Targeting platelets to improve post-thrombotic syndrome?

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Venous thromboembolism (VTE) encompasses deep vein thrombosis (DVT) and its associated complications, which includes pulmonary embolism (PE), when a thrombus embolizes from its initial venous location to the lungs, and also postthrombotic syndrome (PTS) when the vein wall becomes fibrosed during the course of thrombus resolution. VTE is one of the leading causes of mortality and morbidity worldwide. Globally, there are 10 million people per year diagnosed with VTE and it is the third leading cardiovascular disease after myocardial infarction and stroke.1 Notably, while other cardiovascular diseases have been on the decline, the incidence of DVT is still increasing.2 In the United States, the management of VTE-related events cost an estimated $7 to $10 billion per year for 375 000 to 425 000 newly diagnosed patients, whereas in the United Kingdom, it represents £640 million per year for ~100 000 patients.3,4 Anticoagulants remain the treatment of choice in DVT to limit thrombus extension and prevent its recurrence. Whereas they have contributed to improved outcomes for DVT patients, they also incur elevated bleeding risk, and particularly in cases when the thrombus has reached an appreciable size, they have limited efficacy while still increasing the bleeding risk.5,6 Between 20% and 50% of patients diagnosed with DVT develop PTS long-term sequelae such as leg pain, swelling, cramps, itching, and ulcers for the most severe cases (5%-10%).7 There is currently no treatment fully alleviating these symptoms, although evidence suggests direct oral anticoagulants, in particular rivaroxaban, reduce incidence of PTS compared with vitamin K antagonists.7,8 Therefore, identifying novel strategies to specifically target PTS after DVT are certainly warranted.

The formation of a pathological thrombus and its resolution in DVT can be defined by three main stages. The initiation of a venous thrombus usually starts in the valve pockets of the large veins and also postthrombotic syndrome (PTS) when the vein wall becomes fibrosed during the course of thrombus resolution. VTE is one of the leading causes of mortality and morbidity worldwide. Globally, there are 10 million people per year diagnosed with VTE and it is the third leading cardiovascular disease after myocardial infarction and stroke.1 Notably, while other cardiovascular diseases have been on the decline, the incidence of DVT is still increasing.2 In the United States, the management of VTE-related events cost an estimated $7 to $10 billion per year for 375 000 to 425 000 newly diagnosed patients, whereas in the United Kingdom, it represents £640 million per year for ~100 000 patients.3,4 Anticoagulants remain the treatment of choice in DVT to limit thrombus extension and prevent its recurrence. Whereas they have contributed to improved outcomes for DVT patients, they also incur elevated bleeding risk, and particularly in cases when the thrombus has reached an appreciable size, they have limited efficacy while still increasing the bleeding risk.5,6 Between 20% and 50% of patients diagnosed with DVT develop PTS long-term sequelae such as leg pain, swelling, cramps, itching, and ulcers for the most severe cases (5%-10%).7 There is currently no treatment fully alleviating these symptoms, although evidence suggests direct oral anticoagulants, in particular rivaroxaban, reduce incidence of PTS compared

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models of thrombosis have been invaluable for our understanding of DVT as such studies are generally not possible in human DVT patients. Although the complete ligation of the inferior vena cava (IVC) offers reproducible thrombus in size, the partial ligation model is thought to more closely resemble the human scenario where perturbation of blood flow in the venous valves pockets plays a crucial role in DVT initiation.\(^5\)\(^2\)\(^6\)\(^7\) Importantly, mouse thrombi in the stenosis IVC model are remarkably similar histologically to thrombi extracted from human DVT patients.\(^1\)\(^4\)

The role of platelets in early events of DVT have been established. Indeed, depletion of platelets in mice led to protection in the IVC stenosis model.\(^1\)\(^4\) Platelets are thought to facilitate leukocyte recruitment in particular neutrophils are one of the first cells present at the site of injury.\(^1\)\(^4\) The importance of the von Willebrand factor A1-GPlb\(\alpha\) axis in DVT has been demonstrated by various studies showing that inhibiting that interaction provide protection in this model.\(^1\)\(^4\)-\(^1\)\(^8\) disrupting that interaction was also accompanied with a greatly diminished number of leukocytes recruited at the site of stenosis.\(^1\)\(^4\)-\(^1\)\(^5\) More recently, von Willebrand factor-primed platelets have been shown to interact with neutrophils via SLC44A2,\(^1\)\(^9\) one of two susceptibility loci recently identified for VTE by genome wide association studies.\(^2\)\(^0\)-\(^2\)\(^1\) Additional roles for platelets in DVT have been highlighted during the propagation of the thrombus in DVT via P-selectin and HMGB1 released from activated platelets, which promotes leukocyte recruitment, activation, and subsequent NET formation.\(^1\)\(^4\),\(^2\)\(^2\)-\(^2\)\(^5\) In this issue of the Journal of Thrombosis and Haemostasis, DeRoo et al explored the role of platelets in later stages of DVT. Their results are exciting and uncover a novel role for platelets in thrombus maturation, fibrosis, and remodeling of the injured vein.

In this study, the authors used the stenosis model of the IVC to evaluate the role of platelets in later stages of venous thrombosis. First, they established at which time point postsurgery thrombus formation, resolution, and vein wall remodeling occurs. Thrombus length and volume gradually decreased from 2 to 16 days. However, there was no difference between days 8 and 2, suggesting that thrombus resolution was limited. Thrombus fibrosis measured as collagen content was significantly present 2 weeks postsurgery, whereas fibrosis in the vein was already evident after a week. These timings are in line with those of the stasis model in which maximal thrombus formation has been reported to be reached around 2 to 4 days and thrombus resolution initiated around 2 weeks postinduction.\(^5\),\(^2\)\(^8\) To evaluate the role of platelets in thrombus resolution and later stages of DVT, the authors therefore chose to deplete platelets in mice subjected to partial ligation of the IVC 2 days postsurgery, after the formation of a sizable thrombus. Although the reduction in platelet counts did not affect the size or length of the thrombus 10 days after IVC stenosis, it had an appreciable effect in the thrombus and vessel wall architecture. Indeed, collagen content was reduced by \(-50\%\) in the thrombus of mice injected with platelet-depleting anti-GP Ib\(\alpha\) antibodies compared with control mice. In addition to diminished thrombus fibrosis in these mice, a significant decrease in smooth muscle cell invasion was also detected in their thrombi. Another important finding from this study by DeRoo et al is that a significant reduction in the intimal thickening of platelet-depleted compared with control mice was observed, suggesting platelets not only influence thrombus maturation and resolution but also vein wall remodeling. The authors speculate that platelets influence these processes via molecules such as TGF-\(\beta\), bFGF, or PDGF that are released upon platelet activation.

These results could have potentially important clinical implications because it is known that as venous thrombi progress into resolution stages and fibrosis increases, they are generally more resistant to pharmacological therapy.\(^2\)\(^6\) As mentioned previously, slower thrombus resolution and recanalization combined with vein wall thickening are associated with development of PTS.\(^2\)\(^6\),\(^2\)\(^7\) Targeting platelets after DVT onset may therefore facilitate thrombus resolution without posing the risk of subsequent excessive vein wall remodeling. Importantly, thrombi analyzed at day 10 postsurgery were significantly smaller than at day 2, indicative that thrombus resolution was initiated in platelet-depleted mice as seen for control mice. As stated by the authors, however, this time point represents one of the limitations of the study because thrombus resolution was likely not complete as the thrombus length and volume at day 16 were 3.2 mm and 3.4 mm\(^3\), respectively, and at day 10 were estimated to be 7.4 mm and 13.1 mm\(^3\) (similar to those found at day 8 for the control mice). Based on the very promising results obtained at day 10, further evaluation of the therapeutic value to target platelets after thrombus formation in the DVT stenosis model at a later timepoint is warranted. Perhaps defining the mechanism by which platelet depletion decreases thrombus fibrosis, smooth muscle cell invasion, and vein wall thickening should be established first so that one is not limited by the immune response associated with administration of antibodies for efficient platelet depletion.

Anucleated platelets continue to amaze us by their multifaceted functions. They are increasingly recognized as immune cells that orchestrate various inflammatory conditions or fight infections. Although their role in thrombosis had been more traditionally associated with the arterial system, it is becoming clear that they also play a key role in venous thrombosis. Although we are a long way away from fully understanding exactly how platelets contribute to later stages of DVT, the manuscript by DeRoo et al provides exciting perspectives on developing new therapeutic options to prevent PTS during the thrombus resolution phase in DVT.

CONFLICT OF INTEREST
All authors declare that they have no conflicts of interest to declare.

AUTHOR CONTRIBUTION
Isabelle I. Salles-Crawley developed, wrote, and proofread this commentary.

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