JACC: BASIC TO TRANSLATIONAL SCIENCE
PUBLISHED BY ELSEVIER ON BEHALF OF THE AMERICAN COLLEGE OF CARDIOLOGY FOUNDATION.

VOL. 5, NO. 4, 2020

EDITORIAL COMMENT

Shining a Light on Venous Thromboembolism*

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Preclinical studies are important grounds to develop and test hypotheses and novel approaches for translation to human disease. A number of distinct pathologic processes can result in venous thromboembolism. Studies using murine models have shed light on contributions from multiple cell types. Roles have been identified for endothelial and platelet activation, neutrophil extracellular trap generation, myeloid-derived tissue factor release, and erythrocyte-mediated thrombus stabilization. Critical roles have also been described for activation of the inflammasome resulting in maturation of the paradigmatic inflammatory cytokine, interleukin-1β, in venous thrombogenesis (1).

Several models to examine venous thrombogenesis and resolution have been described, each with strengths and limitations. To help investigators select an appropriate model to test their hypotheses, several professional societies recently published a consensus statement with detailed methodologies, advantages, and disadvantages of the most widely used models of deep vein thrombosis (DVT) (2). In this issue of *JACC: Basic to Translational Science*, Okano et al. (3) describe an intriguing new model of venous thromboembolism in the femoral and saphenous veins. The most commonly used murine models of DVT involve manipulation of the infrarenal inferior vena cava (IVC) by stopping or altering blood flow or inducing free radical generation. Studying thrombosis in IVC provides sufficient tissue to examine both the vein wall and thrombus itself. However, the IVC lacks valves and does not replicate flow dynamics seen in human superficial and deep veins with valves where thrombosis often occurs.

Initial DVT models involve interrupting blood flow in the infrarenal IVC by complete ligation of the vein and all branch vessels (1,2). The static column of blood generated coagulates upstream of the ligation site. Thrombi generated by this "stasis" model are rich in erythrocytes and fibrin and are reproducible in size. This model can be used to study coagulation in gene-modified mice and for following pharmacologic interventions for thrombus resolution days-to-weeks after surgery. Although this model of stasis DVT in mice does not typically result in pulmonary thromboembolism, rats subjected to IVC ligation under hypoxic, hyperbaric conditions developed thrombi downstream of the ligature in the direction of flow with embolism to the lungs. Specific cell-depletion experiments to date suggest that platelets, neutrophils, and monocytes may not play significant roles in thrombogenesis in this model of DVT. Therefore, investigators interested in these cellular inputs to thrombosis should consider an alternate approach.

A modification of this model involves restricting but not occluding the IVC to reduce vein diameter to approximately 10% of its normal size while

*Editorials published in *JACC: Basic to Translational Science* reflect the views of the authors and do not necessarily represent the views of *JACC: Basic to Translational Science* or the American College of Cardiology.

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The authors attest they are in compliance with human studies committees and animal welfare regulations of the authors' institutions and Food and Drug Administration guidelines, including patient consent where appropriate. For more information, visit the *JACC: Basic to Translational Science* author instructions page.

ISSN 2452-302X https://doi.org/10.1016/j.jacbts.2020.03.004

JACC: BASIC TO TRANSLATIONAL SCIENCE VOL. 5, NO. 4, 2020
APRIL 2020:357–9

minimizing endothelial injury (1,2). Narrowing the IVC lumen generates turbulent blood flow patterns and activation of platelets, neutrophils, and monocytes as they interact with the endothelium (4). Platelets play essential roles in DVT under these conditions, monocytes release tissue factor–bearing microvesicles, whereas neutrophils contribute to thrombogenesis through several mechanisms including formation of extracellular chromatin traps and reactive oxygen species generation. This "stenosis" model of DVT has inherent variability in thrombus frequency and size, recapitulating the clinical condition. It is typically used to study thrombus accretion and propagation beyond the first several hours of thrombogenesis. Thrombus and vessel tissue is sufficiently sized for analysis. Notably, these DVTs have histologic features characteristic of human thrombi, with alternating layers of erythrocyte-rich "red" and fibrin- and platelet-rich "white," typically described as "lines of Zahn." Intravital microscopy following IVC restriction can help shed light on cellular recruitment and interactions during initial thrombogenesis. Significant limitations include the large number of animals needed for studies due to variability in thrombus incidence and size and the need for sophisticated equipment for image acquisition due to the depth of the IVC. This model has also recently been suggested to result in pulmonary artery thrombi, although it is unclear whether this is a result of thromboembolism from the IVC or in situ pulmonary arterial coagulation, which may result from procoagulant microvesicles or soluble mediators.

Free radical–induced thrombosis models using chemical or electrical triggers have been used in large and small veins. Application of iron(III) chloride to the adventitial surface of the vein can be used to trigger thrombosis, although this may cause vessel wall tissue damage. Alternate models deliver a small 1.5 to 3 volt current directly to the external surface of the saphenous or femoral veins for short periods of time. This rapidly induces thrombosis in these valve-containing veins, which can be used for acute or chronic thrombus formation and resolution. Another advantage of this model is the small vein diameter that makes these vessels well-suited for application of real-time intravital microscopy to study cellular and thrombus dynamics. A limitation of this method of DVT formation is that the thrombi generated are rich in platelets and fibrin, reminiscent of "white clot" observed in arterial thrombi rather than those seen in DVT. Another recently developed model involving electrolytic injury involves running a current through a needle inserted into the IVC to generate intraluminal free radicals and subsequent thrombosis. Side branches are ligated for consistent flow dynamics. This model has several advantages, including thrombus formation in all experiments, which reduces the number of animals required for each experiment. In addition, thrombus size can be controlled by altering current and time of exposure, which gives investigators significant flexibility to test prothrombotic conditions and antithrombotic interventions. As with other surgical models, the electrolytic IVC injury model requires technical proficiency for consistent results.

Each model carries strengths and weaknesses, making model selection critical to test specific hypotheses. Okano et al. (3) describe a new method of venous thrombosis that starts with ligation of the femoral vein to create disturbed flow. The investigators discovered that whereas disturbed flow alone did not generate a thrombus, intravital imaging with fluorescein isothiocyanate–dextran and filtered epifluorescent light triggered coagulation in the imaged segments within minutes. They also found no contribution to thrombogenesis from fluorescent dyes with differing quantum efficiencies as thrombi were seen even if dye was added after light exposure. Clots formed in vein valves, where disturbed flow dominated after downstream ligation. These thrombi were predominantly composed of erythrocytes and fibrin. Although neutrophils and platelets were also recruited to the thrombotic milieu, their role is unclear. Neutrophils were recruited to the center of the thrombus. Treatment with deoxyribonuclease did not alter thrombus size, leading the investigators to conclude that neutrophil extracellular traps are not pathogenic in this model of DVT. However, it is unclear whether deoxyribonuclease can passively penetrate thrombi, and the granulocyte and antimicrobial proteins decorating neutrophil extracellular traps may still be local effectors. Platelet deposition likely alters local flow dynamics and increases thrombus accretion. The presented data also suggest DVT size can be modified by varying light intensity and duration. Notably, thrombosis in the veins could be triggered by longer light exposure even in the absence of flow disturbance. Although the mechanism for filtered light-induced thrombosis in this model is unknown, it has several advantages. First, DVT can be induced in limb veins without chemical or laser-induced injury of the vessel wall. Second, intravital visualization of thrombus dynamics in the earliest stages of DVT is an important strength of this model. Although thrombosis in the mesenteric and cremasteric venules is an excellent tool for intravital

microscopy, differences in gene expression and flow dynamic patterns in these microvascular beds limit extrapolation of observations to larger veins of interest. Third, investigators can use the "tunability" of thrombus size by light exposure to study prothrombotic conditions or therapeutic interventions. Fourth, deligation of the femoral vein led to restored venous return and embolization of the thrombi to the lungs providing a much-needed model to study acute pulmonary embolism. Several questions remain about this platform that investigators will note, including the lack of mechanism by which filtered light induces thrombosis. Light at many spectral wavelengths is known to induce changes in cellular processes, which have not yet been elucidated in this model. Photocoagulation with lasers has been used to treat retinal and other diseases, presumably by thermal injury. Erythrocytes have been found to contribute to venous thromboembolism in several ways, including supporting thrombin generation, expression of surface phosphatidylserine, interacting with other cells, and altering fibrin structure and stability (5). It is unclear whether these cellular processes are enrolled in this erythrocyte-dependent model. Furthermore, low-resolution imaging with epifluorescence precludes real-time individual cell tracking although these can be defined ex vivo. Finally, the significance of engagement by neutrophils and platelets remain unknown in this model of thrombosis.

The new platform developed by Okano et al. (3) to study venous thrombosis offers distinct strengths to gain new insights into the earliest stages of thrombogenesis. Careful selection of the most appropriate preclinical tool among the available models will greatly help investigators generate important insights to develop new approaches for this highly prevalent and morbid disease.

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KEY WORDS animal models, coagulation, deep vein thrombosis, pulmonary embolism, venous thrombosis