

Venous Thromboembolism: Mechanisms, Treatment, and Public Awareness

Mechanisms of Venous Thrombosis and Resolution

Thomas W. Wakefield, Daniel D. Myers, Peter K. Henke

Abstract—Venous thromboembolism is a significant health care problem in the US. In this review, the unique role of inflammation to the venous thrombotic process is emphasized as well as the potential role of abnormalities of fibrinolytic mechanisms to the thrombotic process. Inflammation influences not only thrombogenesis but also thrombus resolution and vein wall remodeling, and these interactions are also discussed. Knowledge of molecular and immunologic mechanisms for venous thrombosis and its resolution should allow for the future development of targeted therapies. (*Arterioscler Thromb Vasc Biol.* 2008;28:387-391)

Key Words: venous thrombosis ■ inflammation ■ selectins ■ plasminogen ■ fibrosis

Venous thromboembolism (VTE) is a significant health care problem in the United States, with an estimated 900 000 cases of deep venous thrombosis (DVT) and pulmonary embolism (PE) yearly, with approximately 300 000 deaths.¹ For the past 150 years, thoughts on the pathogenesis of VTE centered on Virchow's triad of stasis, changes in the vessel wall, and thrombogenic changes in the blood. However, in the early 1970s, through the pioneering theories of Gwendolen Stewart, a relationship between thrombosis and inflammation was suggested. Stasis by itself, although an important factor, is usually not enough to produce thrombosis and should be considered a permissive factor in thrombogenesis for the other events that are required for thrombosis to occur (M. Meissner, personal communication, 2005). In this review, we will discuss particular molecular and immunologic pathways for venous thrombosis and emphasize the role of inflammation in the process of thrombogenesis and thrombus resolution.

Endothelium and Hemostasis

Through its ability to express procoagulants and anticoagulants, vasoconstrictors, and vasodilators, as well as key cell adhesion molecules and cytokines, the endothelium has emerged as one of the pivotal regulators of hemostasis. Under normal conditions, endothelial cells sustain a vasodilatory and local fibrinolytic state in which coagulation, platelet adhesion, and activation, as well as inflammation and leukocyte activation, are suppressed. A nonthrombogenic endothelial surface is maintained through a number of mechanisms including: (1) endothelial production of thrombomodulin (TM) and subsequent activation of protein C; (2) endothelial expression of heparan sulfate and dermatan sulfate which

accelerate anti thrombin and heparin cofactor activity; (3) constitutive expression of tissue factor pathway inhibitor (TFPI); and (4) local production of tissue plasminogen activator (tPA) and urokinase-type plasminogen activator (uPA). In addition, the elaboration of NO, prostacyclin, and interleukin (IL)-10 by endothelium inhibits the adhesion and activation of leukocytes and produces vasodilation.²

In contrast, during states of endothelial disturbances, whether physical (eg, vascular trauma) or functional (eg, sepsis), a prothrombotic and proinflammatory state of vasoconstriction is supported by the endothelial surface.² Release of platelet activating factor (PAF) and endothelin-1 promotes vasoconstriction,³ whereas production of von Willebrand factor (vWF), tissue factor (TF), plasminogen activator inhibitor (PAI)-1, and Factor V augment thrombosis.² Additionally, in response to endothelial injury, endothelial cells are activated, resulting in increased surface expression of certain cell adhesion molecules (such as P-selectin or E-selectin), promoting the adhesion and activation of leukocytes. This event initiates and amplifies inflammation and thrombosis (Figure 1).

Inflammation and Thrombosis

Inflammation and thrombosis are interrelated. For example, inflammation increases TF, platelet reactivity, fibrinogen, and leads to exposure of increased levels of phosphatidylserine, while decreasing TM and inhibiting fibrinolysis (by increasing PAI-1).⁴ We have used both a rat and mouse model of inferior vena cava (IVC) thrombosis in studies of the basic mechanisms of thrombogenesis and thrombus resolution. Cell adhesion molecules (CAMs) allow leukocyte transmigration, and selectins (P and E-selectin) are integrally involved in thrombosis. Selectins are the first upregulated glycoproteins

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From the Section of Vascular Surgery, Department of Surgery (T.W.W., D.D.M., P.K.H.) and Unit for Laboratory Animal Medicine (D.D.M.), University of Michigan, Ann Arbor.

Correspondence to Thomas W. Wakefield, MD, CVC 5463, Cardiovascular Center, University of Michigan, 1500 E. Medical Center Drive, SPC 5867, Ann Arbor, MI 48109-5867. E-mail thomasww@umich.edu

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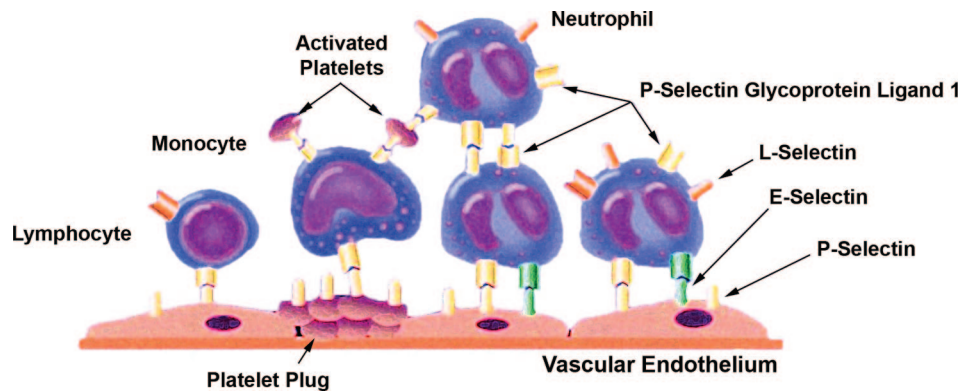


Figure 1. Immediately after endothelial cell injury, endothelial cells and platelets are activated promoting the expression of cell adhesion molecules. This vascular response promotes leukocyte rolling and tethering onto the endothelium that initiates an inflammatory event which can lead to thrombosis. (Modified from Myers DD et al, *Front Biosci* 2005;10:2752.)

on activated endothelial cells and platelets. The cell adhesion molecule P-selectin has been found upregulated in the vein wall as early as 6 h after thrombus induction, whereas E-selectin has been found upregulated at day 6 after thrombosis.⁵

Microparticles (MPs) are involved in the thrombotic process and the amplification of thrombosis. MPs are small (less than 1 micrometer, about the size of a bacterium), phospholipid vesicles shed from platelets, leukocytes, and endothelial cells in a calcium dependent fashion.^{6–8} MPs are a normal constituent of blood and can be isolated from plasma by ultracentrifugation. MPs lack DNA and recent evidence suggests they may carry RNA,⁹ and they are protein rich. Subpopulations of MPs rich in TF and phosphatidylserine have been identified.^{10,11} Several circulating markers of inflammation once thought to be soluble are actually carried by MPs.¹² Lipid rafts are sphingolipid ordered, cholesterol-rich microdomains floating within the more fluid cell surface bilayer (the “fluid mosaic”).¹³ Rafts and raft derived MPs can concentrate TF in cavaolae where it is stored with TFPI.¹⁴ Fusion of MPs with activated platelets results in decryption of TF and the initiation of thrombosis.¹⁵

Venous stasis and ischemia results in the upregulation of P-selectin which localizes prothrombotic MPs to the area of stasis and promotes DVT formation.^{16–18} The P-selectin receptor P-Selectin Glycoprotein Ligand 1 (PSGL-1) is expressed on leukocytes and platelets, as well as on their derived MPs. MPs coexpressing TF and leukocyte markers have been shown to accumulate in growing thrombi in a P-selectin:PSGL-1-dependent fashion.^{19,20} P-selectin:PSGL-1 interactions also stimulate the production of thrombotic MPs from leukocytes, particularly monocytes along with platelets and endothelial cells.^{21,22} These prothrombotic MPs express TF and possess a phosphatidylserine rich anionic surface capable of assembling complexes of the coagulation cascade.²³ They are concentrated back into the area of thrombus formation (for example, MPs also express on their surface PSGL-1 which then can bind to upregulated P-selectin on platelet surfaces in the thrombus⁹) leading to thrombus amplification (Figure 2). MPs have been found to normalize tail bleeding times in hemophilic mice,²¹ and human pericardial-derived MPs expressing TF have been demonstrated to increase thrombosis in a rat venous stasis

model.²⁴ The importance of P-selectin:PSGL-1 to venous thrombosis likely depends on the nature of the stimulus and the role of TF, which is normally abundant in the outer portion of the vessel wall. With significant vascular injury and the exposure of vein wall TF, this TF is likely more important in the thrombotic process than the TF that is brought to the point of thrombogenesis by activated MPs.²⁵ Further, monocyte-derived MPs deliver TF to areas of injury and inflammation by binding to P-selectin mobilized to the surface of activated platelets and endothelial cells, resulting in the generation of fibrin. In patients with DVT, MPs have been found elevated²⁶ as well as have platelet-leukocyte conjugates.²⁷

MPs are not only prothrombotic but also appear to inhibit fibrinolysis. PAI-1 is stored in the α -granules of quiescent platelets.²⁸ PAI-1 is a potent inhibitor of tPA and uPA which are largely responsible for the initiation of fibrinolysis.²⁹ On activation, MPs shed from platelets express PAI-1 and these MPs are localized to the growing thrombus via P-selectin:PSGL-1 interactions. In this manner, platelet MPs are not only prothrombotic but also inhibit fibrinolysis, delaying thrombus resolution and facilitating thrombus growth.³⁰

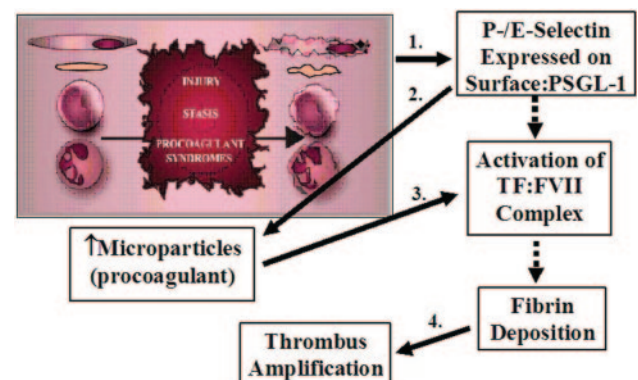


Figure 2. Proposed mechanism of the role of microparticles. (1) With stimulation, selectins are upregulated and bind to PSGL-1 on leukocytes and platelets; (2) Microparticles which are procoagulant are produced, especially from monocytes but also from platelets and endothelial cells; (3) These microparticles then are concentrated back into the area of thrombosis; (4) This then leads to thrombus amplification. (Modified from Myers DD et al, *Front Biosci* 2005;10:2753.)

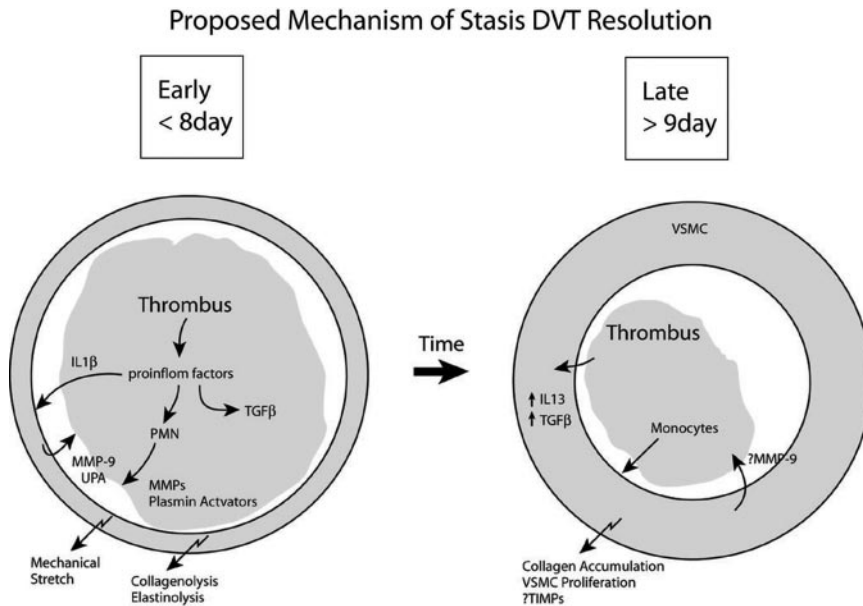


Figure 3. Early time postthrombosis is characterized by thrombolysis that is leukocyte dependent, primarily neutrophils, and driven by a proinflammatory cytokine milieu. This transitions overtime to a monocyte predominant thrombus environment, with plasmin- and MMP-mediated thrombus breakdown. Coincident with the thrombus changes are early collagenolytic (and likely elastinolysis) changes, followed by later vein wall fibrosis. (Reproduced with permission from Henke PK, *Vascular* 2007; 15:369.)

Plasminogen Activators and Thrombolysis

In addition to natural anticoagulants such as protein C and protein S, physiological clot formation is balanced by a constant process of thrombolysis to prevent pathological intravascular thrombolysis. The central fibrinolytic enzyme is plasmin, a serine protease generated by the proteolytic cleavage of the proenzyme plasminogen. Its main substrates include fibrin, fibrinogen, and other coagulation factors. Plasmin also interferes with vWF-mediated platelet adhesion by proteolysis of GpIb.³¹ Activation of plasminogen occurs through several mechanisms.

Plasminogen activators are serine proteases that activate plasminogen, by cleavage of a single arginine-valine peptide bond, to the enzyme plasmin. Plasminogen activation provides localized proteolytic activity.^{32–34} In plasma, PAI-1 is the primary inhibitor of plasminogen activators. It is secreted in an active form from liver and endothelial cells and stabilized by binding to vitronectin (Vn). PAI-1 levels are elevated by hyperlipidemia, and PAI-1 elevation appears to synergize with Factor V Leiden genetic abnormalities. It is plausible that elevated PAI-1 could suppress fibrinolysis and increase thrombolysis, hence increasing the clinical manifestations of DVT, although studies on the role of elevated levels of PAI-1 to venous thrombolysis have been contradictory.^{35,36}

In humans, recent studies have evaluated the role of genetic polymorphisms, particularly the 4G/5G insertion/deletion in the promoter region, which affects transcription rates. The highest levels of PAI-1 have been noted in those individuals carrying the 4G/4G polymorphism. One study found an increased odds ratio of 5.5x for DVT with the 4G allele, increased even greater when combined with concurrent Factor V Leiden.³⁷ A second study found an 8.14 $\times$ increased risk elevation in patients with the 4G allele combined with other thrombophilic markers,³⁸ whereas PE was increased in 4G/4G patients with protein S deficiency (odds ratio 4.5 $\times$).³⁹

The degradation of fibrin polymers by plasmin ultimately results in the creation of fragment E and 2 molecules of fragment D which, during physiological thrombolysis, are released as a

covalently linked dimer (d-dimer).⁴⁰ Clinically, detection of d-dimer in the circulation is a marker for ongoing clot formation and fibrinolysis. An elevated d-dimer level after successful treatment of DVT is one biomarker that has been found to accurately predict an ongoing risk of recurrent VTE.⁴¹

Thrombus Resolution and Vein Wall Remodeling

Despite prophylaxis, patients may present clinically with a formed DVT of variable age. DVT resolution resembles wound healing, and involves both profibrotic growth factors, collagen deposition, matrix metalloproteinase (MMP) expression and activation (Figure 3). The fact that leukocytes invade the thrombus in a specific sequence suggests their importance in normal thrombus resolution.⁴² The first cell type in the thrombus is the neutrophil (PMN). Although PMNs may cause vein wall injury, they are essential for early thrombus resolution by promoting both fibrinolysis as well as collagenolysis.^{43,44} In a rat model of stasis DVT, neutropenia was associated with larger thrombi at 2 and 7 days, and was correlated with increased thrombus fibrosis and significantly lower thrombus levels of both uPA and MMP-9.⁴⁵

The monocyte is likely the most important cell for later DVT resolution. Monocyte influx into the thrombus peaks at day 8 after thrombogenesis and correlates with elevated monocyte chemotactic protein-1 (MCP-1) levels, one of the primary CC chemokines that direct monocyte chemotaxis and activation,⁴⁶ and which has also been associated with DVT resolution.⁴⁷ Targeted deletion of CC receptor-2 (CCR-2 KO) in the mouse model of stasis thrombolysis was associated with late impairment of thrombus resolution, probably via impaired MMP-2 and MMP-9 activity. We also found that CCR-2 KO mice with stasis thrombolysis supplemented with exogenous gamma interferon (INF) had full restoration of thrombus resolution, in part attributable to recovery of MMP-2 and MMP-9 activities without an increase in thrombus monocyte influx.⁴⁸

As the thrombus resolves, a number of proinflammatory factors are released into the local environment, including IL-1 β (IL-1 β) and tumor necrosis factor (TNF)- α .⁴² The cellular sources of these different mediators have not been specifically defined but likely include leukocytes and smooth muscle like cells within the resolving thrombus. The leukocyte kinetics in the vein wall after DVT are similar to what is observed in the thrombus, with an early influx of PMNs followed by monocytes. Based on our model of stasis DVT in the rat, elastinolysis seems to occur early, as measured by an increase in vein wall stiffness, persists through 14 days, and is accompanied by elevated MMP-2 and MMP-9 activities. However, early vein wall collagenolysis rather than deposition seems to occur within the first 7 days, representing an acute response to injury.⁴⁹

Linking inflammation to fibrosis, recent data demonstrates that inhibition of the inflammatory response can decrease vein wall fibrosis. In a rat study in which animals were treated with either LMWH or an oral inhibitor to P-selectin 2 days after establishment of thrombosis using an IVC stenosis model, we found that the inhibitor to P-selectin significantly decreased vein wall injury (independent of thrombus size), as measured by vein wall tensiometry (stiffness), intimal thickness score, IL-13 levels, MCP-1 levels, and platelet-derived growth factor- β (PDGFB) levels.⁵⁰

Associated with the early biomechanical injury from DVT is an elevation of profibrotic mediators, including transforming growth factor (TGF)- β , IL-13, and MCP-1. These are present within the vein wall and thrombus and may drive the fibrotic response. For example, IL-13 promotes the expression of MCP-1. Although exogenous MCP-1 may hasten DVT resolution, it promotes organ fibrosis in vivo. The profibrotic growth factor TGF β is also present in the thrombus and is activated with normal thrombolysis.⁵¹ This factor may be a key mechanism promoting vein wall fibrosis. Late fibrosis has been observed in our mouse model of DVT with a significant increase in vein wall collagen after stasis thrombogenesis.⁵² Correlating with this increase in fibrosis is an increase in collagen I and III gene expression, as well as an increase in MMP-2 and MMP-9 gene expression and activity. Thus, early vein wall injury is associated with active matrix remodeling that seems to promote net fibrosis. Taken together, therapeutic advances to alleviate postthrombotic vein wall damage will need to take into account what processes are occurring in relation to DVT age.

Disclosures

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References

- Heit JA, Cohen AT, Anderson FJ. Estimated annual number of incident and recurrent, non-fatal venous thromboembolism (VTE) events in the US. *Blood*. 2005;106:11.
- Becker BF, Heindl B, Kupatt C, Zahler S. Endothelial function and hemostasis. *Zeitschrift Fur Kardiologie*. 2000;89:160–167.
- Gross PL, Aird WC. The endothelium and thrombosis. *Semin Thromb Hemost*. 2000;26:463–478.
- Esmon CT. Inflammation and thrombosis. *J Thromb Haemost*. 2003;1:1343–1348.
- Myers D Jr, Farris D, Hawley A, Wroblewski S, Chapman A, Stoolman L, Knibbs R, Strieter R, Wakefield T. Selectins influence thrombosis in a mouse model of experimental deep venous thrombosis. *J Surg Res*. 2002;108:212–221.
- Gilbert GE, Sims PJ, Wiedmer T, Furie B, Furie BC, Shattil SJ. Platelet-derived microparticles express high affinity receptors for factor VIII. *J Biol Chem*. 1991;266:17261–17268.
- Mesri M, Altieri DC. Endothelial cell activation by leukocyte microparticles. *J Immunol*. 1998;161:4382–4387.
- Sabatier F, Roux V, Anfossio F, Camoin L, Sampol J, Dignat-George F. Interaction of endothelial microparticles with monocytic cells in vitro induces tissue factor-dependent procoagulant activity. *Blood*. 2002;99:3962–3970.
- Deregibus MC, Cantaluppi V, Calogero R, Lo Iacono M, Tetta C, Biancone L, Bruno S, Bussolati B, Camussi G. Endothelial progenitor cell derived microvesicles activate an angiogenic program in endothelial cells by a horizontal transfer of mRNA. *Blood*. 2007;110:2440–2448.
- Falati S, Liu Q, Gross P, Merrill-Skoloff G, Chou J, Vandendries E, Celi A, Croce K, Furie BC, Furie B. Accumulation of tissue factor into developing thrombi in vivo is dependent upon microparticle P-selectin glycoprotein ligand 1 and platelet P-selectin. *J Exp Med*. 2003;197:1585–1598.
- Martinez MC, Tesse A, Zibairi F, Andriantsitohaina R. Shed membrane microparticles from circulating and vascular cells in regulating vascular function. *Am J Physiol Heart Circ Physiol*. 2005;288:H1004–H1009.
- Ahn ER, Lander G, Jy W, Bidot CJ, Jimenez JJ, Horstman LL, Ahn YS. Differences of soluble CD40L in sera and plasma: implications on CD40L assay as a marker of thrombotic risk. *Thromb Res*. 2004;114:143–148.
- Singer SJ, Nicolson GL. The fluid mosaic model of the structure of cell membranes. *Science*. 1972;175:720–731.
- Sevinsky JR, Rao LV, Ruf W. Ligand-induced protease receptor translocation into caveolae: a mechanism for regulating cell surface proteolysis of the tissue factor-dependent coagulation pathway. *J Cell Biol*. 1996;133:293–304.
- Osterud B. The role of platelets in decrypting monocyte tissue factor. *Semin Hematol*. 2001;38(4 Suppl 12):2–5.
- Myers DD, Hawley AE, Farris DM, Wroblewski SK, Thanaporn P, Schaub RG, Wagner DD, Kumar A, Wakefield TW. P-selectin and leukocyte microparticles are associated with venous thrombogenesis. *J Vasc Surg*. 2003;38:1075–1089.
- Myers DD, Wakefield TW. Inflammation-dependent thrombosis. *Front Biosci*. 2005;10:2750–2757.
- Polgar J, Matuskova J, Wagner DD. The P-selectin, tissue factor, coagulation triad. *J Thromb Haemost*. 2005;3:1590–1596.
- Siddiqui FA, Desai H, Amirkhosravi A, Amaya M, Francis JL. The presence and release of tissue factor from human platelets. *Platelets*. 2002;13:247–253.
- Giesen PL, Rauch U, Bohrmann B, Kling D, Roque M, Fallon JT, Badimon JJ, Himmer J, Riederer MA, Nemerson Y. Blood-borne tissue factor: another view of thrombosis. *Proc Natl Acad Sci U S A*. 1999;96:2311–2315.
- Hrachovinova I, Cambien B, Hafezi-Moghadam A, Kappelmayer J, Camphausen RT, Widom A, Xia L, Kazazian HH Jr, Schaub RG, McEver RP, Wagner DD. Interaction of P-selectin and PSGL-1 generates microparticles that correct hemostasis in a mouse model of hemophilia A. *Nat Med*. 2003;9:1020–1025.
- Andre P, Hartwell D, Hrachovinova I, Saffaripour S, Wagner DD. Procoagulant state resulting from high levels of soluble P-selectin in blood. *Proc Natl Acad Sci U S A*. 2000;97:13835–13840.
- Jy W, Horstman LL, Wang F, Duncan RC, Ahn YS. Platelet factor 3 in plasma fractions: its relation to microparticle size and thromboses. *Thromb Res*. 1995;80:471–482.
- Biro E, Sturk-Maquelin KN, Vogel GM, Meuleman DG, Smit MJ, Hack CE, Sturk A, Nieuwland R. Human cell-derived microparticles promote thrombus formation in vivo in a tissue factor-dependent manner. *J Thromb Haemost*. 2003;1:2561–2568.
- Day SM, Reeve JL, Pedersen B, Farris DM, Myers DD, Im M, Wakefield TW, Mackman N, Fay WP. Macrovascular thrombosis is driven by tissue factor derived primarily from the blood vessel wall. *Blood*. 2005;105:192–198.
- Rectenwald JE, Myers DD Jr, Hawley AE, Longo C, Henke PK, Guire KE, Schmaier AH, Wakefield TW. D-dimer, P-selectin, and microparticles: novel markers to predict deep venous thrombosis. A pilot study. *Thromb Haemost*. 2005;94:1312–1317.

27. Chirinos JA, Heresi GA, Velasquez H, Jy W, Jimenez JJ, Ahn E, Horstman LL, Soriano AO, Zambrano JP, Ahn YS. Elevation of endothelial microparticles, platelets, and leukocyte activation in patients with venous thromboembolism. *J Am Coll Cardiol*. 2005;45:1467–1471.
28. Booth NA, Simpson AJ, Croll A, Bennett B, MacGregor IR. Plasminogen activator inhibitor (PAI-1) in plasma and platelets. *Br J Haematol*. 1988;70:327–333.
29. Horrevoets AJ. Plasminogen activator inhibitor 1 (PAI-1): in vitro activities and clinical relevance. *Br J Haematol*. 2004;125:12–23.
30. Podor TJ, Singh D, Chindemi P, Foulon DM, McKelvie R, Weitz JJ, Austin R, Boudreau G, Davies R. Vimentin exposed on activated platelets and platelet microparticles localizes vitronectin and plasminogen activator inhibitor complexes on their surface. *J Biol Chem*. 2002;277:7529–7539.
31. Adelman B, Michelson AD, Loscalzo J, Greenberg J, Handin RI. Plasmin effect on platelet glycoprotein Ib-von Willebrand factor interactions. *Blood*. 1985;65:32–40.
32. Dano K, Andreasen PA, Grondahl-Hansen J, Kristensen P, Nielsen LS, Skriver L. Plasminogen activators, tissue degradation, and cancer. *Adv Cancer Res*. 1985;44:139–266.
33. Vassalli JD, Sappino AP, Belin D. The plasminogen activator/plasmin system. *J Clin Invest*. 1991;88:1067–1072.
34. Kohler HP, Grant PJ. Plasminogen-activator inhibitor type 1 and coronary artery disease. *N Engl J Med*. 2000;342:1792–1801.
35. Schulman S, Wiman B. The significance of hypofibrinolysis for the risk of recurrence of venous thromboembolism. Duration of Anticoagulation (DURAC) Trial Study Group. *Thromb Haemost*. 1996;75:607–611.
36. Crowther MA, Roberts J, Roberts R, Johnston M, Stevens P, Skingley P, Patrassi GM, Sartori MT, Hirsh J, Prandoni P, Weitz JJ, Gent M, Ginsberg JS. Fibrinolytic variables in patients with recurrent venous thrombosis: a prospective cohort study. *Thromb Haemost*. 2001;85:390–394.
37. Akar N, Yilmaz E, Akar E, Avcu F, Yalcin A, Cin S. Effect of plasminogen activator inhibitor-1 4G/5G polymorphism in Turkish deep vein thrombotic patients with and without FV1691 G-A. *Thromb Res*. 2000;97:227–230.
38. Segui R, Estelles A, Mira Y, Espana F, Villa P, Falco C, Vaya A, Grancha S, Ferrando F, Aznar J. PAI-1 promoter 4G/5G genotype as an additional risk factor for venous thrombosis in subjects with genetic thrombophilic defects. *Br J Haematol*. 2000;111:122–128.
39. Zoller B, Garcia de Frutos P, Dahlback B. A common 4G allele in the promoter of the plasminogen activator inhibitor-1 (PAI-1) gene as a risk factor for pulmonary embolism and arterial thrombosis in hereditary protein S deficiency. *Thromb Haemost*. 1998;79:802–807.
40. Hassouna HI. Laboratory evaluation of hemostatic disorders. *Hematol Oncol Clin North Am*. 1993;7:1161–1249.
41. Palareti G, Cosmi B, Legnani C, Tosetto A, Brusi C, Iorio A, Pengo V, Ghirarduzzi A, Pattacini C, Testa S, Lensing AW, Tripodi A. D-dimer testing to determine the duration of anticoagulation therapy. *N Engl J Med*. 2006;355:1780–1789.
42. Wakefield TW, Strieter RM, Wilke CA, Kadell AM, Wroblewski SK, Burdick MD, Schmidt R, Kunkel SL, Greenfield LJ. Venous thrombosis-associated inflammation and attenuation with neutralizing antibodies to cytokines and adhesion molecules. *Arterioscler Thromb Vasc Biol*. 1995;15:258–268.
43. Varma MR, Varga AJ, Knipp BS, Sukheepod P, Upchurch GR, Kunkel SL, Wakefield TW, Henke PK. Neutropenia impairs venous thrombosis resolution in the rat. *J Vasc Surg*. 2003;38:1090–1098.
44. Stewart GJ. Neutrophils and deep venous thrombosis. *Haemostasis*. 1993;23 Suppl 1:127–140.
45. Varma MR, Moaveni DM, Dewyer NA, Varga AJ, Deatrick KB, Kunkel SL, Upchurch GR Jr, Wakefield TW, Henke PK. Deep vein thrombosis resolution is not accelerated with increased neovascularization. *J Vasc Surg*. 2004;40:536–542.
46. Hogaboam CM, Steinhauser ML, Chensue SW, Kunkel SL. Novel roles for chemokines and fibroblasts in interstitial fibrosis. *Kidney Int*. 1998;54:2152–2159.
47. Humphries J, McGuinness CL, Smith A, Waltham M, Poston R, Burnand KG. Monocyte chemotactic protein-1 (MCP-1) accelerates the organization and resolution of venous thrombi. *J Vasc Surg*. 1999;30:894–899.
48. Henke PK, Pearce CG, Moaveni DM, Moore AJ, Lynch EM, Longo C, Varma M, Dewyer NA, Deatrick KB, Upchurch GR Jr, Wakefield TW, Hogaboam C, Kunkel SL. Targeted deletion of CCR2 impairs deep vein thrombosis resolution in a mouse model. *J Immunol*. 2006;177:3388–3397.
49. Henke PK, Varma MR, Moaveni DK, Dewyer NA, Moore AJ, Lynch EM, Longo C, Deatrick CB, Kunkel SL, Upchurch GR Jr, Wakefield TW. Fibrotic injury after experimental deep vein thrombosis is determined by the mechanism of thrombogenesis. *Thromb Haemost*. 2007;98:1045–1055.
50. Myers DD Jr, Henke PK, Bedard PW, Wroblewski SK, Kaila N, Shaw G, Meier TR, Hawley AE, Schaub RG, Wakefield TW. Treatment with an oral small molecule inhibitor of P selectin (PSI-697) decreases vein wall injury in a rat stenosis model of venous thrombosis. *J Vasc Surg*. 2006;44:625–632.
51. Grainger DJ, Wakefield L, Bethell HW, Farndale RW, Metcalfe JC. Release and activation of platelet latent TGF β in blood clots during dissolution with plasmin. *Nature Med*. 1995;1:932–937.
52. Deatrick KB, Eliason JL, Lynch EM, Moore AJ, Dewyer NA, Varma MR, Pearce CG, Upchurch GR, Wakefield TW, Henke PK. Vein wall remodeling after deep vein thrombosis involves matrix metalloproteinases and late fibrosis in a mouse model. *J Vasc Surg*. 2005;42:140–148.