Targeting platelets to improve post-thrombotic syndrome?

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‘The role of platelets in thrombus fibrosis and vessel wall remodeling after venous thrombosis”

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Venous thromboembolism (VTE) encompasses deep vein thrombosis (DVT) and its associated complications, which includes pulmonary embolism (PE), when a thrombus embolises from its initial venous location to the lungs, and also post-thrombotic 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 US, the management of VTE-related-events cost an estimated $7-10 billion per year for 375,000-425,000 newly diagnosed patients while in the UK 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 whilst still increasing the bleeding risk.[5, 6] Between 20% to 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 DOACs, in particular rivaroxaban, reduces incidence of PTS compared to 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 valves pockets of the large veins and involves the combination of different factors including alteration and/or reduction in blood flow that may trigger local hypoxia, endothelial cell dysfunction and elevated hypercoagulability.[9, 10] Within the first week (stage 1), the thrombus is composed mostly of red blood cells with few other cells including platelets. At this stage, the thrombus is firmly attached to a small area at the vessel wall initiation site. Progressively, fibrin becomes more prominent within the thrombus with evidence of immune cells infiltration and endothelial cells covering partially the thrombus (stage 2 - ~7 weeks). After a year (stage 3), the remaining

tissue contains mostly collagen produced by invading fibroblasts with few leukocytes.[11] The mechanisms that drive thrombus resolution are not fully understood but involves various inflammatory cells including neutrophils, macrophages and T-cells but also endothelial cells that drives fibrinolysis, collagenolysis and neovascularisation.[5] Importantly, thrombus maturation and restoration of vessel patency often goes hand in hand with vessel wall fibrosis and intimal thickening. Poor thrombus resolution is one of the strongest predictors of PTS while venous hypertension caused by venous reflux also contributes to PTS symptoms.[6, 7, 12] Murine 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.[13] Importantly, mouse thrombi in the stenosis IVC model are remarkably similar histologically to thrombi extracted from human DVT patients.[14]

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.[14] Platelets are thought to facilitate leukocyte recruitment in particular neutrophils are one of the first cells present at the site of injury.[14] The importance of the VWF A1-GPIbα axis in DVT has been demonstrated by various studies showing that inhibiting that interaction provide protection in this model.[14-18] Disrupting that interaction was also accompanied with a greatly diminished number of leukocytes recruited at the site of stenosis.[14, 15] More recently, VWF-primed platelets have been shown to interact with neutrophils via SLC44A2,[19] one of two susceptibility loci recently identified for VTE by genome wide association studies. [20, 21] Additional roles for platelets in DVT have been highlighted during the propagation of the thrombus in DVT via P-selectin and HMBG1 released from activated platelets which promotes leukocyte recruitment, activation and subsequent NET formation. [14, 22-25]. 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 post-surgery thrombus formation, resolution and vein wall remodelling 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 post-surgery, while fibrosis in the vein was already

evident after a week. These timings are in line with those of the stasis model where maximal thrombus formation has been reported to be reached around 2-4days and thrombus resolution initiated around 2 weeks post-induction.[5, 26] 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 post-surgery, 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 post 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-GPIbα antibodies compared to 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 to control mice was observed, suggesting platelets not only influence thrombus maturation and resolution but also vein wall remodelling. The authors speculate that platelets influence these processes via molecules such as TGFβ, bFGF or PDGF that are released upon platelet activation.

These results could have potentially important clinical implications since it is known that as venous thrombi progress into resolution stages and fibrosis increases, they are generally more resistant to pharmacological therapy.[26] As mentioned above, slower thrombus resolution and recanalization combined with vein wall thickening are associated with development of PTS.[26, 27] Targeting platelets after DVT onset may therefore facilitate thrombus resolution without posing the risk of subsequent excessive vein wall remodelling. Importantly, thrombi analysed at day 10 post-stenosis 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, this time point however represents one of the limitations of the study as 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 recognised 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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