Editorial

Modeling Venous Thrombosis In Vitro
More Than Just (Valve) Pocket Change

Alisa S. Wolberg

Venous thrombosis (VT) and pulmonary embolism (collectively called venous thromboembolism) affect >1 million people annually, in the United States and in Europe.[1] Approximately 30% of venous thromboembolism patients die within 30 days of onset, usually after the development of pulmonary embolism. One-third to one-half of venous thromboembolism patients develop recurrent VT or suffer debilitating long-term morbidity, including chronic pain, edema, and intractable venous leg ulcers (post-thrombotic syndrome).[1–4]

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Our current understanding of VT is based on a conceptual model in which a combination of abnormalities in the blood (proteins and cells), blood vessels (endothelial cell activation and vessel architecture), and blood flow (disturbed flow or stasis) contribute to venous thrombus formation (so-called Virchow's Triad). Although contributions of plasma hypercoagulability to thrombosis have been well-characterized, the integrated biochemical and biophysical contributions of red blood cells (RBCs), platelets, and leukocytes remained poorly defined by either epidemiological or basic research studies. Moreover, although venous thrombi are thought to originate in venous valve pockets[5]—hypoxic regions of low flow immediately adjacent to the valves—prior studies have not interrogated the specific impact of venous valve geometry (shape) or local hemodynamics on thrombus formation. Increased understanding of both hemodynamics and valve geometry to thrombus initiation and propagation requires models that permit simultaneous control of blood composition, vessel morphology, and flow. Development of such a model necessitates an innovative approach that uses interdisciplinary methods including bioengineering, cell biology, and computational fluid mechanics.

In this issue of *ATVB*, Lehmann et al[6] have developed an in vitro experimental system that leverages conventional advantages of microfluidic models with several key advances. Their system uses recalcified human blood in a small device that enables real-time visualization of blood and clot components during tissue factor–initiation thrombus formation. Moreover, the authors have tested different valve geometries and blood composition and used platelet antagonists to investigate cellular and molecular interactions that arise during thrombus formation in a low flow region mimicking the valve pocket. This system produces a thrombus that exhibits alternating platelet- and RBC-rich zones, an intriguing and defining characteristic of venous thrombus morphology in vivo. This property suggests this system captures the pathophysiologic sequence of events that lead to VT.

Using this system, the authors found that valve geometry, even more than blood velocity, dictates flow patterns within the valve pocket. These patterns have profound effects on the accumulation of blood components within the valve pocket and consequently, on thrombus growth. Their study documents a stepwise process leading to thrombus formation, with several key findings. First, initial fibrin deposition coincides with regions of low wall shear rates. This finding is consistent with prior studies[7] and demonstrates that stasis subverts the antithrombotic effects of flowing blood, allowing fibrin polymerization and promoting initial platelet accumulation. Second, their findings uncover complex interactions between RBCs, platelets, and the vessel wall during thrombus formation: tissue factor at the vessel wall is required for fibrin formation, RBCs are required for platelet accumulation, and platelets are required for fibrin expansion beyond the valve pocket. Ultimately, these interactions between RBCs, platelets, and plasma produce 2-fold larger thrombi compared with the absence of any one of these components. Third, experiments with various platelet antagonists suggest these affect different aspects of thrombus development; protease-activated receptor-1 has only modest effects on thrombus formation, whereas the β_3 integrin mediates platelet accumulation and growth. Interestingly, GPVI (glycoprotein VI) also regulates thrombus growth; in the presence of a GPVI antagonist, platelet aggregation is intact, but platelet accumulation is reduced. The similarly inhibitory effect of D-dimer is consistent with studies demonstrating interactions between GPVI and fibrin, and inhibitory activity of soluble D-dimer on GPVI-mediated platelet activation, aggregation, and spreading on fibrin.[8–10] Ultimately, findings from this study suggest a model (Figure) in which tissue factor drives initial fibrin formation within the low flow niche of the valve pocket. Propelled by currents generated by valve geometry, RBCs then promote platelet accumulation in the valve pocket and adherence on this fibrin. Platelets then activate in a GPVI-dependent manner, express phosphatidylserine, and support thrombin generation, resulting in thrombus growth. Elucidating this sequential mechanism reveals new potential points for intervention to reduce venous thrombus initiation and growth.

The current experimental system has limitations that warrant future development. First, the system does not assess the biomechanical contributions of flexible venous valves. Thus, flow patterns observed here, and therefore, influx and incorporation of blood cells into the thrombus, are likely to differ from

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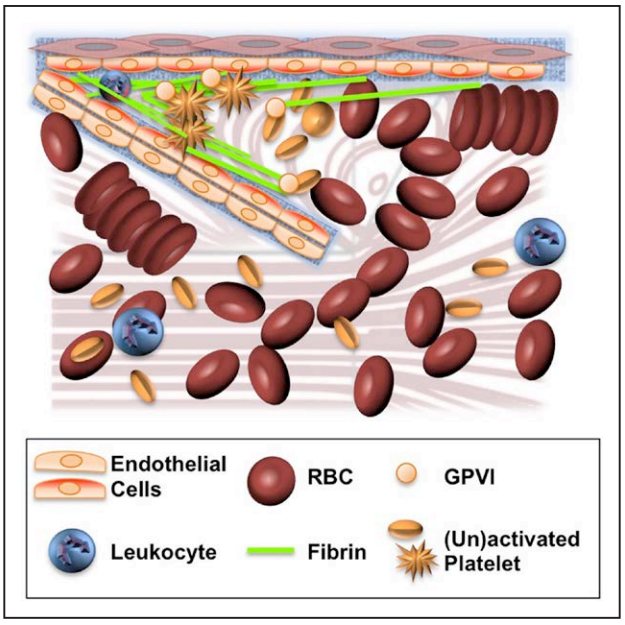

Figure. Conceptual model of venous thrombus formation in a valve. Procoagulant activity on the activated endothelium drives initial fibrin formation within the valve pocket. Platelets then accumulate in the valve pocket in flow- and red blood cell (RBC)-dependent fashion. Platelet activation via GPVI (glycoprotein VI)/fibrin interactions leads to phosphatidylserine expression, thrombin generation, fibrin formation, and thrombus growth.

those arising in vivo. Development of a model that incorporates biomechanical movement recapitulating natural venous values would be an exciting next step. Second, activated endothelium was only modeled by tissue factor. Thus, contributions of other endothelial-associated proteins (eg, von Willebrand factor, thrombomodulin, endothelial protein C receptor, and tissue factor pathway inhibitor) were not evaluated. Because expression of these proteins differs within and adjacent to the valve pocket,[11] determining how this distribution affects thrombus growth along the vessel wall is another important next step. Third, the finding that reconstituted blood (RBCs, platelets, and plasma) behaves similarly as native blood implies a limited role for leukocytes on thrombosis in this model. However, this conclusion is at odds with other studies that detect contributions of leukocyte tissue factor and neutrophil extracellular traps in thrombosis.[12,13] This discord may arise from insensitivity of the current system to mechanisms that occur during so-called immunothrombosis. Finally, the authors did not test the effect of anticoagulants or aspirin on thrombus formation in their system. Because anticoagulants are the primary therapy for preventing VT, it is important to evaluate their effect in this model. Moreover, because clinical trials show low-dose aspirin reduces recurrent VT[14–16] and VT after hip or knee arthroplasty,[17] experiments with aspirin are needed to further elucidate the role of platelets in this system. These next steps are needed to uncover the extent to which findings from this system can be translated to clinical situations.

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Disclosures

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