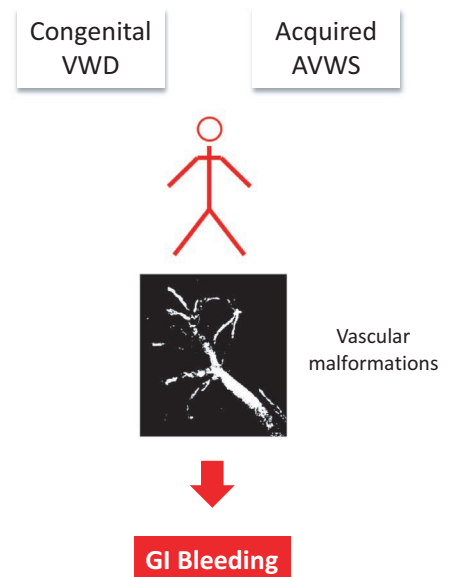
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VWF regulation of angiogenesis and angiodysplasia

Pathways regulating angiogenesis: model



Clinical implications: Angiodysplasia

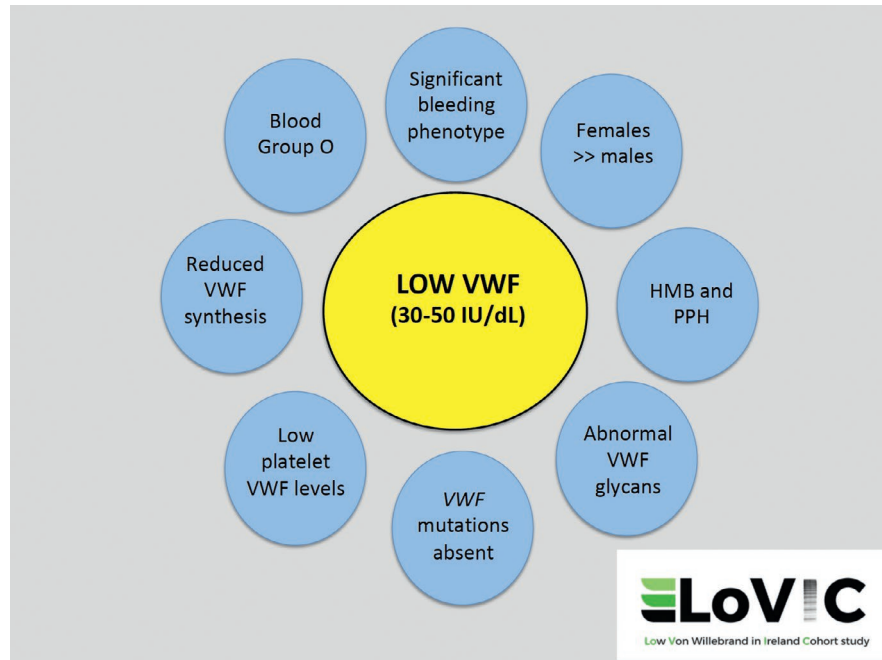


The discovery that the hemostatic protein von Willebrand factor (VWF), best known for mediating platelet adhesion, controls blood vessel formation has provided a novel hypothesis to explain the pathogenesis of vascular malformations (angiodysplasia) and gastrointestinal (GI) bleeding in patients with congenital von Willebrand disease (VWD) or acquired von Willebrand syndrome (AVWS).²⁷ VWF is found predominantly in 3 pools: cellular, plasmatic, and subendothelial; VWF binds to many proteins, some of which regulate angiogenesis. Loss of VWF in endothelial cells (ECs) in vitro and in vivo results in abnormal angiogenesis.²⁸ The mechanism is not completely understood, but is likely to involve angiopoietin-2 (Ang-2), a coregulator of angiogenesis, and possibly other components of the endothelial organelles Weibel-Palade bodies (WPBs). Clinical evidence indicates that high-molecular-weight multimers of VWF are also likely to be involved.²⁹ Understanding the contribution of these pathways will be key to developing new therapeutic approaches and improve patients' treatment.

Insights into low VWF

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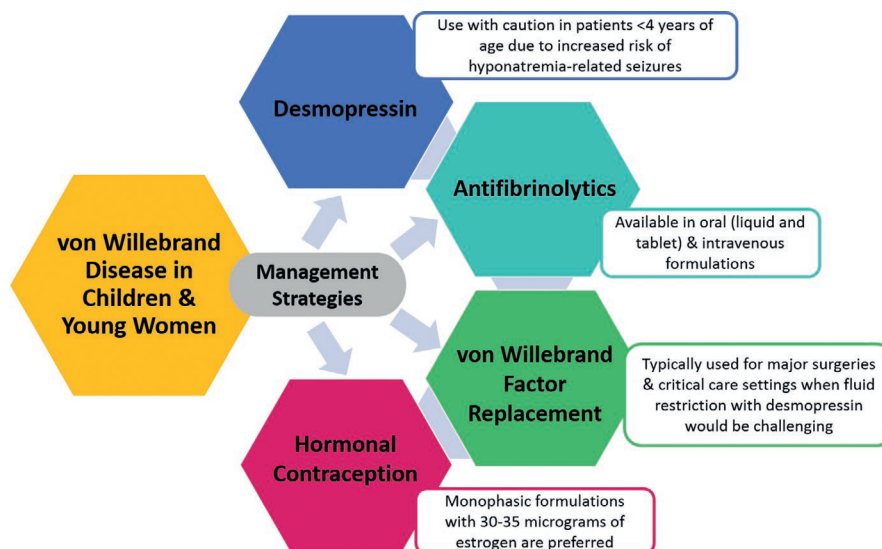


Since the pathophysiology and heritability of low von Willebrand factor (VWF) levels remain poorly understood, diagnosis and management of these patients continues to pose significant clinical challenges. Data from the Low VWF Ireland Cohort (LoVIC) study have demonstrated that blood group O females are overrepresented in those registered with low VWF.³⁰ Despite having modest reductions in plasma VWF:Ag levels, Low VWF patients can have significant bleeding, particularly heavy menstrual bleeding (HMB) and postpartum hemorrhage (PPH).³¹ In the majority of patients, low VWF levels are due to reductions in VWF synthesis that appear to be largely independent of any VWF gene mutations.³⁰ Enhanced VWF clearance may also contribute to pathophysiology in some individuals with low VWF, with abnormal VWF glycosylation involved in the etiology of this increased VWF clearance.³² Importantly however, any reduction in VWF plasma half-life is usually mild and does not significantly impair duration of DDAVP-induced VWF responses.

VWD in children and young women

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This review focuses on 4 management issues commonly faced by clinical providers in the care of children and adolescents with von Willebrand disease (VWD): epistaxis, heavy menstrual bleeding, and preparation for tooth extraction and tonsillectomy. A variety of management strategies are available, including desmopressin, antifibrinolytics, plasma-derived and recombinant factor replacement, hormonal contraception for heavy menses, and local measures for epistaxis.³³ While many aspects of management are similar to hematologic care for adults with VWD, there are pediatric-specific considerations. For example, desmopressin is typically avoided in patients under 4 years of age due to the increased risk of hyponatremia-related seizures. For heavy menstrual bleeding in adolescents, monophasic formulations of oral contraceptives with low-dose estrogen (30–35 micrograms) are preferred over triphasic or ultra-low-estrogen formulations.³⁴ Finally, when planning for tonsillectomy, the risk of delayed bleeding (days 5–12) must be considered.³⁵

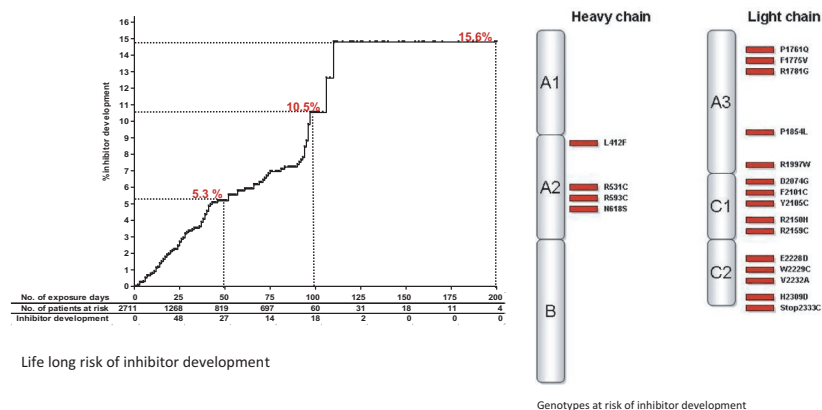
Inhibitor development in nonsevere hemophilia A

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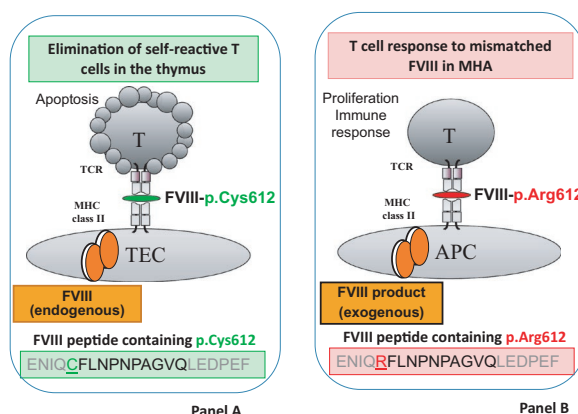


Inhibitor development in non-severe hemophilia A



Life long risk of inhibitor development

Genotypes at risk of inhibitor development



TCR: T cell receptor, MHC: Major Histocompatibility Complex,
 TEC: Thymus endothelial cell, APC: antigen presenting cell,

The development of neutralizing factor VIII (FVIII) antibodies (inhibitors) is a major challenge in patients with nonsevere hemophilia A (NSHA).³⁶ In contrast to severe hemophilia, NSHA patients have a lifelong risk of inhibitor development. In the studies of the INSIGHT consortium, we demonstrated that inhibitors are associated with a deterioration of bleeding phenotype and increased mortality rate.³⁷

F8 genotype is an important risk factor for inhibitor development. Inhibitor development in NSHA is provoked by exposure to a peptide sequence of wild-type FVIII that overlaps with the mutated sequence in endogenous FVIII. The patient's immune system is tolerant to the endogenous peptide (A), but naïve to the wild-type sequence of this specific peptide (B). When this peptide is presented to the T-cell receptor, an immune response will be elicited. Thus, T-cell epitopes in NSHA patients are likely to be associated with the location of the missense mutation. This concept is supported by studies that demonstrate that T-cell epitopes in NSHA inhibitor patients generally include the wild-type sequence overlapping the mutated sequence in endogenous FVIII, indicating that a relatively small sequence mismatch can provoke a T-cell response, subsequently resulting in inhibitor development.³⁸

Bispecific antibodies and advances in non-gene therapy options in hemophilia



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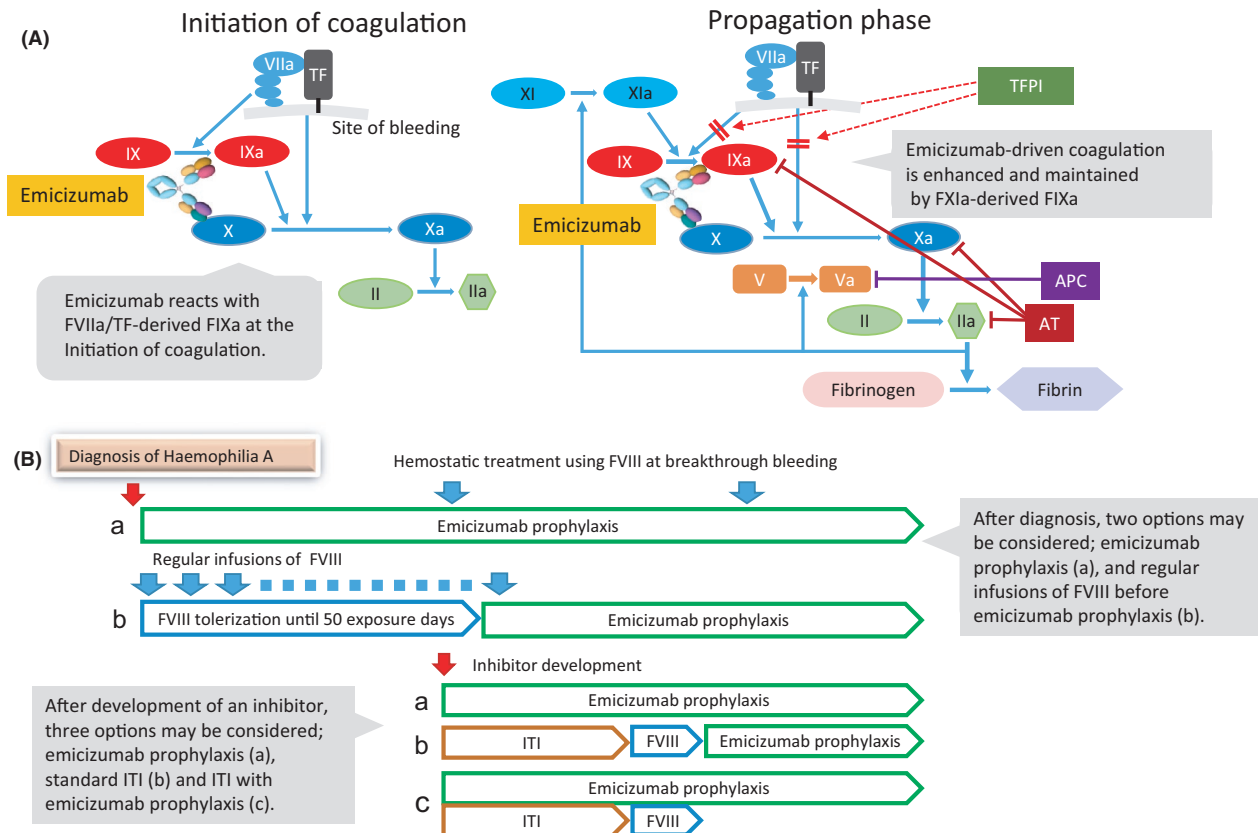


Fig A Emicizumab-driven coagulation reactions

Fig B New options for hemophilia A treatment with emicizumab

Emicizumab is a bispecific antibody recognizing factor IXa (FIXa) and factor X (FX). It promotes activation of FX in the absence of factor VIII (FVIII).³⁹ Emicizumab initially reacts with FIXa mediated by the factor FVIIa (FVIIa)/tissue factor (TF) complex (A). Under physiologic circumstances, FVIIa/TF activity is limited by tissue factor pathway inhibitor (TFPI), but emicizumab-driven FXa and thrombin generation is enhanced in the presence of FIXa derived from FXIa-dependent reactions. Hence, FIXa is supplied through a FXI activation-loop, and emicizumab-driven FXa and thrombin generation is maintained. The products of emicizumab activity are regulated by natural anticoagulants including activated protein C (APC), antithrombin (AT), and TFPI (A). Emicizumab provides a novel therapeutic strategy for hemophilia A with several clinical advantages, including subcutaneous availability, longer half-life, and effectiveness in the presence of an FVIII inhibitor.⁴⁰ The use of emicizumab for early prophylaxis offers the prevention of bleeding and long-term maintenance of intact joints. Several options should be considered on an individual basis, however (B).

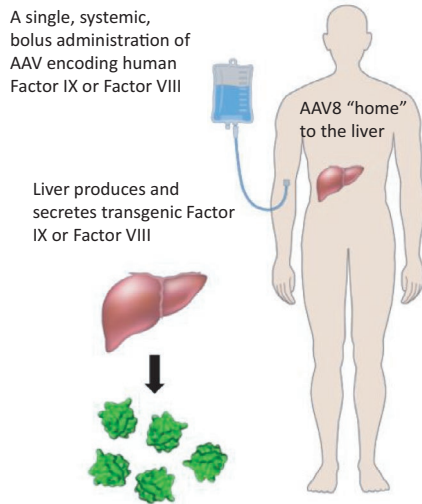
Gene therapy

Amit Nathwani MBChB, FRCP, FRCPATH, PhD¹

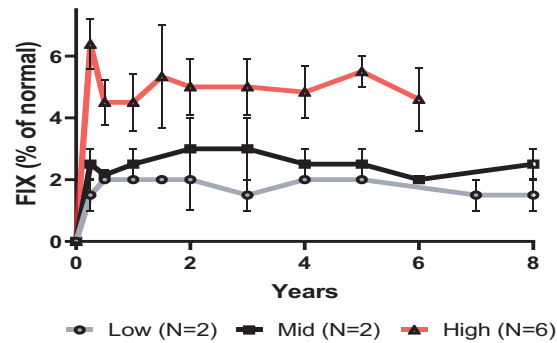
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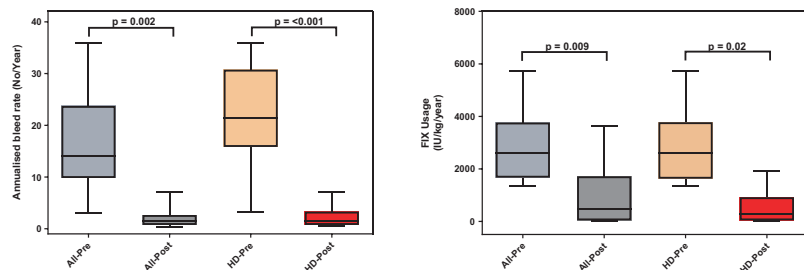
(A) Simple mode of vector delivery



(B) Stable multi-year therapeutic expression of transgenic protein



(C) Persistent reduction in annualized bleed rates (left panel) and Factor concentrate usage (right panel)

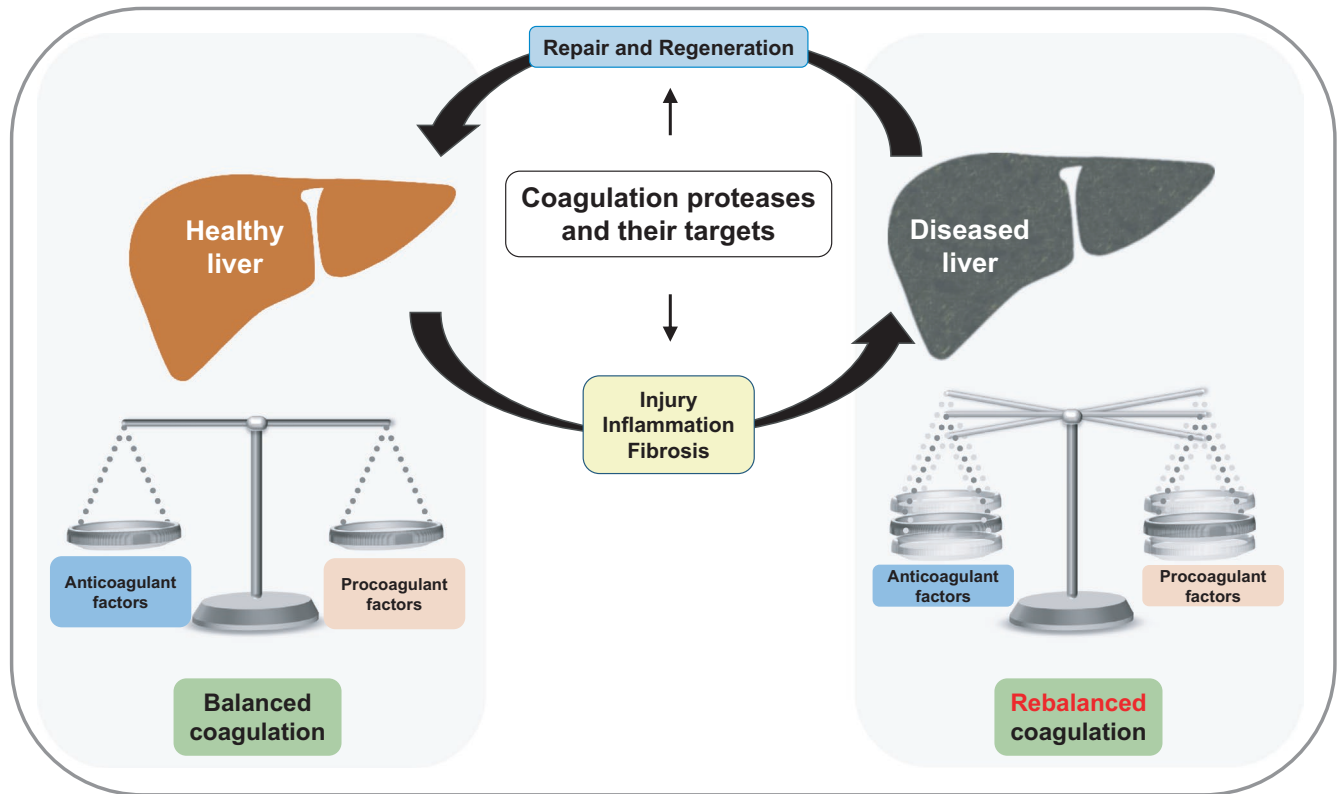


Gene therapy offer the potential for a cure for the hemophilias. Recombinant adeno-associated virus (AAV) vectors are particularly attractive for gene therapy because of their excellent safety profile and ability to mediate efficient transduction of the liver following systemic administration of vector (A). The liver is an attractive site for gene transfer, as it is the site of synthesis of many clotting factors and its ability to induce tolerance to transgenic protein. The first trial to provide clear evidence of efficacy after a single peripheral vein infusion of AAV vector encoding the human factor IX gene in patients with hemophilia B was reported by our group.^{41,42} Longer follow-up of these patients has not unveiled any toxicities, while plasma factor IX levels have remained stable in all 3 dose cohorts over a period extending 8 years (B), resulting in persistent reduction in annualized bleed rates and factor concentrate usage (C). Similar efficacy with AAV vectors has also been reported in haemophilia A.⁴³

Coagulation proteins and liver disease

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The liver synthesizes a majority of pro- and anticoagulant factors. Thus, hepatic dysfunction in acute and chronic liver diseases results in substantial changes in function of the hemostatic system. Importantly, hepatic dysfunction produces a fragile rebalancing of hemostasis, leaving patients with liver disease often prone to both bleeding and thrombosis. Experimental evidence has linked coagulation proteases and their targets (eg, Protease-activated receptors (PARs), fibrinogen) to the development of diseases of high concern including alcoholic and nonalcoholic fatty liver disease, and to end-stage pathologies of liver diseases such as fibrosis/cirrhosis. This connection is under investigation in patients with liver fibrosis. Finally, the role of coagulation factors in liver injury is clearly context dependent, as emerging experimental and clinical evidence links fibrinogen-driven mechanisms to pro-repair and pro-regenerative responses after acute liver injury and partial liver resection.⁴⁴⁻⁴⁶

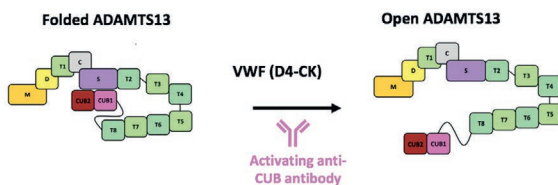
ADAMTS13 and VWF in TTP

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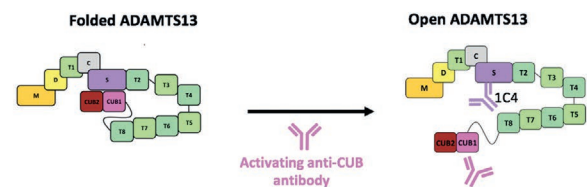


(A) VWF and activating monoclonal anti-ADAMTS13 antibodies open ADAMTS13 structure



ADAMTS13 adopts a folded conformation in which the N-terminal spacer domain interacts with the C-terminal CUB domains, resulting in an autoinhibition of the enzyme. Binding of the VWF D4-CK fragment or activating murine anti-CUB antibodies to the ADAMTS13 CUB domains uncouples the spacer-CUB interaction resulting in an open and more active enzyme (47, 48).

(B) ELISA to detect ADAMTS13 with an open conformation



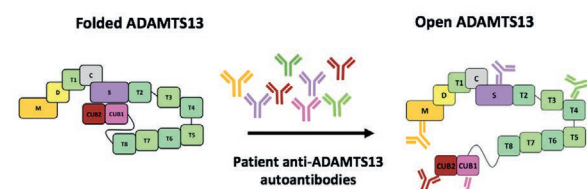
An open ADAMTS13 conformation can be detected in ELISA using a murine anti-spacer antibody (1C4) which is directed against a cryptic epitope in folded ADAMTS13 (49).

(C) ADAMTS13 conformation in healthy donors and iTTP patients



Interestingly, an open ADAMTS13 conformation is linked to the pathophysiology of immune-mediated TTP (iTTP) as the antibody 1C4 could capture ADAMTS13 from acute iTTP plasma but not from healthy donor plasma (49).

(D) iTTP patient anti-ADAMTS13 autoantibodies induce an open ADAMTS13 conformation



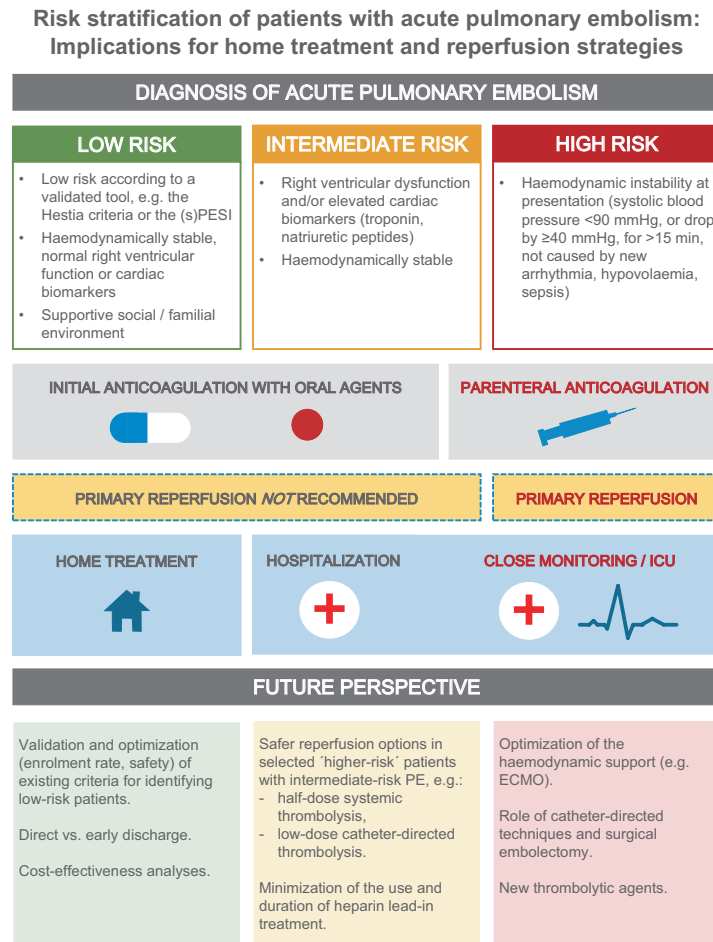
Anti-ADAMTS13 autoantibodies from iTTP patients are the plasma factors that change the folded conformation of healthy donor ADAMTS13 to an open conformation. Anti-ADAMTS13 autoantibodies do not only clear and/or inhibit ADAMTS13, but also induce an open ADAMTS13 conformation in iTTP.

Risk stratification of patients with acute pulmonary embolism (PE): implications for home treatment and reperfusion strategies



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Abbreviations: PE, pulmonary embolism; (s)PESI, (simplified) Pulmonary Embolism Severity Index; ICU, intensive care unit; ECMO, ExtraCorporeal Membrane Oxygenation.

The severity of acute pulmonary embolism (PE) can be estimated based on (1) the presence and degree of right ventricular (RV) dysfunction as assessed by imaging tests, (2) the levels of cardiac biomarkers in the circulation, and (3) demographic and clinical factors.⁵⁰

Patients with hemodynamic instability and cardiogenic shock (high-risk PE) are candidates for systemic thrombolysis or other reperfusion therapy.⁵⁰ On the other hand, primary reperfusion has an unfavorable risk-benefit ratio in patients with intermediate-risk PE; in future clinical trials, safer regimens and techniques (eg, low-dose systemic thrombolysis or catheter-directed reperfusion) will need to be tested as a therapeutic option for selected normotensive patients.⁵¹ Direct oral anticoagulant agents are an effective and safe treatment option for the vast majority of patients with PE.⁵⁰ Potential candidates for early discharge and home anticoagulant treatment can be identified based on the severity of PE, the presence and burden of comorbidities, and a supportive social/family environment.⁵²

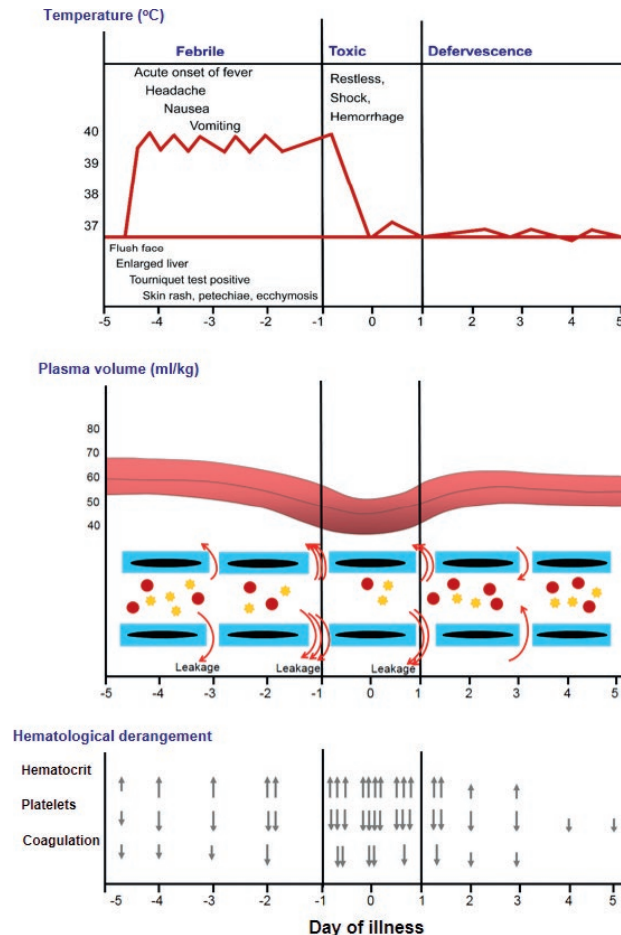
Management strategies for hematological derangement in dengue hemorrhagic fever



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Dengue hemorrhagic fever (DHF) is a clinical diagnosis with 3 stages: febrile, toxic, and defervescence. The febrile stage lasts 2–7 days, followed by the toxic stage of 24–48 hours, and clinical recovery in the defervescence stage. Vasculopathy with positive tourniquet test indicating the increased vascular permeability starts from the early febrile stage. The plasma leakage from the intravascular compartment is most prominent during the toxic stage, resulting in hemoconcentration, hypoproteinemia/hypoalbuminemia, pleural effusion, ascites, threatened shock, and profound shock. The bleeding diathesis is caused by vasculopathy, thrombocytopenia, platelet dysfunction, and coagulopathy. Variable reductions in the activities of several coagulation factors have been reported.⁵³ Low levels of protein C and S and antithrombin III were found to be associated with increased severity of dengue manifestation.⁵⁴ Optimal fluid therapy to maintain the functions of the vital organs during the critical period and effective bleeding control will lead to favorable outcomes.⁵⁵

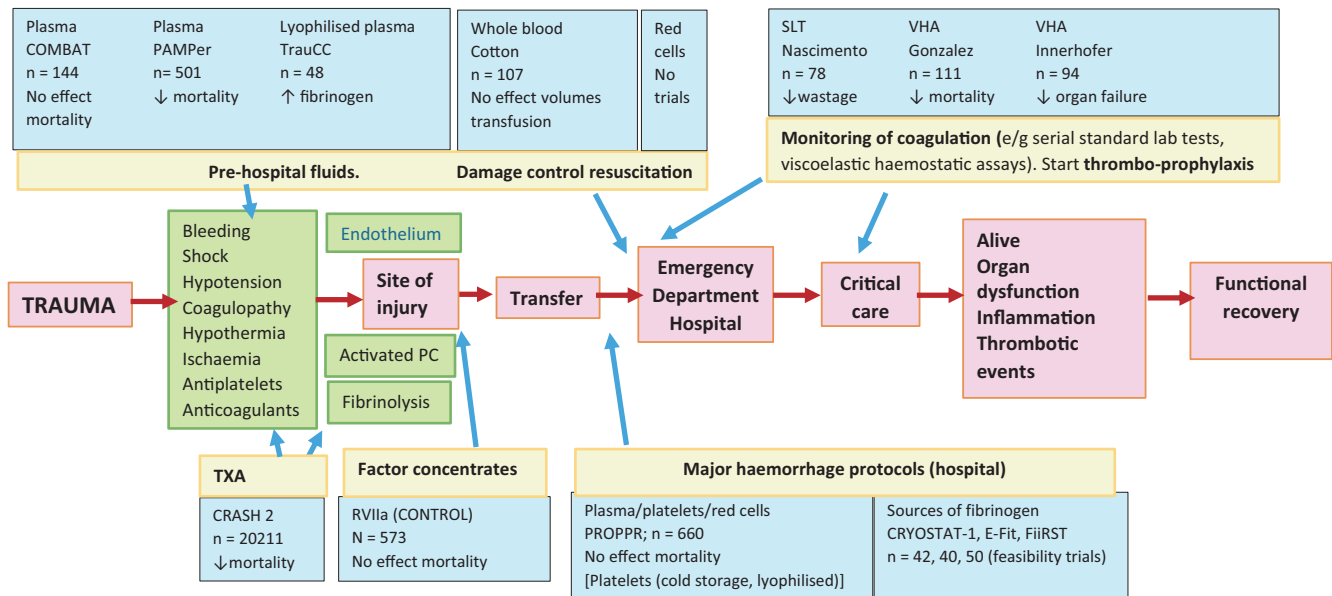
Massive transfusion: algorithm-based or empiric therapy?



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Worldwide, trauma remains a leading cause of death in the younger populations. There has been a paradigm change in our approach to supportive care and transfusion practice for trauma hemorrhage resuscitation, with increased emphasis on timely delivery of “balanced” blood components,⁵⁶ less crystalloids, and use of tranexamic acid. Measures to target trauma-induced coagulopathy are integral to current management approaches, but our understanding of the pathophysiology is incomplete, including the role of endothelial activation and the interactions between hemostatic and inflammatory pathways.⁵⁷ Randomized trials are increasingly informing optimal practice (eg, tranexamic acid, plasma), but the size and methodological quality of all the studies varies very considerably.⁵⁸ Areas of current uncertainty relate to use of platelets, concentrated sources of fibrinogen, and whole blood, in addition to the treatment approaches for older patients taking anticoagulants or antiplatelet agents. It is unclear whether protocols in trauma should be generalized to other hospital clinical settings of major bleeding. SLT, standard laboratory tests; VHA, viscoelastic hemostatic assays.

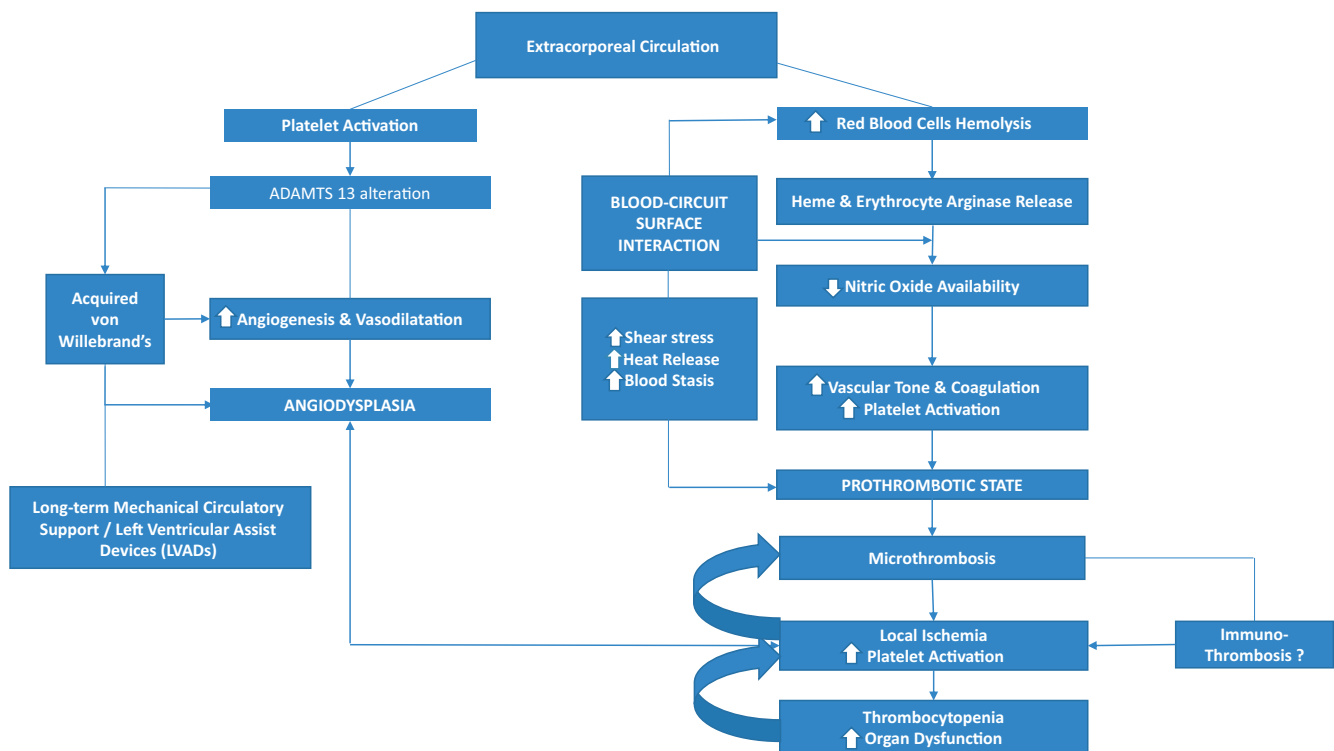
Pathophysiology of coagulopathy during mechanical circulatory support



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Pathophysiology of Coagulopathy during Mechanical Circulatory Support



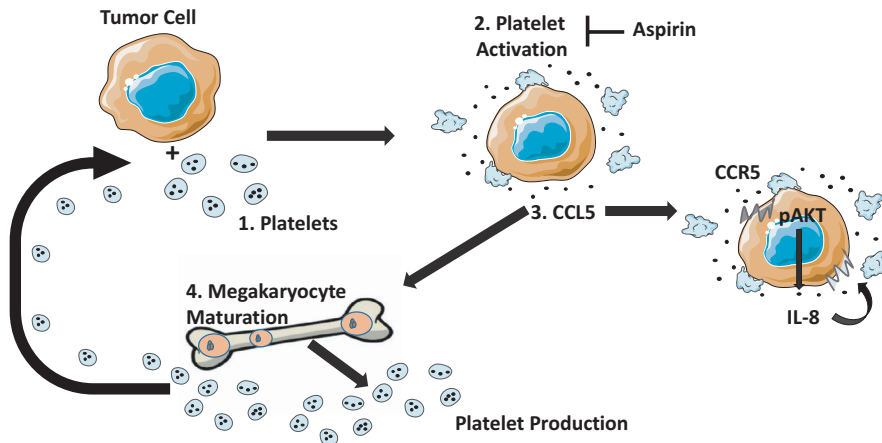
Exposure to extracorporeal circulation circuits and consequent blood-circuit surface interaction triggers a cascade of events that promote both bleeding and thrombosis. Platelet activation is a central event that contributes to both. Platelet activation generates microthromboses and local ischemia, which can have a significant role in organ dysfunction in this setting. Importantly, hemolysis also promotes both a pro-thrombotic and a vasoconstrictive state due to nitric oxide consumption via free hemoglobin.

In addition to the consumption coagulopathy that can result from platelet activation and loss (similar to the coagulopathic stage of disseminated intravascular coagulation), it is well described that exposure of glycoprotein ADAMTS-13 lytic site and proteolysis of high-molecular-weight von Willebrand multimers results in acquired von Willebrand syndrome. This syndrome, along with angiodyplasia, is more comprehensively described with long-term support with left ventricular assist devices (LVADs). Both mechanisms lead to increased risk of bleeding; therefore, bleeding and thrombosis can coexist and complicate the use of anticoagulation in this population. Our understanding of the role of platelets and immunothromboses as important factors in occurrence of short- and long-term organ dysfunction in this unique patient population is still evolving.⁵⁹⁻⁶¹

Crosstalk between platelets and tumor cells

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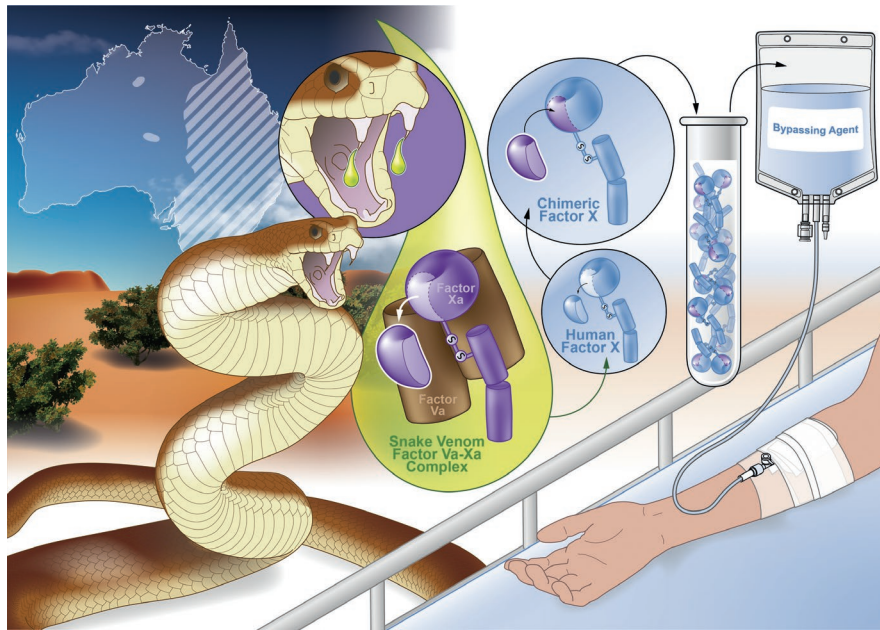
Functional versatility of platelets beyond hemostasis is best exemplified in their resourcefulness in malignancy. Platelets promote metastasis and support tumor growth. Platelet activation and increased production are associated with a poor cancer-related prognosis. It is unknown whether these platelet responses are epiphenomenal or due to malignancy. We have made significant discoveries in understanding platelets regulation of tumor growth. Platelets are activated by tumor cells.⁶² Activated platelets release biologically active protein cargo including cytokines and growth factors that support neovascularization and metastasis. Platelet secretion of these factors can be blocked by platelet-targeted therapies like aspirin.⁶³ One cytokine released upon activation is chemokine ligand 5 (CCL5), which interacts with the chemokine receptor type 5 (CCR5) receptor on tumor cells stimulating the pAKT pathway, leading to tumor cell secretion of interleukin-8.⁶⁴ CCL5 also drives megakaryocytopoiesis. By understanding how tumor cells hijack platelets, we will elucidate the platelet's role in malignancy and determine key regulators of megakaryocytopoiesis.

Factor X variants: from outback to bedside

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The venom of several Australian Elapid snakes, including the common or eastern brown snake (*Pseudonaja textilis*), contains procoagulant factor (F)Xa-Va-like enzymes that, once injected into the bloodstream of the prey, convert prey prothrombin into thrombin.⁶⁵ Both venom proteins comprise exceptional structural and functional features,⁶⁶ with venom FXa carrying specific modifications of the substrate-binding aromatic S4 subpocket within its active site that disrupt high-affinity engagement of the direct FXa-inhibiting oral anticoagulants.⁶⁷ Human FX variants comprising a similarly modified S4 subsite resulting either from a single point mutation at position Phe174 (chymotrypsinogen numbering) or insertion of a unique snake venom FXa 99-loop demonstrated a 10- to 100-fold loss of FXa inhibitor-sensitivity.⁶⁷ As these FX variants are able to restore hemostasis in plasma inhibited by the FXa inhibitors, they have the potential to bypass the direct factor Xa inhibitor-mediated anticoagulation in patients that require restoration of blood coagulation.

Microbiota and cardiovascular risk

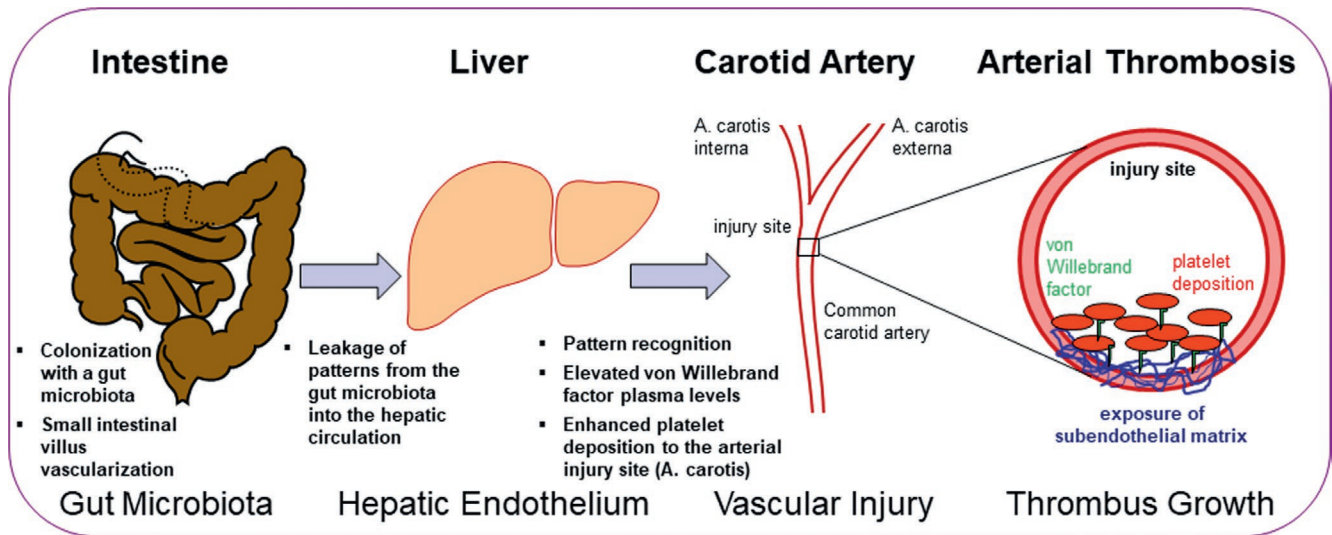


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The gut microbiota is an environmental factor that impacts vascular physiology, locally in the intestine,⁶⁸ but also through remote signaling.⁶⁹ Dependent on diet, numerous mouse studies demonstrated that this gut-resident microbial ecosystem affects atherogenesis.⁷⁰ Atherosclerotic lesion formation can be driven by gut microbial metabolites, such as the choline-metabolite trimethylamine, but also by microbiota-derived microbial-associated molecular patterns that reach the circulation and affect endothelial cell activation and organ-specific immunity.^{69,70} We and others could show a reduced thrombus growth in germ-free mice in various carotid artery injury models, implicating the gut microbiota in arterial thrombosis.^{66,67} The deposition of platelets to the vascular injury site of germ-free mice was diminished due to reduced von Willebrand factor plasma levels and impaired platelet integrin function.⁶⁶ This recent evidence broadens our perception of colonizing gut microbes beyond their established role in bloodstream infections.

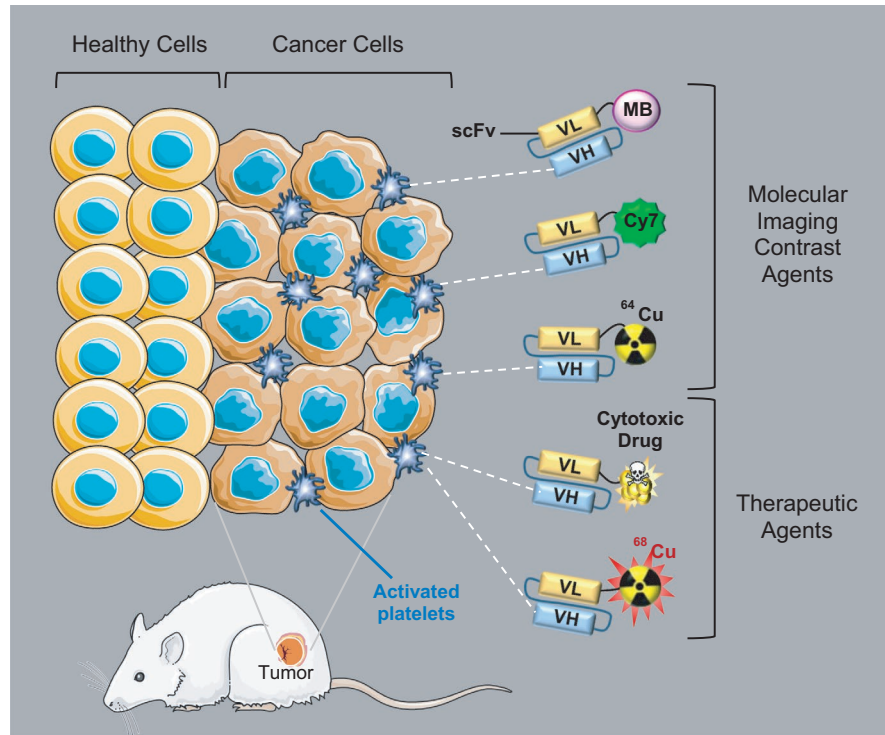
Innovative molecular imaging and drug delivery techniques



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Recently, platelets have attracted major interest in regards to their various roles beyond thrombosis and hemostasis. To study the role of platelets in 2 common diseases, myocardial infarction and cancer, which are major causes of mortality/morbidity, we generated a single-chain antibody (scFv, consisting only of the variable region of the heavy [VH] and light chain [VL]) that specifically binds to activated glycoprotein (GP)IIb/IIIa and thus allows molecular targeting toward activated platelets.

We showed that platelets are highly abundant in areas of cardiac ischemia/reperfusion (I/R). The coupling of the anti-inflammatory, activated platelet-targeted scFv-CD39 construct could indeed fully prevent I/R injury and thus maintain cardiac function.⁷¹

Furthermore, we could show that platelets are highly abundant in tumors and indeed can be used as a target to diagnose cancer, including metastases, either by using ultrasound, fluorescence, or positron emission tomography (PET) imaging (see example in figure).⁷² Most interestingly, targeting platelets with chemotherapeutic drugs or radioactive drugs demonstrates an effective anticancer approach.⁷³

In summary, activated platelets present a highly promising target for diagnostic and therapeutic, or the combination of both, theranostic approaches in I/R and cancer.

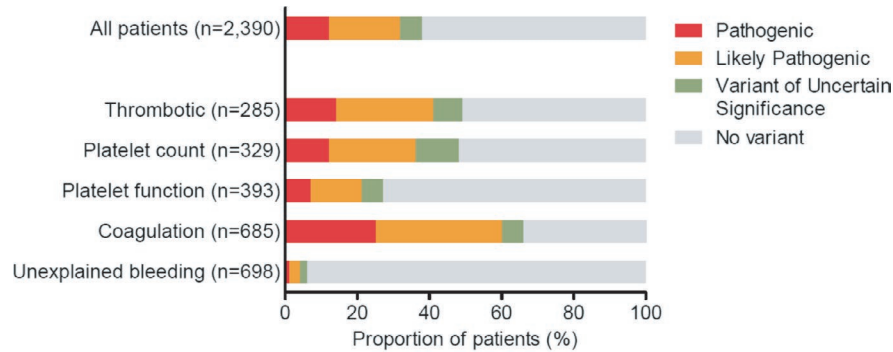
ThromboGenomics



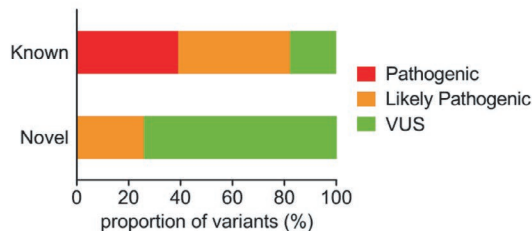
Keith Gomez MD, PhD¹

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Panel A: Genetic Diagnosis Rate by Disorder category in ThromboGenomics



Panel B Classification of Variant Pathogenicity depends on amount of pre-existing data. 'Known' refers to variants previously described in the literature

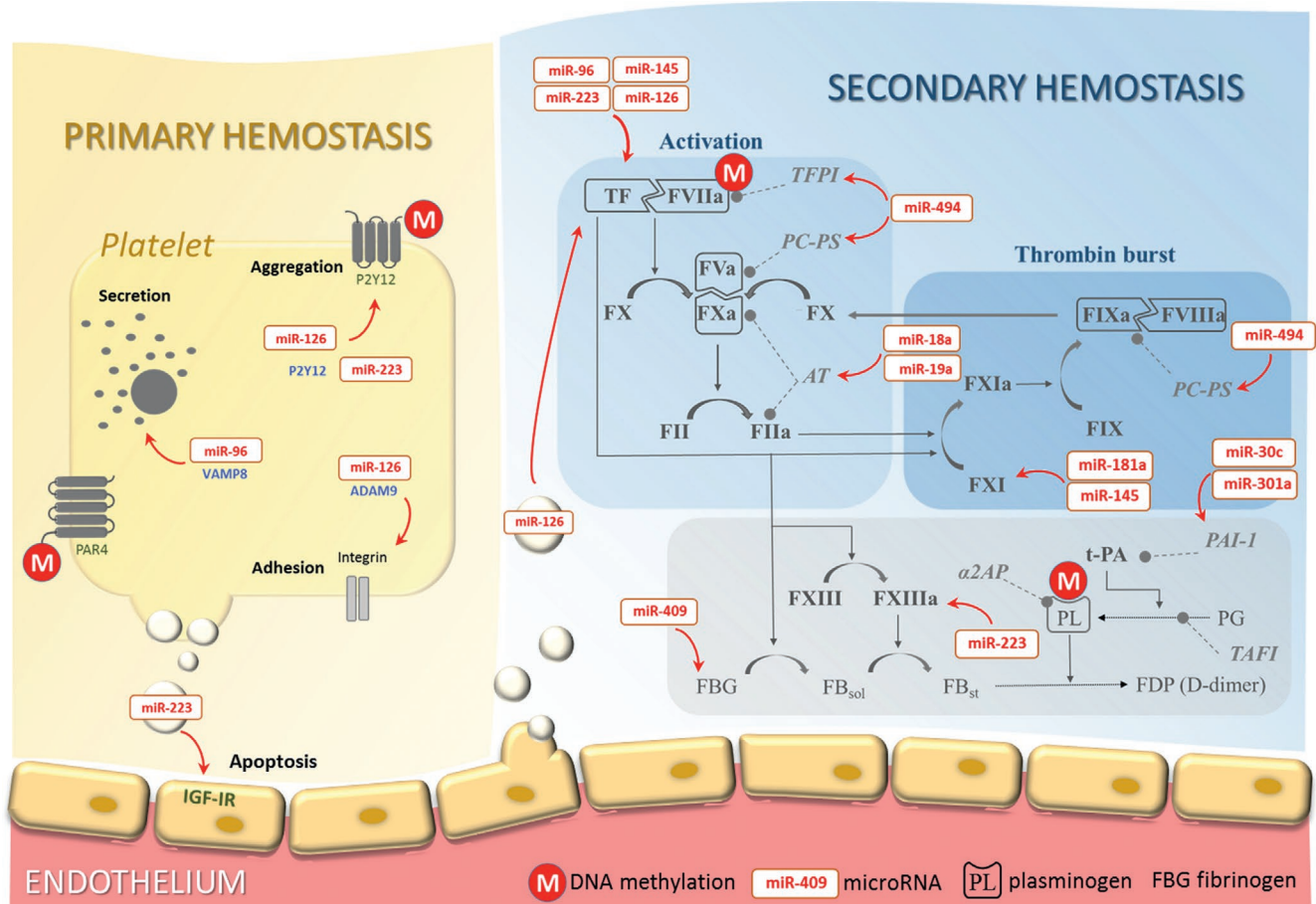


ThromboGenomics was the first high-throughput sequencing (HTS) test covering known genes responsible for monogenic bleeding and thrombotic and platelet disorders. It was introduced in 2015 and has issued reports on 2390 index cases from around the world. The rate of a genetic diagnosis depends on the type of disorder (A). For established monogenic coagulation factor deficiencies, the diagnostic rate was nearly 70%. For heritable thrombotic or platelet disorders, the diagnosis rate was lower. Unexplained bleeding disorders with normal laboratory evaluation and no established molecular mechanism have a low genetic diagnosis rate, perhaps because these have a polygenic or nongenetic etiology. Of 756 unique variants identified, half are novel. Establishing pathogenicity with novel variants is difficult, leading to classification as variants of uncertain significance (VUS; B). VUS can be reclassified as benign or pathogenic through sharing information in online databases. Clinicians need to be aware of this possibility when explaining results to patients.

Epigenetics in hemostasis

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Epigenetics is one of the fastest growing research domains in biomedicine. Recent studies suggested that microRNAs (miRNAs) and DNA methylation may have a clear-cut impact on regulation of the hemostatic balance and, in particular, in development of the prothrombotic state associated with coronary artery disease.⁷⁴ Reliable evidence has been provided that some modifications play a crucial role in triggering endothelial dysfunction, platelet activation, impaired fibrinolysis, and increased values of procoagulant factors, thus opening exciting perspectives for understanding the molecular mechanisms involved in the pathophysiology of hemostasis and also for designing new diagnostic and prognostic disease biomarkers.^{74,75} Epigenetics may also provide complementary, and often more informative, value than that gained from pharmacogenetic and pharmacodynamic studies for predicting response to anticoagulant and antiplatelet therapies.⁷⁶ However, before such promising epigenetic biomarkers will enter clinical practice, multiple challenges need to be addressed, such as the lack of analytical standardization and poor standardization of preanalytical phase.

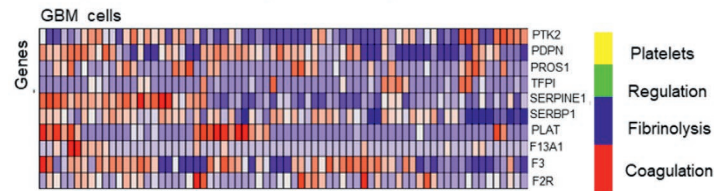
Coagulome, oncogenes, and oncomirs in cancer

Janusz Rak MD, PhD¹

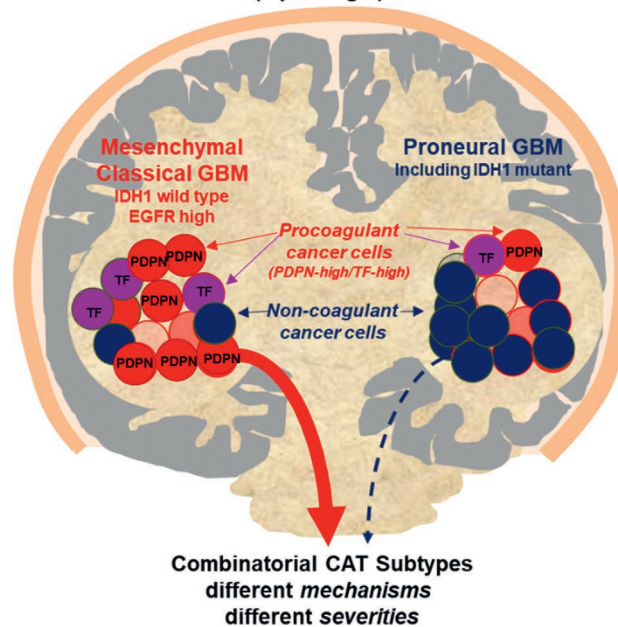
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(A) Single cell mRNA coagulomes in glioblastoma multiforme (GBM)



(B) Combinatorial coagulant heterogeneity model in cancer - GBM (a paradigm)

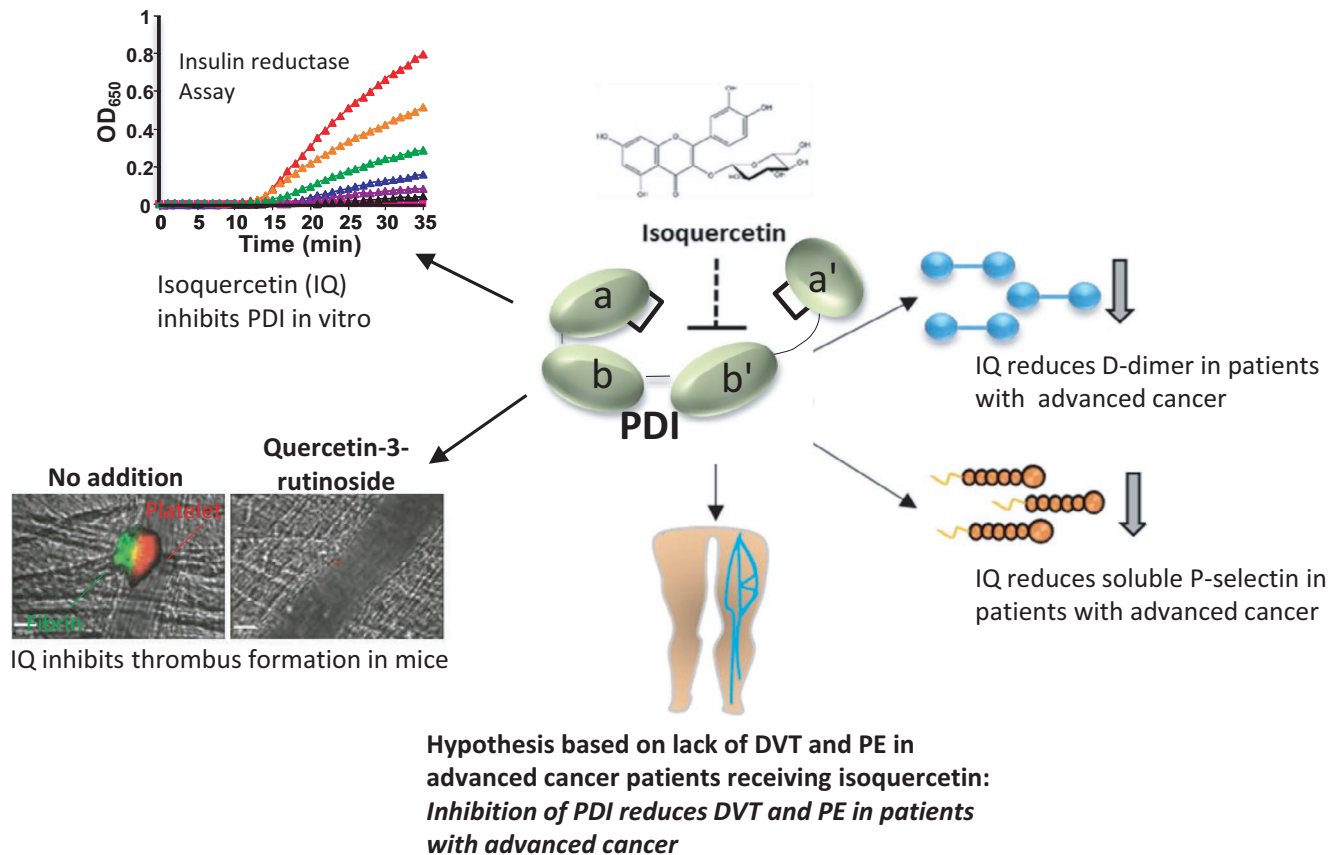


While cancer-associated thrombosis (CAT) represents a complex nexus of cellular, stromal, and microenvironmental influences, it is not an “unspecific” condition. Rather, CAT is a consequence of oncogenic driver events that alter the phenotype of cancer cells and their communication with stroma and the hemostatic system. In glioblastoma multiforme (GBM), this is exemplified by the profile of coagulation-related genes (coagulome), which is a function of molecular subtypes of the disease. Indeed, morphologically similar GBMs (eg, proneural, mesenchymal, or classical) and other brain tumors exhibit different profiles of oncogenes (IDH1, EGFR, oncomirs), as well as coagulomes and risks of thrombosis^{77,78} (A, B). Moreover, each GBM subtype represents a combinatorial mixture of cells with distinct coagulation profiles with enrichment of different effectors, including podoplanin (PDPN), tissue factor (TF), and other regulators.⁷⁹ This coagulant heterogeneity predicts the existence of genetically/epigenetically combinatorial CAT subtypes with different targetable mechanisms and different thrombotic severities.

Thiol isomerases: novel regulators of thrombosis

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Protein disulfide isomerase (PDI) is an essential component of in vivo clot formation. Using a high-throughput screen, we identified rutin and isoquercetin as inhibitors of PDI. These compounds also inhibited in vivo thrombus formation.⁸⁰ Following oral ingestion in humans, isoquercetin inhibited platelet-dependent thrombin generation in a PDI-dependent manner.⁸¹ Isoquercetin was subsequently evaluated in a phase II study of patients with advanced cancer to assess its effect on hypercoagulability.⁸² Isoquercetin ingestion resulted in PDI inhibitory activity in plasma, reduced D-dimer levels, reduced soluble P-selectin levels, and inhibited platelet-dependent thrombin generation ex vivo. While approximately 20% of patients experienced a thrombotic event in a similarly designed study,⁸³ none of the patients receiving isoquercetin demonstrated deep vein thrombosis (DVT) or pulmonary embolism (PE) during this study. Five incidental catheter-associated DVT or superficial clots that did not meet the prespecified venous thromboembolism (VTE) endpoint criteria were detected. There were no severe adverse events attributable to isoquercetin. No major hemorrhages were observed.

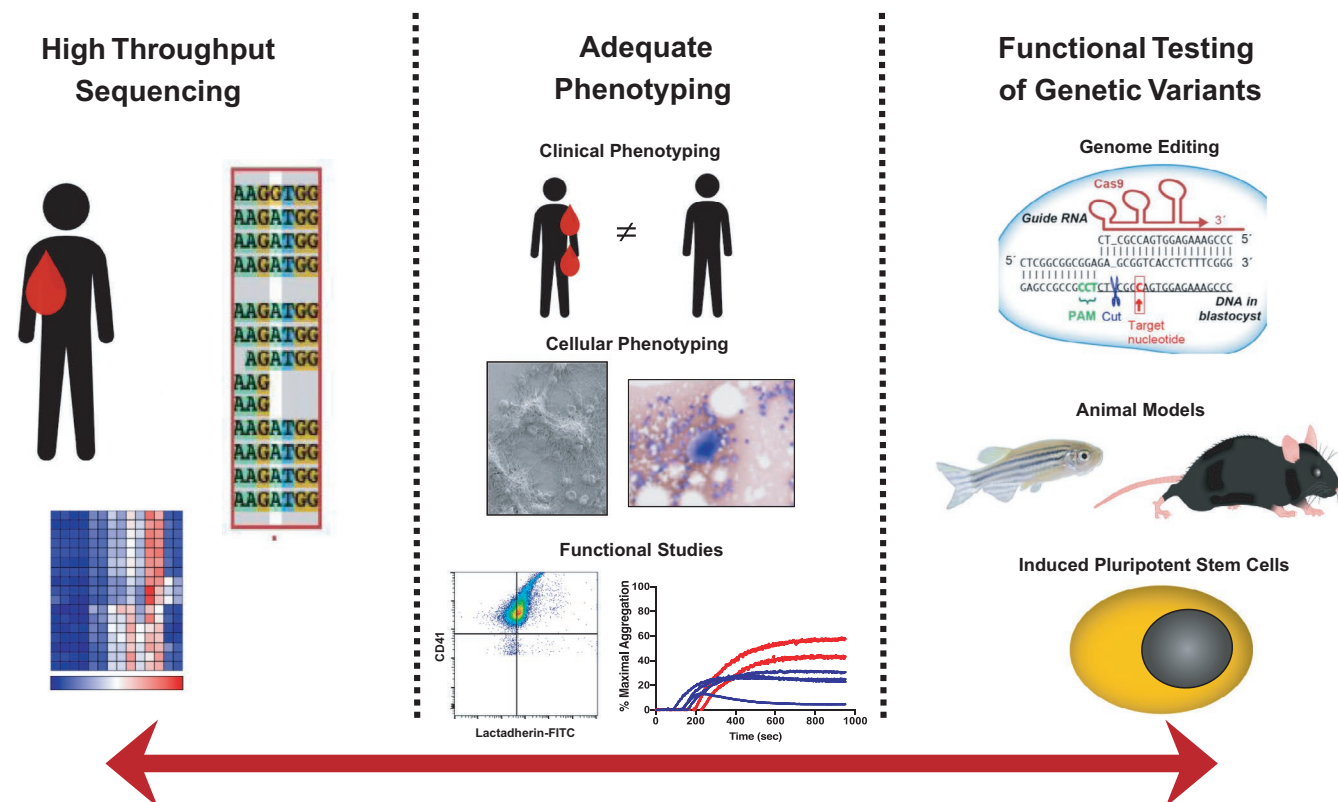
Genomic discovery approaches for inherited bleeding disorders



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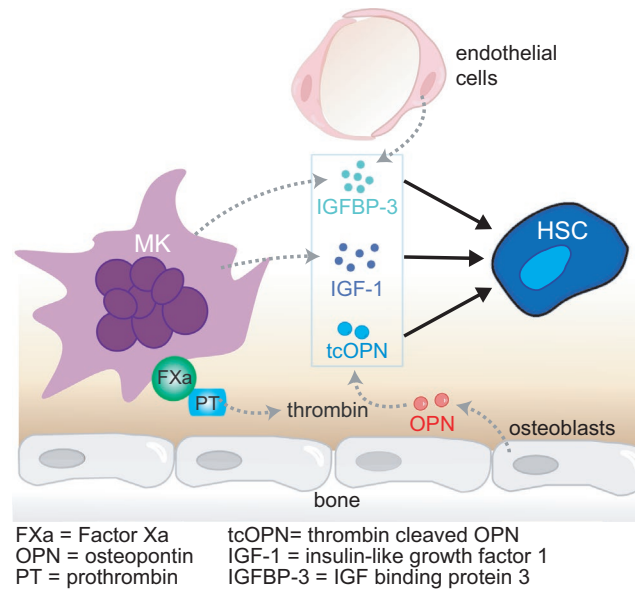


The use of high-throughput sequencing has significantly improved scientists and physicians' ability to characterize the genetic mechanisms of bleeding disorders. Over the past decade, there has been a surge of reports identifying candidate sequence variants not only for single-gene Mendelian diseases but also for complex inherited coagulation and platelet disorders and traits. Large-scale worldwide whole-genome sequencing projects that link genotypes with health records have become invaluable tools in understanding the link between gene mutations and disease. These discoveries have been coupled with genome-wide RNA and chromatin immunoprecipitation sequencing databases, allowing investigators to understand the effect of genetic variants on transcription and chromatin regulation. The recent introduction of efficient genome editing by CRISPR, the generation of relevant animal models and induced pluripotent stem cells from patients have significantly advanced our understanding of the functional consequence of these mutations and their effect on the complex biology of hemostasis.⁸⁴⁻⁸⁶

Interplay between HSCs and megakaryocytes

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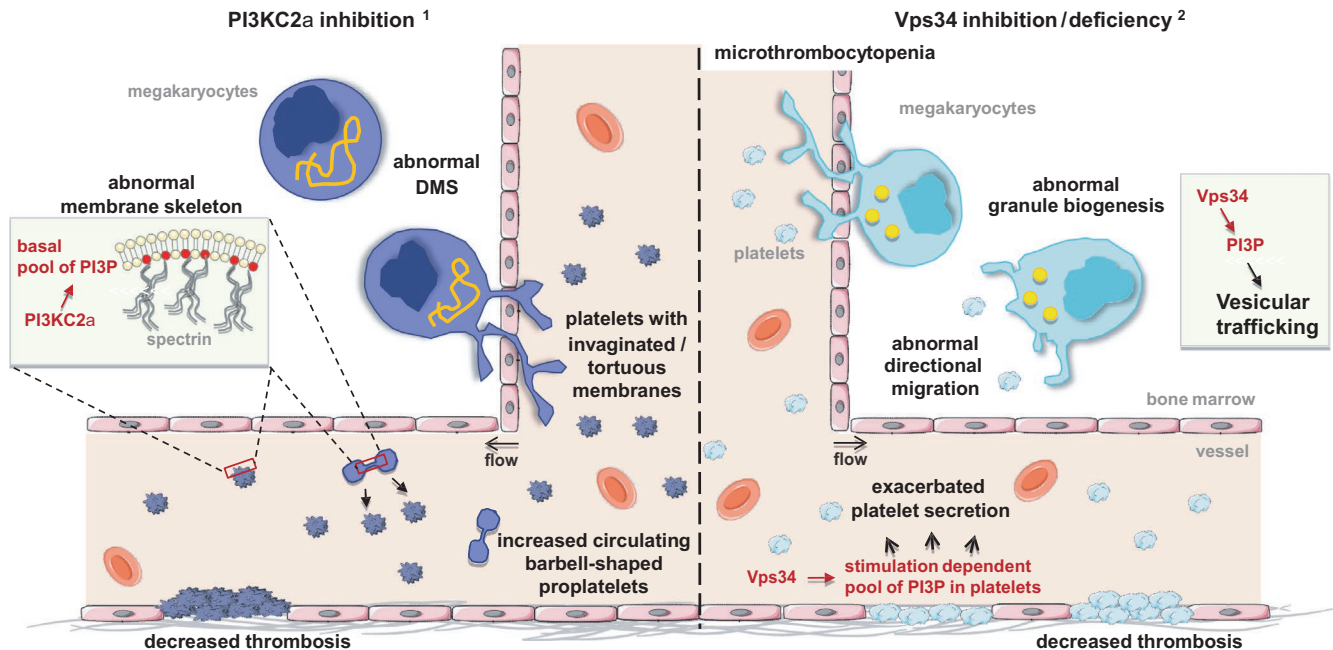


Interactions between hematopoietic stem cells (HSCs) and the cellular and extracellular constituents of the endosteal bone marrow niche are crucial for HSC regulation. However, the precise interactions regulating HSC function remain unclear. Interestingly, we showed that following HSC transplant into nonablated recipients, 68% of transplanted cells lodge within 2 cells of mature megakaryocytes (MKs); large multiploid cells responsible for platelet production that account for approximately 0.1% to 0.2% of marrow cells. MKs have now been identified as key components of the bone marrow (BM) stem cell niche, directly regulating HSC through the maintenance of cell quiescence; which we identified to occur via multiple mechanisms, including thrombin cleaved osteopontin (tcOPN), insulin-like growth factor-1 (IGF-1) and insulin-like growth factor binding protein 3 (IGFBP-3). Furthermore, the absence of mature MKs, coincides with dysregulation of the HSC pool, which in our hands results in a significant increase in marrow HSC and endogenous mobilization.⁸⁷⁻⁸⁹

PI3K function in platelet production

Sonia Severin PhD¹

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The class II PI3K PI3KC2 α and the class III PI3K Vps34 regulate specific pools of their common lipid product, phosphatidylinositol 3 monophosphate (PI3P), and have different implications in platelet production and activation. On one hand, the class II PI3K PI3KC2 α , by regulating a basal pool of PI3P and organizing the spectrin-rich membrane skeleton, is important for maintaining normal platelet membrane morphology/re-modeling that is important for platelet thrombotic capacities.⁹⁰ On the other hand, the class III PI3K Vps34 regulates a stimulation-dependent pool of PI3P involved in control of platelet secretion and arterial thrombus growth. Also, Vps34, by regulating a specific pool of PI3P that controls endocytic/endosomal trafficking, granule biogenesis, and directional migration in megakaryocytes, maintains normal platelet production (platelet granule content and circulating platelet count and size).⁹¹ In summary, PI3KC2 α and Vps34 could be promising targets for antiplatelet therapy as their inhibition may decrease thrombosis without increasing bleeding risk.

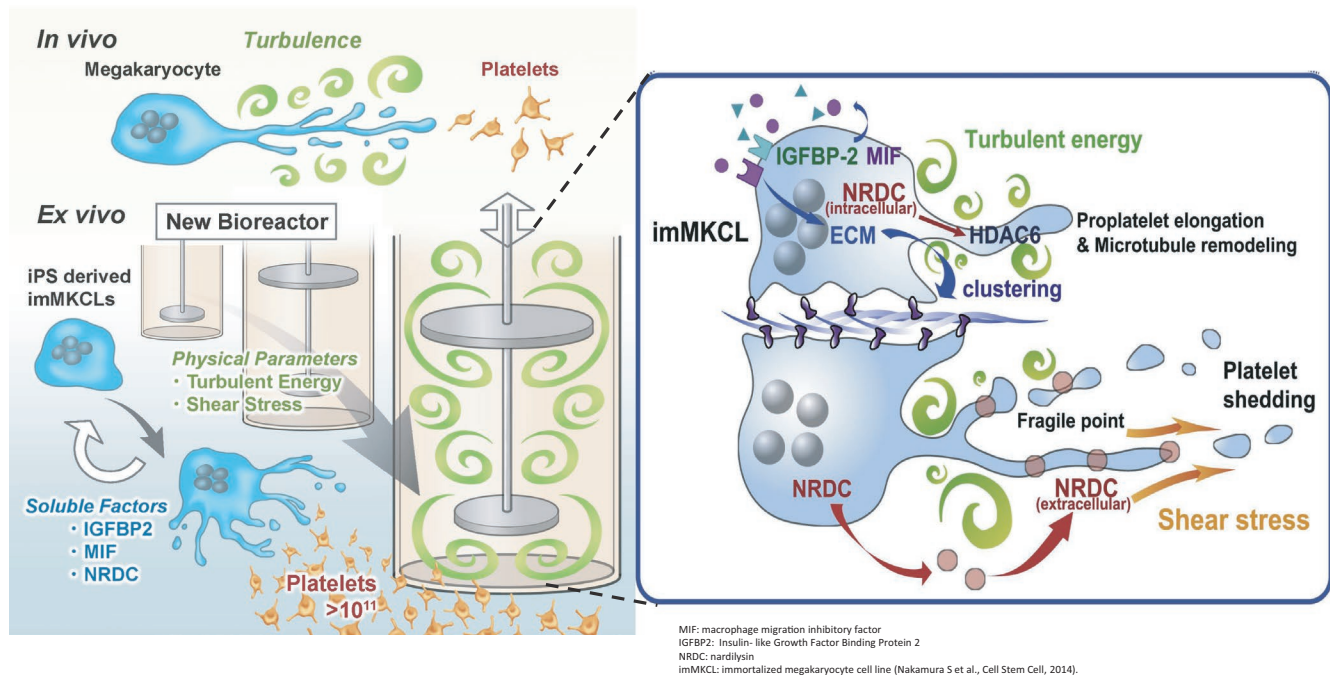
Beyond ex vivo platelet biogenesis

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Turbulence dependent Platelet Generation ex vivo



To overcome the limitations in supply and safety of current platelet transfusion products, the ex vivo production of human platelets using pluripotent stem cells has been developed.⁹² One of strategies is to establish expandable megakaryocyte lines as a source of cyclic guanosine monophosphate (cGMP)-manufacturing platelets.⁹³ Additionally, industrial scaling up of manufacture needs a new concept on the development of bioreactor. Recently, we found by in vivo observation in mice that (1) turbulent flow is present at the active bone marrow megakaryocyte sites, and (2) optimal ranges of turbulent energy and shear stress under turbulent flow conditions are required for ex vivo intact platelet manufacturing at levels of 100 billion order.⁹⁴ We further elucidated the new mechanism whereby turbulent flow released 6 factors from megakaryocytes, where insulin-like growth factor-binding protein 2 (IGFBP2), macrophage migration inhibitory factor (MIF), and nardilysin mostly contributed to platelet shedding. Figure shows development of bioreactor system (left), and hypothesized illustration of platelet biogenesis (right).

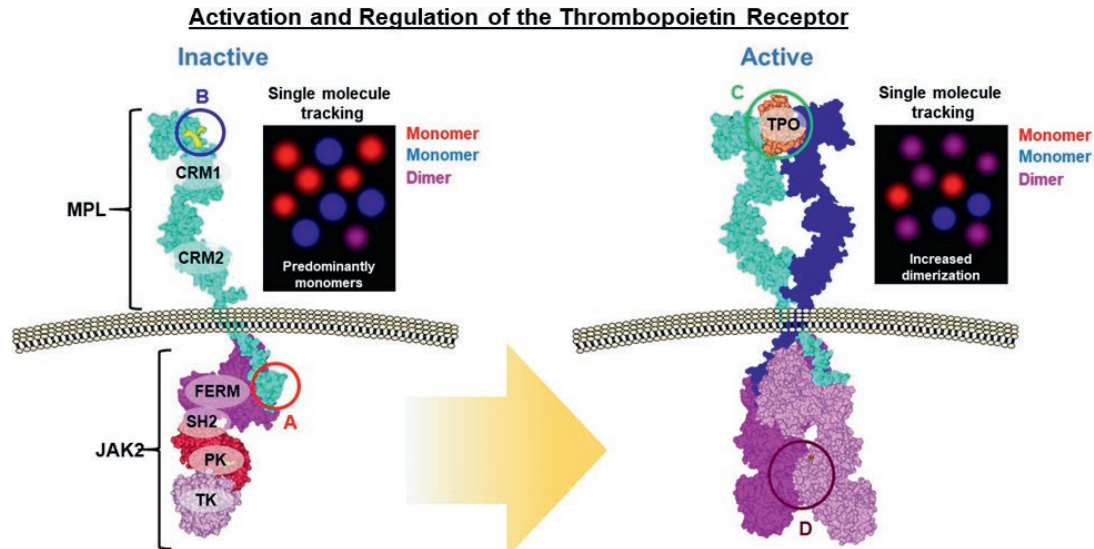
Activation and regulation of the thrombopoietin receptor



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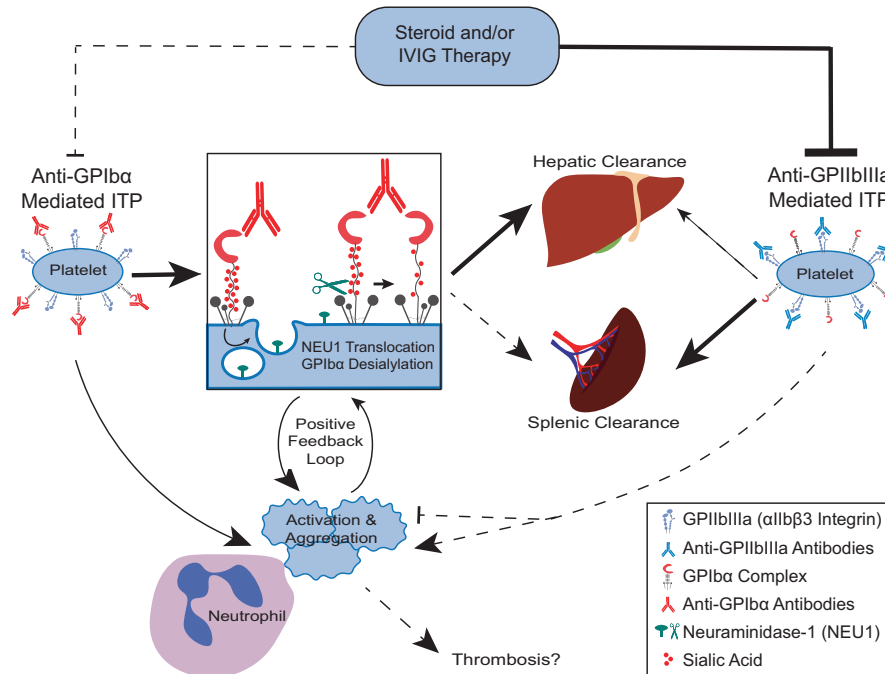
Thrombopoietin (TPO) and its receptor, MPL, are critical for hematopoietic stem cell maintenance and megakaryocyte differentiation. Similar to other homodimeric type I cytokine receptors, MPL lacks intrinsic kinase activity, instead associating with the nonreceptor tyrosine kinase Janus kinase 2 (JAK2) via Box1/2 motifs in the intracellular domain of MPL and the FERM domain of JAK2 (A).⁹⁵ Residues at the N-terminus of the extracellular domain are essential for TPO-binding and receptor glycosylation (B).⁹⁶ The current dogma suggests that MPL exists at the plasma membrane as a preformed dimer.⁹⁷ However, using single imaging, we now have conclusive data that MPL is present predominantly in monomeric form and dimerize following association with TPO (C). Furthermore, we have found that intermolecular interactions between the JAK2 pseudokinase (PK) domains stabilise dimerization (D), which is further potentiated by oncogenic mutations at the PK-PK interface.

Mechanisms of Fc-independent immune thrombocytopenia

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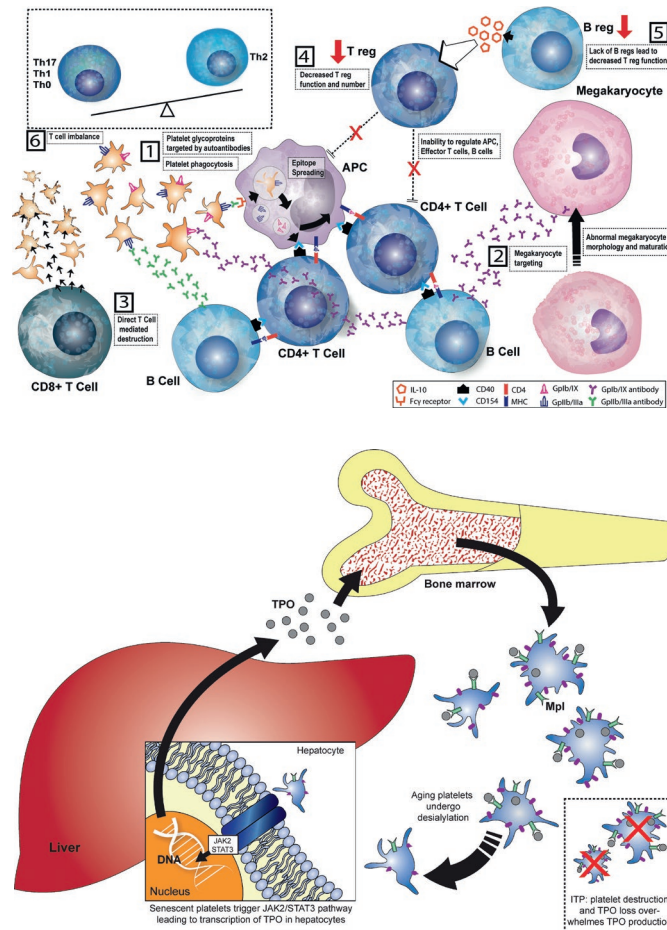


Immune thrombocytopenia (ITP) is an autoimmune bleeding disorder characterized primarily by antibody-mediated platelet destruction. The major platelet autoantigens targeted in ITP are localized on glycoprotein (GP)IIb/IIIa (α IIb β 3 integrin) and the GPIb α complex. Platelet destruction is thought to be mediated by an Fc-dependent pathway, where the Fc-portion of platelet-associated antibodies and Fc receptors on macrophages (eg, in spleen) interact, initiating phagocytosis and opsonized platelet destruction. Interestingly, Dr Nieswandt and our group found that anti-GPIb α antibodies can cause Fc-independent thrombocytopenia, which is more resistant to intravenous immunoglobulin (IVIG) and steroid therapies.^{98,99} We further found that anti-GPIb α and some anti-GPIIb/IIIa antibodies can induce platelet activation, sialidase neuraminidase-1 (NEU 1) translocation and desialylation, leading to platelet clearance in the liver via Ashwell-Morell receptors. This platelet clearance can be inhibited by sialidase inhibitors.¹⁰⁰ These findings shed light on Fc-independent thrombocytopenia, designating desialylation as a potential diagnostic/prognostic biomarker and therapeutic target for refractory ITP treatment.

Immune thrombocytopenia in children

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The pathophysiology of ITP is complex and incompletely understood. There are many mechanisms, which can vary among patients (Top Panel):

1. Autoantibodies target platelet glycoproteins. Opsonized platelets bind Fcγ receptors on antigen presenting cells (APCs), resulting in phagocytosis. APCs express platelet glycoproteins, which are recognized by CD4⁺ T cells. T-cell clones interact with B cells and propagate antibody production.¹⁰¹
2. Antibodies can target megakaryocytes, reducing mature megakaryocytes and causing abnormal maturation.¹⁰¹
3. Cytotoxic T cells can target platelets.¹⁰²
4. T-regulatory cells are decreased in number and function.¹⁰²
5. B-regulatory cells are decreased and less responsive to cytokines.¹⁰²
6. T-cell balance is shifted toward CD4⁺ Th₀/Th₁ and Th₁₇ activation and decreased Th₂ responses.^{101,102}

Hepatic platelet clearance may be an Fc-independent mechanism of platelet clearance (Bottom Panel).¹⁰³ GP1b antibodies can induce Fc-independent platelet clearance through desialylation. Desialylated platelets bind hepatic Ashwell-Morell receptors, then undergo phagocytosis and subsequent removal from circulation.

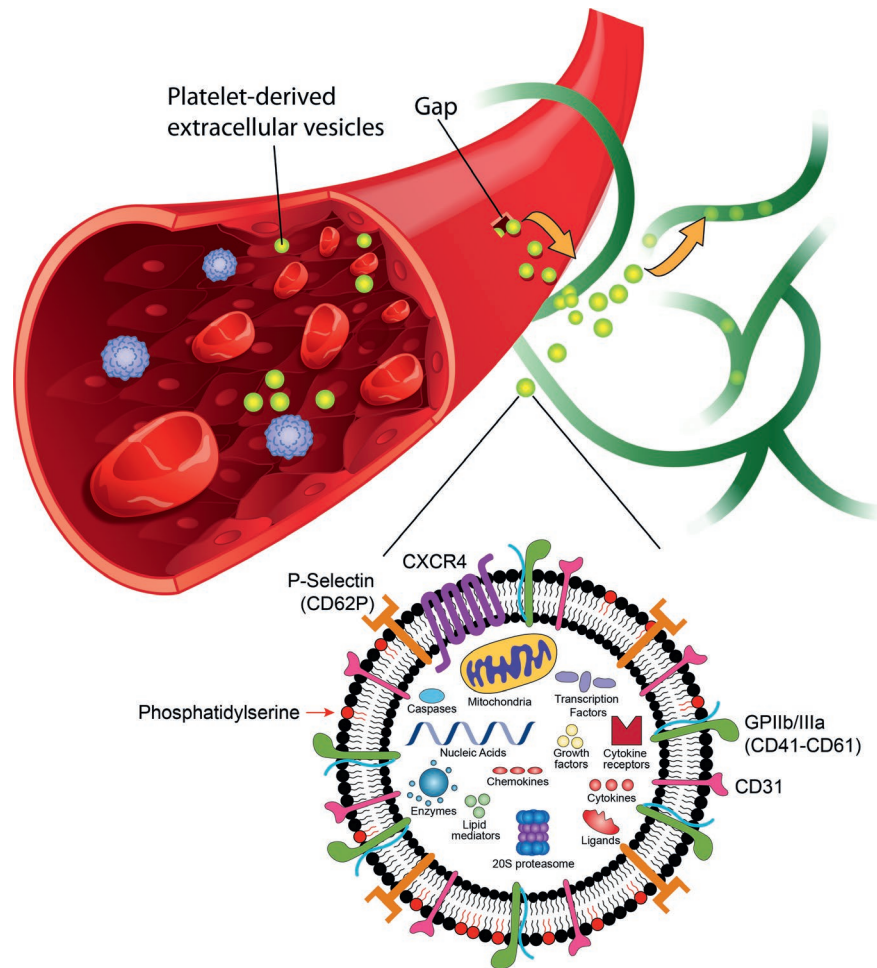
Figure design by Taylor Kim MD

Platelet-derived extracellular vesicles disseminate platelet organelles in blood and lymph

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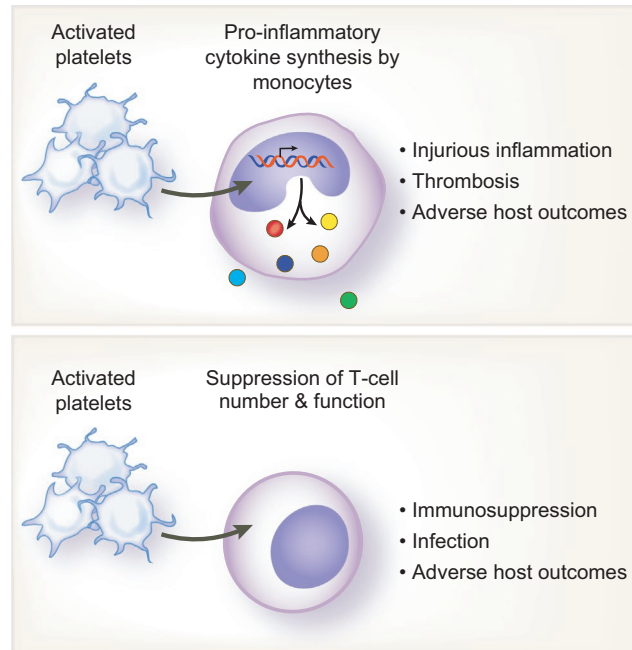
Platelet extracellular vesicles (EVs), also known as microparticles, harbor platelet receptors, and a subpopulation of them express surface phosphatidylserine. Platelet EVs contain a vast repertoire of molecules, such as cytokines, growth factor, lipid mediators, transcription factors, enzymes, and nucleic acids (eg, messenger RNA and microRNA).¹⁰⁴ Moreover, some platelet EVs contain mitochondria and recent investigations highlight the presence of functional 20S proteasome. During inflammation, the blood vasculature (in red in the schematic) is more leaky and favors the egress of the platelet EVs outside the blood vessels through gaps formed between endothelial cells.¹⁰⁵ However, exactly how EVs escape the blood circulation and the exact location of the egress is not completely understood. While platelets are absent in lymph, platelet EVs are very abundant in lymphatic circulation (in green in the schematic) during inflammation.¹⁰⁶ As platelet EVs contain organelles and molecules from platelets, platelet EVs can disseminate platelet components in tissue locations normally unreachable by platelets through lymph.

Influence of platelets on other cells: mechanisms and consequences



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Activated platelets interact with and signal to leukocytes. (Top panel) Platelet interactions with monocytes, through P-selectin and PSGL1, triggering proinflammatory cytokine synthesis by monocytes. In some settings, this may contribute to thromboinflammation and injurious host responses. (Bottom panel) During sepsis, platelets may signal to T cells, leading to suppression of T-cell number and function, and contributing to the risk of secondary infections and adverse host outcomes. (Illustration by Diana Lim)¹⁰⁴⁻¹⁰⁶

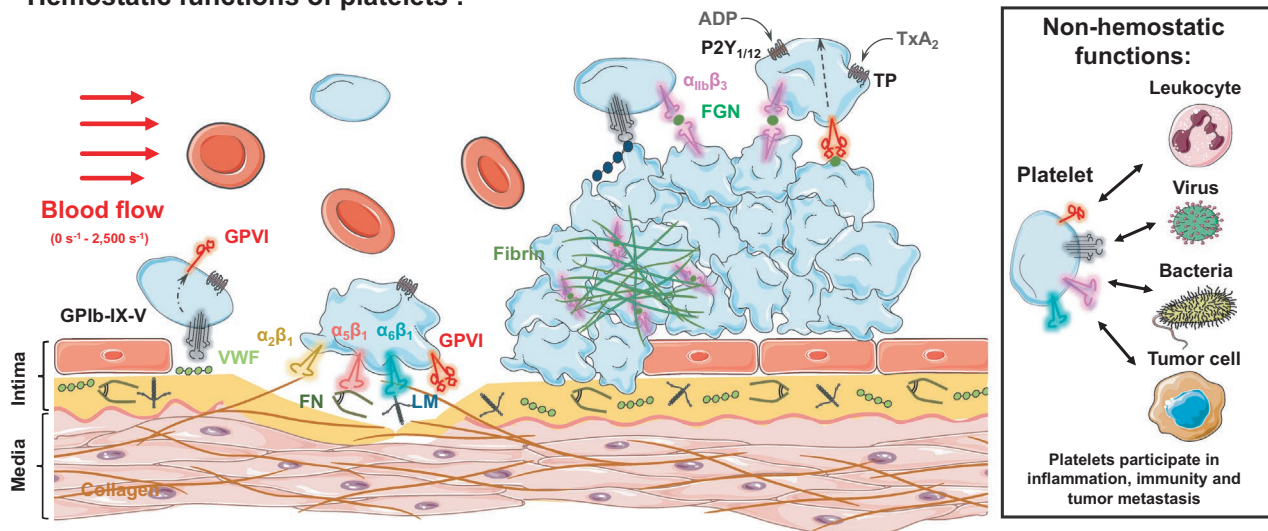
The role of platelet adhesion receptors in hemostasis and beyond

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Hemostatic functions of platelets :



Platelet adhesion at site of injury is challenged by the rheological conditions and the complex composition of the vessel wall. Under elevated blood flow (>1000/s), the glycoprotein (GP)Ib-IX-V complex is the only receptor that supports platelet recruitment to immobilized von Willebrand factor (VWF). Under low shear, this receptor teams up with integrins to sustain their adhesion and initiate activation sufficient to dimerize GPIIb/IIIa, which in turn binds collagen and initiates strong activation, directly through its signaling cascade and indirectly through released ADP and thromboxane A₂ (TxA₂). The GPIb-IX-V complex and integrin α IIb β 3 are also instrumental in thrombus growth, allowing platelet recruitment through VWF and fibrinogen bound to activated platelets, respectively.¹¹⁰ GPIIb/IIIa plays a central role in thrombus buildup through its ability to promote and sustain platelet activation.¹¹¹ The ability of platelets to interact with their adhesion receptors to counter receptors on pathogens, immune cells, or tumor cells also contributes to their nonhemostatic functions. FN, fibronectin; FGN, fibrinogen; LM, laminins.

Platelet protease-activated receptors (PARs): function and targeting



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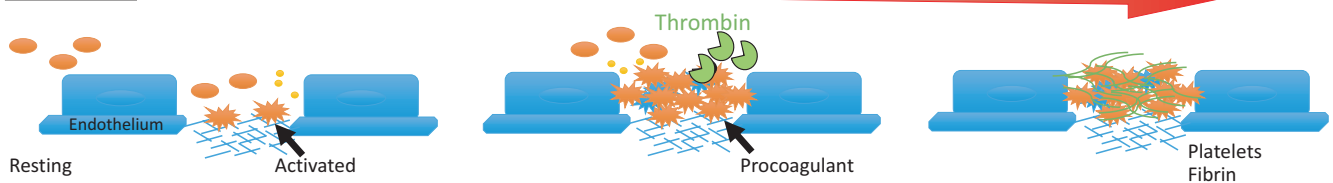
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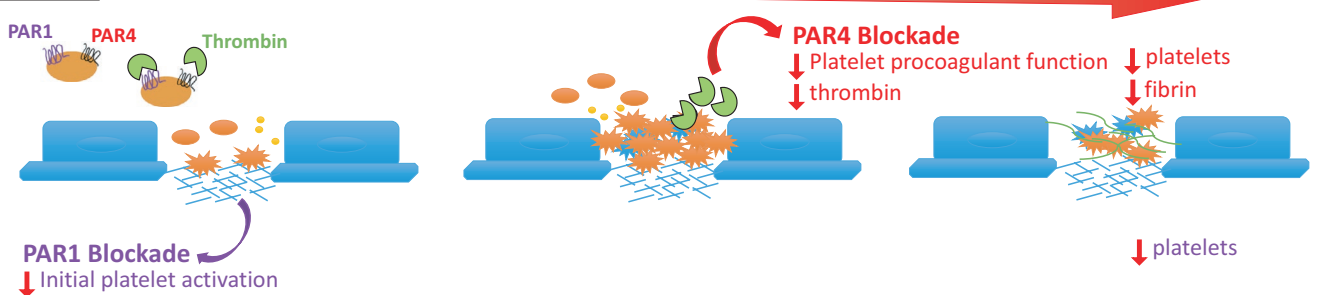
Platelet activation



Thrombosis



Thrombin

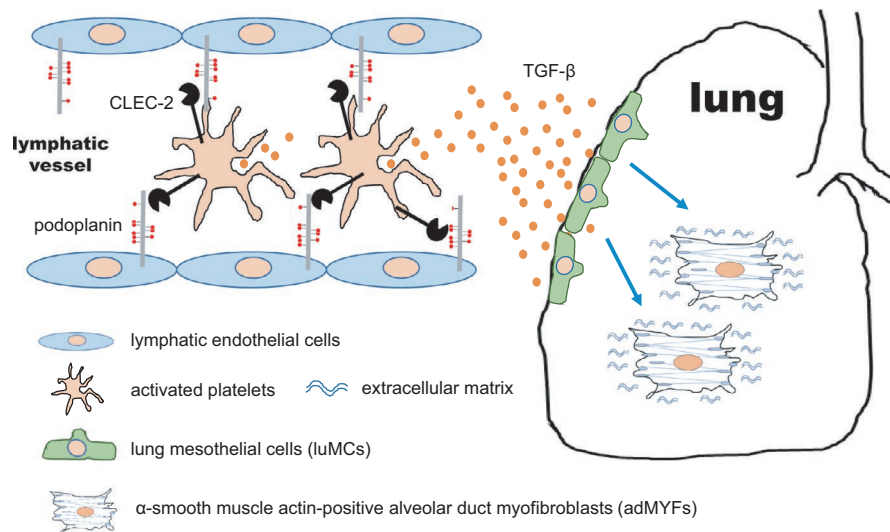


Thrombin-induced platelet activation is mediated via the two protease-activated receptors, PAR1 and PAR4, and is central to arterial thrombosis. Both PARs are targets of antiplatelet drugs: a PAR1 antagonist (vorapaxar) is approved for prevention of myocardial infarction and peripheral arterial disease,¹¹² while PAR4 antagonists are in clinical trial.¹¹³ PAR1 is a higher-affinity thrombin receptor but blockade of this receptor with vorapaxar increases bleeding when administered in addition to standard-of-care therapy. In contrast to all other current antiplatelet approaches, including PAR1, PAR4 blockade prevents thrombosis by inhibiting the late-stage platelet activation events of phosphatidylserine exposure and platelet procoagulant function.¹¹⁴ Early platelet responses to low thrombin concentrations are triggered by PAR1 and are more important for hemostasis, while later platelet responses to higher thrombin concentrations are triggered by PAR4 and are more important for thrombosis, suggesting that PAR4 is a target for safe and effective antiplatelet therapy with a unique mechanism of action.

Platelet CLEC-2 and lung development

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We have shown that platelets facilitate lung development through the interaction between the platelet activation receptor C-type lectin-like receptor-2 (CLEC-2), and its ligand membrane protein, podoplanin.¹¹⁵⁻¹¹⁷ CLEC-2 deletion in mouse platelets led to neonatal lethality due to lung malformation and respiratory failure. In the primary alveolar septa of these embryos, α -smooth muscle actin-positive alveolar duct myofibroblasts (adMYFs) were almost absent, which was caused by abnormal differentiation of lung mesothelial cells (luMCs) into adMYFs. In the developing lung, podoplanin expression was detected in alveolar epithelial cells, luMCs, and lymphatic endothelial cells (LECs), but only specific deletion of podoplanin from LECs resulted in neonatal lethality and lung malformation. These lung abnormalities were also observed after thrombocytopenia or transforming growth factor- β (TGF- β) depletion in fetuses. We propose that the interaction between platelet CLEC-2 and LEC podoplanin stimulates differentiation of luMCs into adMYFs through TGF- β released from activated platelets.

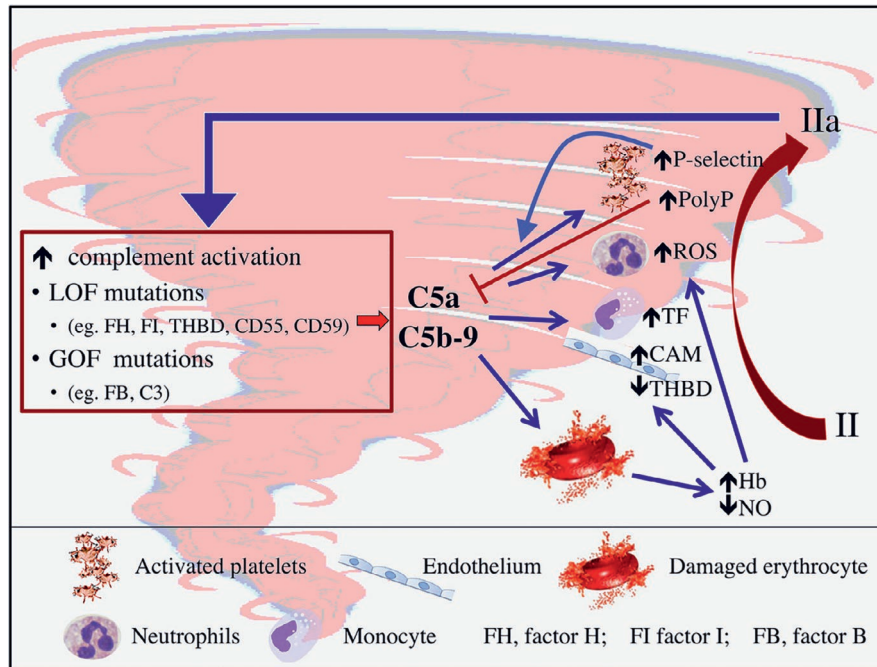
Molecular links between coagulation and innate immunity



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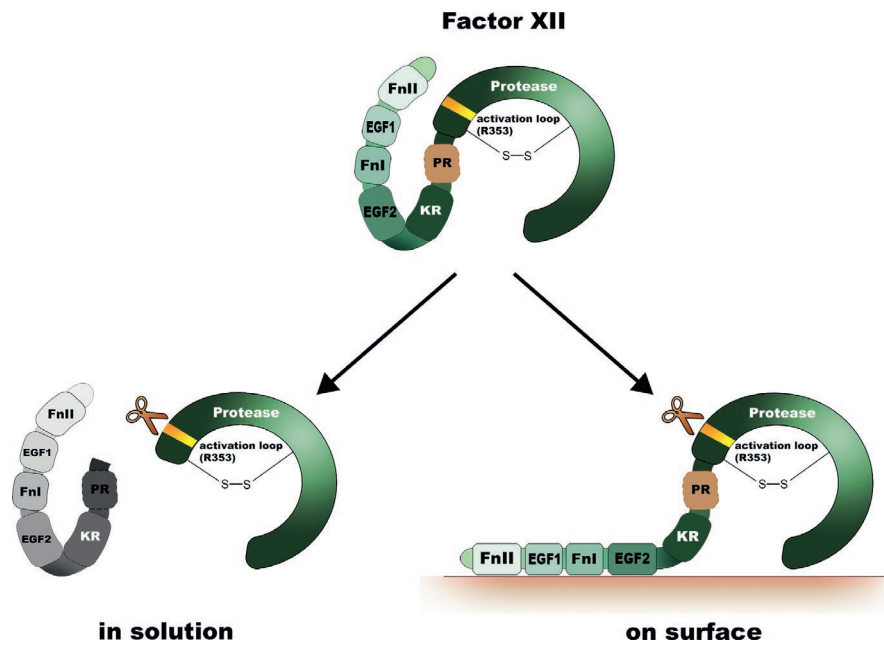


Inflammatory, infectious, malignant, metabolic, and thrombotic diseases often feature simultaneous excess activation of complement and coagulation.¹¹⁸ Mechanisms by which a positive feedback loop escalates to generate damaging amounts of thrombin (IIa), are exemplified in atypical hemolytic uremic syndrome (aHUS) and paroxysmal nocturnal hemoglobinuria (PNH), caused by gain-of-function (GOF) and loss-of-function (LOF) mutations in complement proteins.^{119,120} Following an ill-defined trigger, complement activations leads to excess formation of biologically active C5a and C5b-9. These induce prothrombotic and proinflammatory effects including exposure of tissue factor (TF), leukocyte adhesion molecules (CAM) and P-selectin, release of reactive oxygen species (ROS) and polyphosphate (PolyP), reduced thrombomodulin (THBD) expression, erythrocyte release of hemoglobin (Hb), and reduced bioavailability of nitric oxide (NO). These disturbances converge to cause a sustained increase in thrombin generation, with widespread microvascular thrombosis and further activation of complement. Without rapid and appropriate intervention, this positive feedback loop persists and escalates to cause multiorgan damage.

Contact pathway activation: an unfolding story

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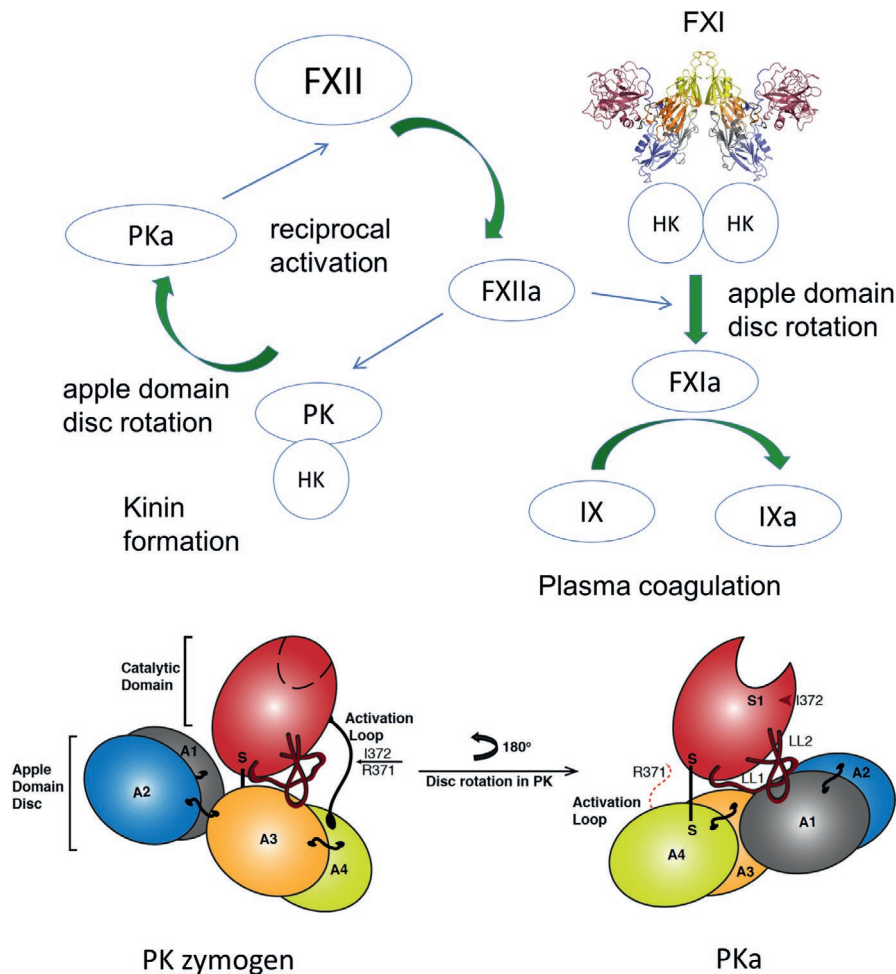
The contact pathway depends on factor XII (FXII) activation. FXII changes conformation during surface binding and becomes susceptible to activating cleavage.¹²¹ This suggests that the activation loop of FXII is usually shielded in solution, protecting it from misdirected activation. Human mutations can disturb this mechanism and cause disease. These are mostly located in or around the proline-rich region of FXII. At least 3 separate mutations make FXII vulnerable to enzymatic truncation, which exposes the activation loop.^{122,123} Subsequent accelerated FXII activation causes hereditary angioedema, an acute inflammatory disorder caused by excessive production of bradykinin. We have now identified a new, chronic inflammatory disorder that is caused by a FXII mutation. The mutation influences the conformation of FXII, leading to continuous exposure of the activation loop and constitutive low-grade systemic contact activation. In conclusion, the conformation of FXII is of critical importance for control over the contact pathway.

Scissors indicate activating cleavage events, which can for example be executed by plasma kallikrein, activated FXII or plasmin. The unfolding of FXII has (so far) been demonstrated to expose its activation loop on dextran sulfate polymers (indicated by "Surface"). EGF-1, EGF-like domain 1; EGF-2, EGF-like domain 2; FnI, fibronectin type 1 domain; FnII, fibronectin type II domain; KR, Kringle domain; PR, proline-rich region; Protease, protease domain.

Structure and function of FXI/FXII

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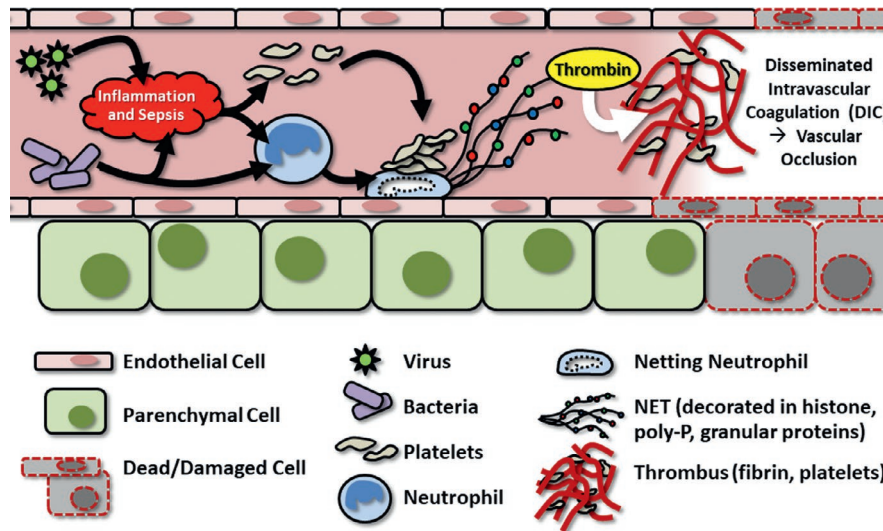


The contact activation system (CAS) is central to the crosstalk between coagulation and inflammation and contributes to diverse disorders affecting the cardiovascular system.¹²⁴ CAS initiation contributes to thrombosis but is not required for hemostasis and can trigger plasma coagulation via the intrinsic pathway (factor XI [FXI]). Activation of contact factor FXII can initiate proteolytic cleavages of FXI and prekallikrein (PK). PK is a plasma protease that performs a double cleavage of substrate kininogen to release the nonapeptide bradykinin (BK). The crystal structure of the activated full length PKa reveals a remarkable conformational change of apple domain disc rotation when compared to the homologous zymogen structure of coagulation FXI.¹²⁵ Apple domain disc rotation may underlie key processes of auto-activation, FXII reciprocal activation and substrate recruitment. The in-depth knowledge of the 3-dimensional structure of the contact factors provides a platform to understand the molecular basis of contact activation and develop novel therapeutics.

Platelets, NETs, and coagulation

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In response to a wide variety of infections and inflammatory injury, both platelets and neutrophils are activated and recruited to the site of insult. This cellular activation can be the result of direct interaction with the pathogen (or pathogen-associated molecular patterns [PAMPs]) or secondarily via cytokines and damage-associated molecular patterns (DAMPs) released from immune sentinels (ie, Kupffer cells) or damaged host tissues.¹²⁶ These activated neutrophils adhere to the vasculature and interact with circulating platelets via integrins. Platelets aggregate on the surface of the neutrophil, providing further activating signals that trigger the release of neutrophil extracellular traps (NETs).¹²⁷ Nets are interwoven structures of extracellular DNA decorated in both nuclear (histone) and granular proteins (neutrophil elastase, myeloperoxidase, etc). Although NETs capture, sequester, and kill pathogens, this potent mix of cytotoxic proteins can damage host tissues and can directly activate thrombin.¹²⁸ This NET-induced thrombin leads to the generation of thrombi within the microvasculature, obstructing blood flow and resulting in ischemic tissue damage leading to organ dysfunction and adverse patient outcomes.

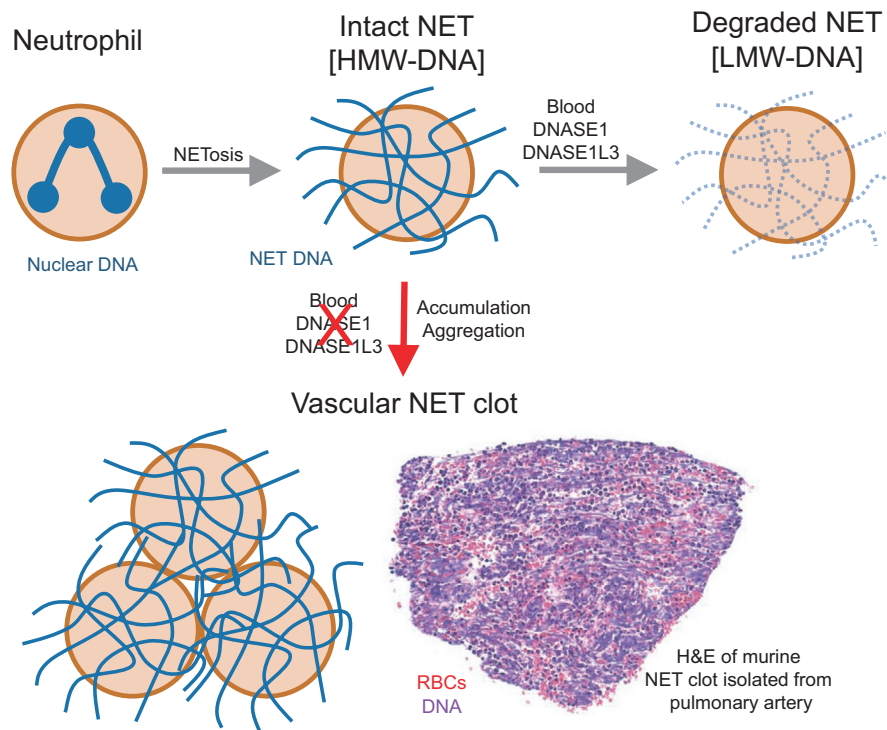
Circulating DNases prevent vascular occlusion by neutrophil extracellular traps



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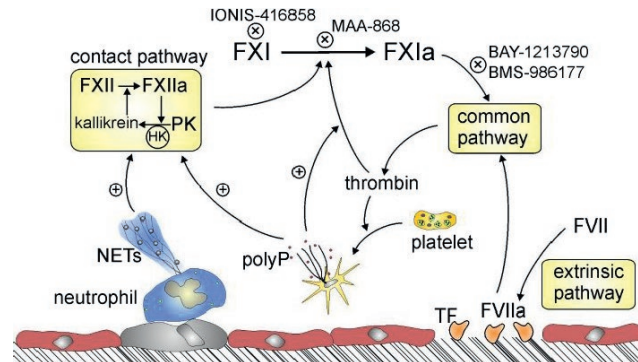


Host factors limit inflammation to prevent tissue injury and mortality. Neutrophils are the first leukocytes recruited to sites of infection and sterile inflammation. At infected sites, neutrophils generate extracellular traps (NETs) to immobilize and neutralize pathogens.¹²⁹ NETs are lattices of high-molecular-weight DNA (HMW-DNA) that carry biologically active molecules. In addition to their antimicrobial function, NETs can harm host cells. Indeed, excessive NET formation (NETosis) is associated with numerous inflammatory and autoimmune diseases.¹³⁰ To prevent disease, blood contains 2 DNA degrading enzymes, DNase1 and DNase1-LIKE 3 (DNase1L3), which cleave HMW-DNA of NETs into low-molecular-weight DNA (LMW-DNA).¹³¹ In the absence of circulatory DNases, intravascular NETs accumulate and aggregate during neutrophilic inflammation. Clots of NETs are sufficient to occlude blood vessels and cause ischemic organ injury in models of sterile neutrophilia and septicemia. Therapeutic targeting of NET clots may limit vascular occlusion and host injury in inflammatory diseases.

Clinical trials with factor XI inhibitors

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The contact pathway can be activated by polyphosphate (polyP) and neutrophil extracellular traps (NETs) released from activated platelets and neutrophils, respectively. In the presence of polyanions, factor (F) XI also is activated by thrombin generated via the extrinsic pathway when tissue factor (TF) is exposed. Thus, FXI bridges the contact and common pathways and allows feedback activation from the extrinsic pathway. FXI inhibitors currently undergoing phase 2 evaluation include the antisense oligonucleotide IONIS-416858 that inhibits FXI biosynthesis, MAA-868, a monoclonal antibody that binds FXI and prevents its activation, BAY-1213790, a monoclonal antibody that inhibits FXIa and BMS-986177, a small molecule that targets the active site of FXIa. (Figure adapted from Weitz JI and Fredenburgh JC. *Front. Med.* 4:19. <https://doi.org/10.3389/fmed.2017.00019>).

Factor XI-directed strategies currently undergoing phase 2 evaluation

Class	Compounds	Mechanisms of action	Administration	Onset/Offset
Antisense oligonucleotide	IONIS-416858	Reduces hepatic synthesis of FXI by inducing catalytic degradation of FXI mRNA	Parenteral	Slow onset and offset
Monoclonal antibodies	BAY1213790, MAA868	Suppress FXIa generation and/or inhibit FXIa activity	Parenteral	Rapid onset and slow offset
Small molecule	BMS-986177	Binds to the catalytic domain of FXIa	Oral	Rapid onset and offset

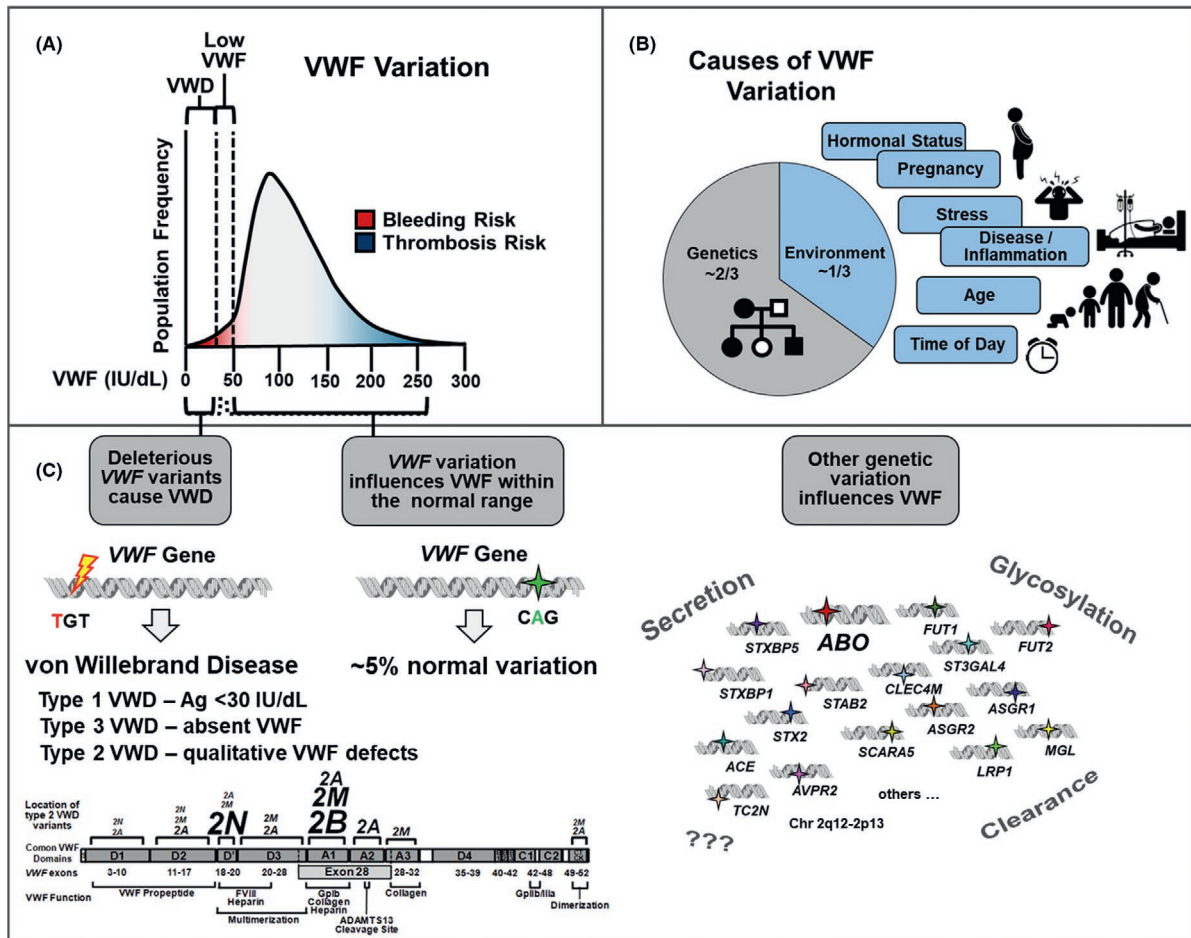
Modifiers and genetics of VWF



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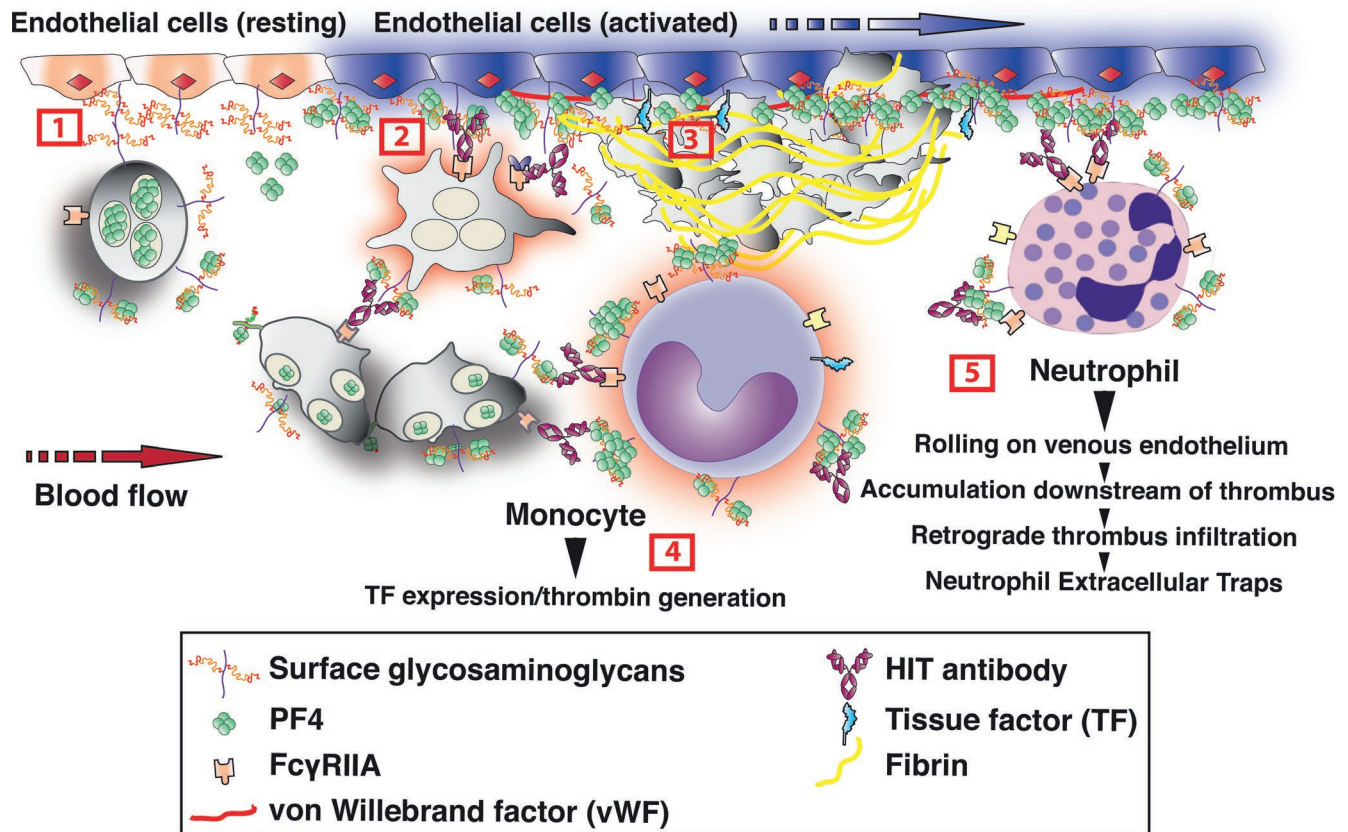


(A) Plasma von Willebrand factor (VWF) antigen levels vary widely in the normal population (50–200 IU/dL). (B) About one-third of this variation is due to environmental/acquired conditions. Some are dramatic, such as in pregnancy, when VWF increases by > 200% late in gestation. Other variation can be more subtle (eg, with aging). The other two-thirds of VWF variation is attributable to genetic causes. (C) In von Willebrand disease (VWD), deleterious VWF gene variants (indicated by a lightning bolt) are commonly identified, particularly in patients with severe disease. Non-disease-causing DNA variants (indicated by stars) can also influence VWF. VWF gene variation accounts for approximately 5% of VWF variation the population. ABO blood group accounts for approximately 25% to 30% of VWF variation. Numerous other non-VWF and non-ABO genes have been implicated to influence VWF through multiple mechanisms including secretion, glycosylation, and clearance. However, a proportion of VWF variation remains unexplained.¹³⁵⁻¹³⁷

Endothelial cell contribution to the pathology in HIT

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Heparin-induced thrombocytopenia (HIT) is a prothrombotic disorder caused by immune complexes that activate platelets, monocytes, neutrophils and vascular endothelium. The glycocalyx expressed on healthy endothelial cells¹³⁸ offers a vast antithrombotic surface that is disrupted by inflammation and vascular disease. Platelet factor 4 (PF4) released from platelets activated on inflamed/injured endothelium binds to endothelial glycosaminoglycans (GAGs), generating targets for HIT antibodies.¹³⁹ This in turn leads to expression of tissue factor and adhesion of platelets. Activated endothelium also releases von Willebrand factor, which binds PF4 and HIT antibodies, promotes platelet adhesion and additional PF4 release, creating a feed-forward prothrombotic pathway.¹⁴⁰ HIT immune complexes assembling on PF4 bound to GAGs expressed on monocytes signal through Fcγ receptors to stimulate production of thrombin that transactivates platelets in concert with their direct activation by HIT antibodies. Neutrophils activated by HIT antibodies adhere to venous endothelium, accumulate downstream of the evolving thrombus, and contribute to venous thrombosis at least partially through a peptidyl arginine deiminase 4 (PAD4)-dependent pathway.

Venous thromboembolic risk assessment in hospitalized medical patients

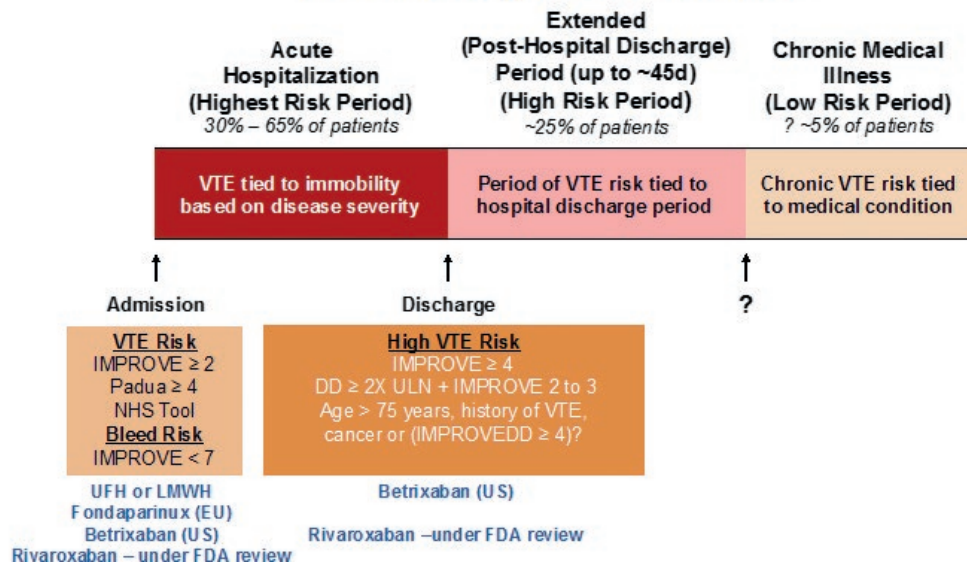


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Individualized, Risk-Adapted Thromboprophylaxis in Medically Ill Patients



Acutely-ill hospitalized medical patients are at risk of venous thromboembolism (VTE), both in-hospital and in the immediate postdischarge period. Although inpatient thromboprophylaxis reduces VTE risk by 50% to 60%, “universal” thromboprophylaxis as recommended by some guidelines, is challenging because of perceived higher risk of bleeding and lower risk of VTE in practice than seen in clinical trials.¹⁴¹ Population-based studies reveal that “universal” hospital-only thromboprophylaxis does not reduce the burden of VTE in this population.¹⁴¹ Five large randomized placebo-controlled trials of extended postdischarge thromboprophylaxis have had mixed results in demonstrating net clinical benefit in hospitalized medical patients, but analyses in key subgroups did reveal net clinical benefit.¹⁴² Recent VTE and bleeding risk assessment models, some of which incorporate D-dimer, have shown good model discrimination and calibration.¹⁴¹ Using these models to provide individualized, risk-adapted thromboprophylaxis has promise to reduce VTE, bleeding, and perhaps other vascular events in this population.¹⁴¹

Is there an ethnic difference in the risk of bleeding complications with the use of antithrombotic agents?

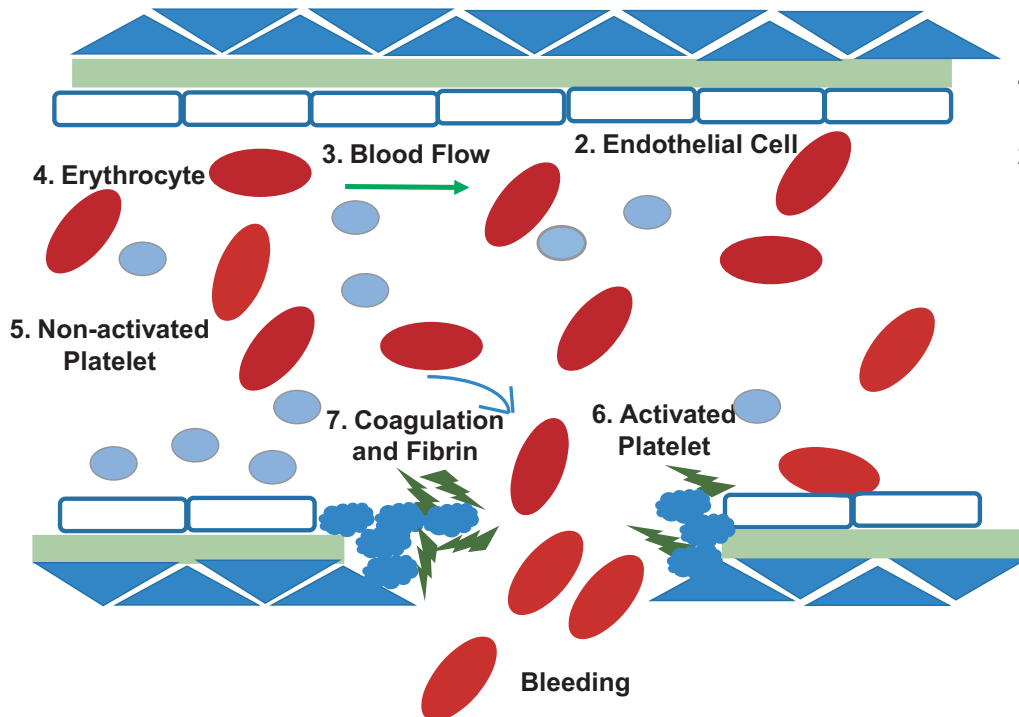


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1. Vessel Wall



Differences in East Asians vs Caucasian

1. Weaker intra-cranial vessel wall in Asian
2. Less production of nitric oxide in Asian
7. Less pro-coagulation due to lack of factor V Leiden in Asian

No Differences

3. Blood flow?
4. Erythrocytes?
- 5, 6. Platelets?

There is a strong perception that the efficacy and safety of antithrombotic therapy is not homogenous across the globe. Recent clinical research indicates that this perception is at least partly right, especially for East Asian patients. Indeed, intracranial hemorrhage occurred more frequently with the use of vitamin K antagonists (VKAs) in East Asians.¹⁴⁴ Thus, a lower prothrombin time (PT)-international normalized ratio (INR) target is recommended for warfarin use among the elderly in East Asia. Lower-dose rivaroxaban is also recommended in Japan. For antiplatelet agents, lower doses were used for the majority of P2Y₁₂ inhibitors in Japan. Accumulating clinical evidence strongly suggests the presence of ethnic or regional difference in the risk of atherosclerosis,¹⁴⁵ thrombosis, and serious bleeding events with the use of antithrombotic agents. Extensive future research is necessary to clarify the major contributors to the higher bleeding rates with these treatments in East Asians.

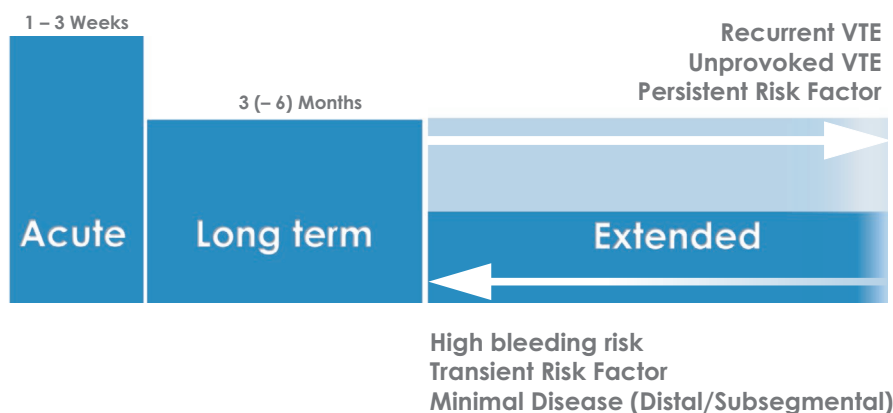
Which patients should receive long-term anticoagulation? What dose?



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Duration of Anticoagulation for VTE



Patients with venous thromboembolism (VTE) that is unprovoked or related to persistent risk factors such as cancer or major thrombophilia are at high risk for recurrent VTE. These patients benefit from extended anticoagulant treatment. Patients with VTE that is provoked by a transient risk factor, with distal deep vein thrombosis or subsegmental pulmonary embolism, or patients at increased bleeding risk, should not continue anticoagulant treatment beyond 3 months.^{146,147}

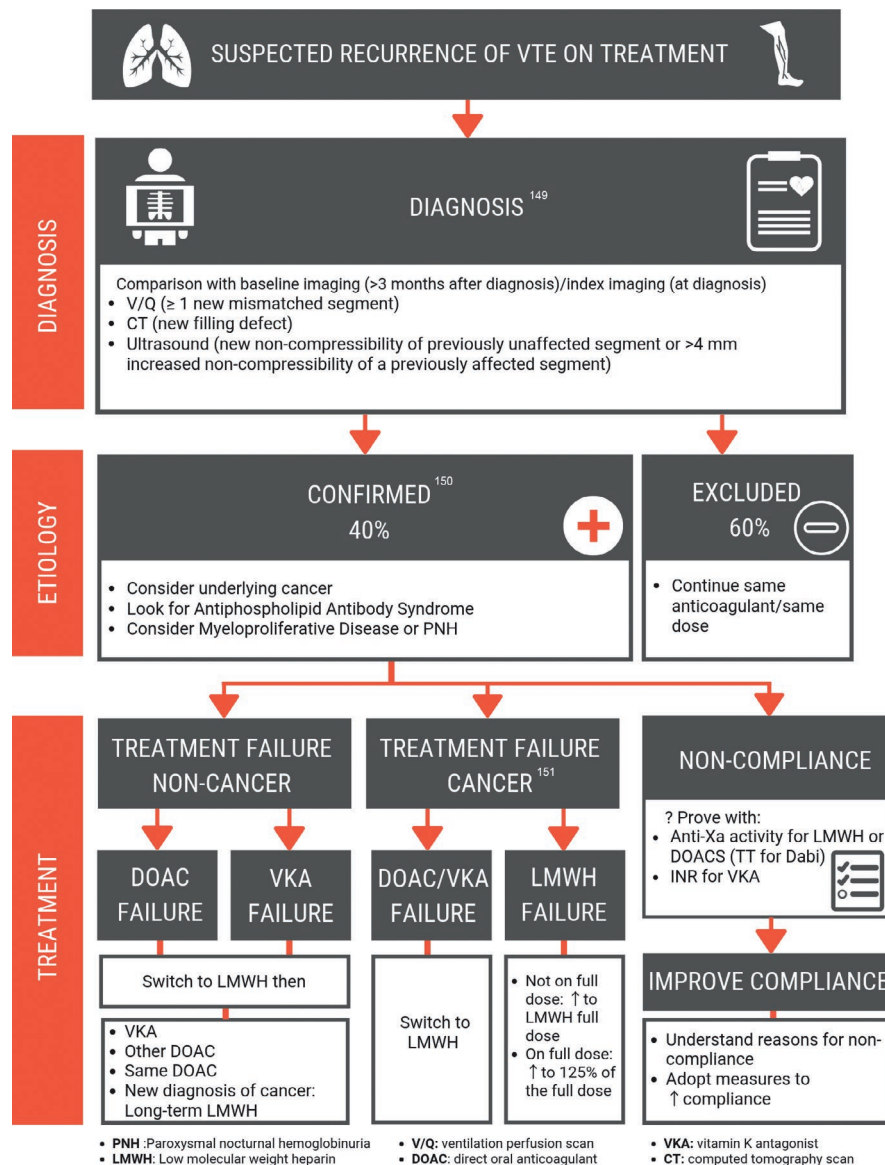
Direct oral anticoagulants are preferred over vitamin K antagonists for most patients because of the lower risk of major bleeding and their convenience. For long-term anticoagulation, the reduced dose of apixaban and rivaroxaban was as effective as the treatment dose to prevent recurrences.¹⁴⁸ This reduced dose harmonizes dosing regimens for primary and secondary prevention of VTE. However, patients with the highest risk of recurrent VTE, such as patients with active cancer or antiphospholipid syndrome, were not included in these trials.¹⁴⁸

Recurrent VTE on anticoagulant therapy: what next?



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VTE risk assessment in pregnancy

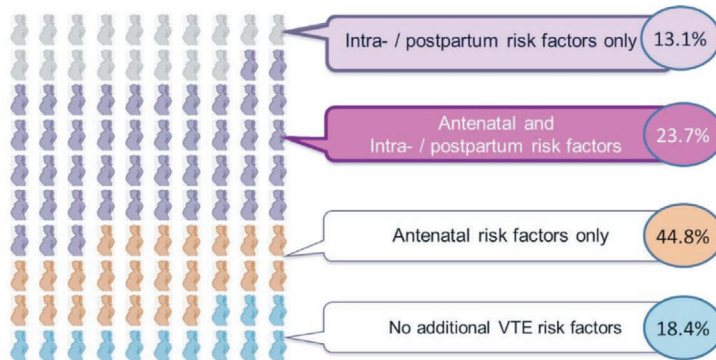
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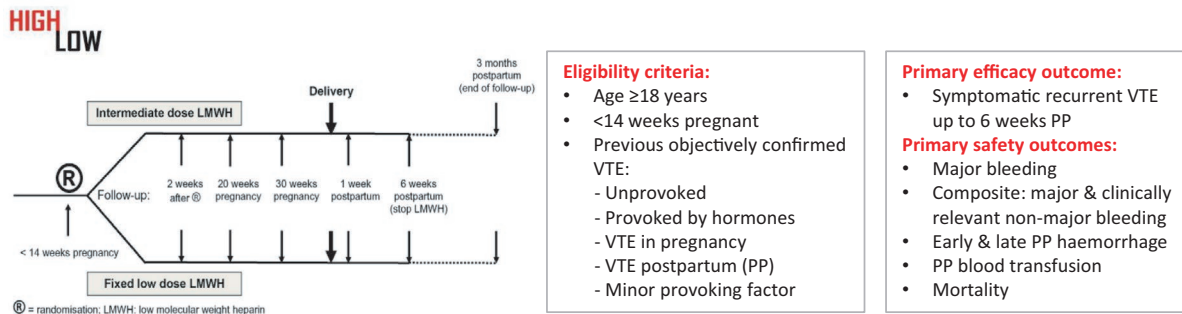


VTE risk assessment in pregnancy

(A) Timing of VTE risk factors during pregnancy and postpartum



(B) Highlow RCT (www.highlowstudy.org; NCT01828697*)



Venous thromboembolism (VTE) is a leading cause of maternal death in developed countries: In the United Kingdom and Ireland, thrombosis/thromboembolism resulted in 1.39 (0.95–1.96) mortalities per 100 000 maternities during 2014–2016.¹⁵² VTE risk increases during pregnancy and reaches a peak during the postpartum period.¹⁵³ This baseline risk increases further with additional VTE risk factors, many of which are common and dynamic (A).¹⁵⁴ One of the strongest risk factors, prior unprovoked VTE (or VTE provoked by a hormonal risk factor), is rare but because of the very high associated VTE risk, thromboprophylaxis is indicated both antenatally and during the postpartum period. The optimal thromboprophylaxis regimen is being evaluated in a randomized trial (www.highlowstudy.org; NCT01828697) (B). Risk assessment and detection of VTE risk factors in pregnant and postpartum women is essential: (1) It can save lives and (2) is feasible even in busy maternity units, through multidisciplinary collaboration.

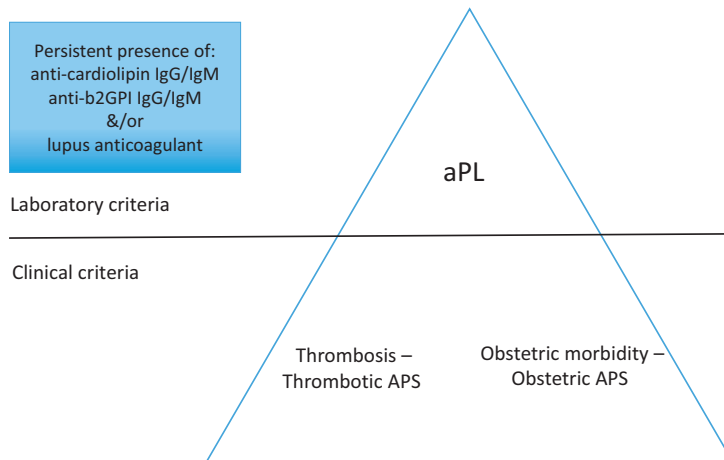
Obstetric antiphospholipid syndrome

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Obstetric antiphospholipid syndrome



Current standard of care treatment for obstetric APS	Evidence
Low dose aspirin	RCTs of varying quality
Low molecular weight heparin	RCTs of varying quality

Possible treatment options for obstetric APS	Evidence
Low dose prednisolone	Observational data
Azathioprine	Anecdotal data
Pravastatin	Observational data
Hydroxychloroquine	Observational data

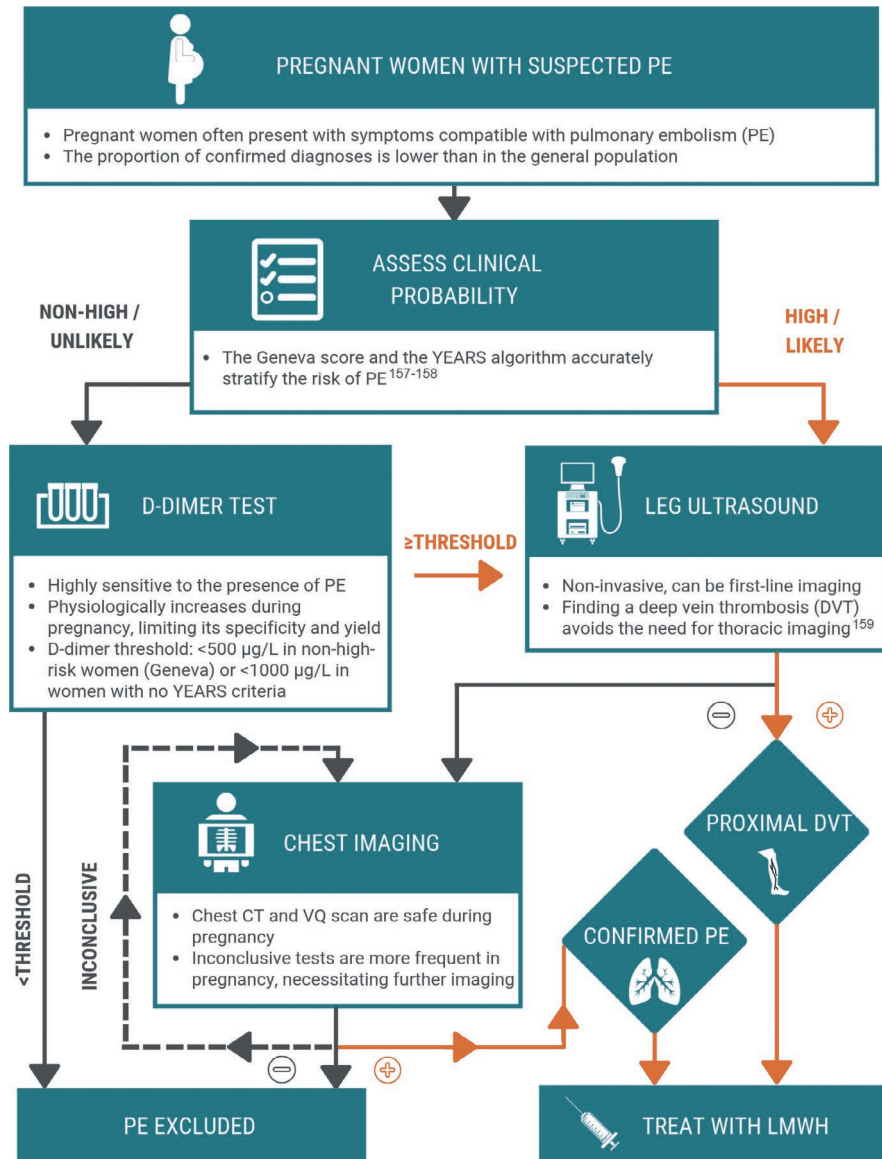
Antiphospholipid syndrome (APS) is an autoimmune disease characterized by the persistent presence of antiphospholipid (aPL) antibodies, including lupus anticoagulant, anticardiolipin antibodies IgG/IgM and anti- β 2-glycoprotein1 antibodies IgG/IgM.¹⁵⁵ APS can present with a variety of clinical phenotypes, such as venous or arterial thrombosis and thrombosis in the microvasculature (thrombotic APS) as well as obstetric complications (obstetric APS). The pathophysiologic hallmark is thrombosis, but other factors such as complement activation might be important.

Pregnancy morbidity includes unexplained recurrent early pregnancy loss, fetal death, and late obstetric manifestations such as pre-eclampsia, premature birth, or fetal growth restriction associated with placental insufficiency. Current treatment to prevent obstetric morbidity is based on low-dose aspirin and/or low-molecular-weight heparin and has improved pregnancy outcomes to achieve successful live birth in >70% of pregnancies. Although hydroxychloroquine and pravastatin might further improve pregnancy outcomes, prospective clinical trials are required to confirm these findings.¹⁵⁶

Diagnosis of PE in pregnancy

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Anticoagulation in pregnancy in women with a mechanical heart valve



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Maternal risk



Fetal risk

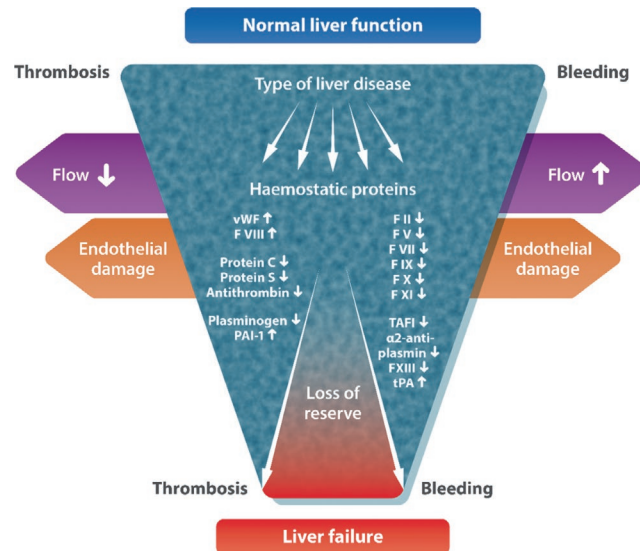
Management of pregnant women with mechanical heart valves is extremely challenging. Options include vitamin K antagonists (VKAs) throughout pregnancy, therapeutic-dose low-molecular-weight heparin (LMWH) during the first trimester, then warfarin in the second and third trimesters, with transition to LMWH and then unfractionated heparin in preparation for birth, or LMWH throughout pregnancy.¹⁶⁰ VKAs are the most effective anticoagulants in minimizing the risk of complications from valve thrombosis but cross the placenta with adverse fetal effects. Adverse fetal outcomes are minimized with LMWH, but valve thrombosis can occur. Anticoagulant-related maternal bleeding can also occur.¹⁶¹ No option is without risk, and women with mechanical heart valves must be involved in the decision making.¹⁶² Local health service capacity (eg, ability to monitor anti-Xa levels) should also be considered, and involvement of an experienced multidisciplinary team is essential.

Hemostasis in liver disease

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A concomitant loss of reserve in the pro- and anticoagulant systems is often seen in liver failure secondary to a reduced synthetic capacity of the hepatocytes. This leads to a fragile balance between bleeding and thrombosis (narrows the distance between these 2 endpoints in the figure). However, additional risk factors are very important for the actual development of bleeding or thrombosis, including disturbed vascular flow and endothelial dysfunction. Low vascular flow increases the risk for portal vein thrombosis, and rupture of varicose veins in portal hypertension causes gastrointestinal bleeding. The specific type of liver disease is also of importance; for example, coagulopathy is more commonly reported in metabolic liver disease, vitamin K-deficiency bleeding is often due to cholestasis, and thrombosis occurs more frequently in liver abscess. Consequently, several aspects need to be considered when evaluating the risk of bleeding and thrombosis in children with liver disease.¹⁶³⁻¹⁶⁵

ISTH STATE OF THE ART SPEAKERS

Christopher M. Ward, Robert K. Andrews, Stefano Barco, Elisabeth M. Battinelli, Eric Boilard, Mettine H. A. Bos, Ampaiwan Chuansumrit, Edward M. Conway, Nicola Curry, Daniel Cutler, Elisa Danese, Simon F. De Meyer, J. Mauricio Del Rio, Jenny Despotovic, Jorge Di Paola, John Eikelboom, Jonas Emsley, Koji Eto, Karin Fijnvandraat, Robert Flaumenhaft, Matthew Flick, Tobias A. Fuchs, Keith Gomez, Shinya Goto, Justin R. Hamilton, Ian S. Hitchcock, Craig N. Jenne, Jill M. Johnsen, Lining A. Ju, Grégoire Le Gal, Gregory Y. H. Lip, Jim Luyendyk, Coen Maas, Maria Magnusson, Pierre Mangin, Alan Mast, Claire McLintock, Sébastien Miranda, Amit Nathwani, Heyu Ni, Fionnuala Ní Áinle, Susie Nilsson, Tor Ny, Sarah H. O'Brien, James S. O'Donnell, Karlheinz Peter, Janusz Rak, Anna M. Randi, Lubica Rauova, Christoph Reinhardt, Marc Rodger, Matthew Rondina, Karen Schreiber, S. Severin, Midori Shima, Alex Spyropoulos, Simon J. Stanworth, Katsue Suzuki-Inoue, Mounneh Thomas, Nagaharu Tsukiji, Tetsumei Urano, Karen Vanhoorelbeke, Peter Verhamme, Jeffrey Weitz, Alisa Wolberg, Dominica Zentner, Cheng Zhu.

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