The Significance of Endothelial Heterogeneity in Thrombosis and Hemostasis

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ABSTRACT

The endothelium is recognized today as a functional and dynamic component of the body that plays an important part in health and disease. This article briefly reviews the role of endothelium in thrombosis and hemostasis. There is a paradigm shift from one that regards the endothelium as one single entity to the concept that the endothelium is heterogeneous. Thrombotic complications in many disorders show a predilection to specific locations, despite the fact that a hypercoagulable state affects the entire body. Likewise, bleeding is more commonly encountered in certain anatomical locations than in others. Recent observations of the heterogeneous distribution of coagulation and fibrinolytic factors in the endothelium and the adaptation of the endothelium to various stimuli may provide an explanation for the diverse phenotypes. Recognition of this changing paradigm is helpful to for the diagnosis and management of bleeding and thrombotic complications. It also helps in the understanding of the pathogenesis of many bleeding and thrombotic disorders, and in the development of new drug designs for site-specific therapy.

KEYWORDS: Endothelium, thrombosis, hemostasis, hemorrhage

In 1865, Wilhelm His first introduced the term *endothelium* to describe the mesoderm-derived lining of internal body cavities.[1] Since then, our perception of this lining has undergone major changes. The term is now applied solely to the lining of blood vessels and lymphatics. Its characteristics and functions are also better understood. Along with this new understanding is the realization that the endothelium is a highly metabolic and dynamic tissue that is constantly responding to changes in its environment. Endothelial cells play a major role not only in many physiological functions but also in the pathogenesis of many pathological disorders. Endothelial phenotypes are uniquely adapted to their local tissue environment. They are influenced by a wide variety of stimuli ranging from biochemical cues (e.g., inflammatory cytokines). This new concept of the endothelium is a departure from the previously accepted notion that endothelium is an inert lining and "all endothelial cells are alike." From clinical observations, in vitro endothelial cell culture studies as well as in vivo investigations in animals, it is clear that the endothelium is heterogeneous, both in its anatomical and functional characteristics. These observations have been previously reviewed in detail.[2–8] In this article, we add new observations that support this concept and show that it also applies to hemostasis and thrombosis.

PHYSIOLOGICAL CONDITIONS

The normal endothelium has a large surface of ~350 m^2 with a mass of ~110 g.[9] The structure of normal endothelium is different in different locations of the

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body. For example, the endothelium can be continuous with tight junction between cells, as found in the central nervous system, lymph nodes, and muscles; fenestrated, with absorption, secretion, and filtration functions, as seen in endocrine glands, gastrointestinal tract, and kidneys; or discontinuous, where intercellular gaps are 0.1 to 1 μm in diameter, as seen in the liver sinusoids, bone marrow, and spleen.[9,10] As another example, the endothelium in arteries is structurally different from that in the veins. This particular topic is reviewed by Tse and Stan in this issue of *Seminars in Thrombosis and Hemostasis*.[11] The endothelium is highly dynamic, adapting to its microenvironment, as affected by spatial and temporal changes in biomechanical and biochemical factors.[12] Further, the endothelium is not simply a passive lining but rather an active participant of many body functions including maintenance of vascular tone, regulating exchange of fluids, and trafficking of cellular components between the intravascular and extravascular compartments, maintaining fluidity of blood and adapting to changes in the blood flow and fluid dynamics. Changes in its environment can activate the endothelium, as detected by specific markers. Under certain conditions, this response may be exaggerated, causing endothelial dysfunction that ultimately leads to disease.

CLINICAL OBSERVATIONS ON SITE OF THROMBOSIS

Most venous thrombosis occurs in the deep veins of the lower extremities in patients with increased risk factors, which can include hereditary thrombophilia and acquired hypercoagulable states, ranging from antiphospholipid syndrome to the adverse effect of drugs such as oral contraceptives.[13] However, thrombotic complications in many disorders present themselves in specific locations, as listed in Table 1. The reasons for this site predilection are not always clear and are reviewed in the following sections.

Cerebral Vein and Sinus Thrombosis

Thrombosis in the cerebral vein and sinus thrombosis is distinguished by its occurrence in young adults and children, its predominance in the female, and its anatomy. Blood from the cerebral veins drains into various sinuses, which become confluent and in turn empty into the internal jugular vein. Thrombosis of the cerebral veins causes edema, venous infarction, and sometimes hemorrhage; sinus thrombosis results in intracranial hypertension due to increased venous pressure and imparted absorption of the cerebrospinal fluid. In most patients with this complication, thrombosis of both the cerebral veins and sinuses occurs.[14] The most frequent location of thrombosis is the transverse sinuses (86%), followed by superior sagittal sinus (62%) and the straight sinus (18%).[15] Some of the predisposing causes are discussed here.

In acute lymphoblastic leukemia (ALL), thrombotic complications are frequent, with reported incidence of 1.7 to 36.7%[16,17] in children, depending on the choice of chemotherapeutic agents, the use of central venous access catheters, and the definition of thrombosis. A recent meta-analysis[18] indicated an incidence of 5.2%. Remarkably, among those with thrombosis, about half (53.8%) occurred in the central nervous system, most commonly sino-venous thrombosis with cerebral infarction in 9.9%.

Although ALL is less common in adults, thromboembolic events are still a major complication with an incidence of 5.9%. In contrast to childhood ALL, deep vein thrombosis in adults occurs mostly in the lower limbs (38.9%).[19] The major factors contributing to the thrombotic risk are the presence of a central venous catheter and the use of treatment protocols using L-asparaginase, which inhibits hepatic synthesis of both the coagulant and anticoagulant proteins. However, the recovery of these proteins following the cessation of L-asparaginase therapy takes place at different rates. The return of anticoagulant level, especially antithrombin, lags behind recovery of the coagulants, resulting in an increased thrombotic risk.[16,20–22] Because these contributory thrombogenic factors are present in both adults and children with ALL, it is intriguing why there is a predilection of thrombotic complications for the cerebral veins in children. A possible explanation is that the anatomical structure may be more prone to thrombosis in this location at a young age. This is supported by the observations that cerebral sino-venous thrombosis is more common in neonates than in young children.[23]

Cerebral venous and sinus thrombosis are also the preferred sites of thrombosis in other hypercoagulable states[14,24] (Table 1). Among women with cerebral venous and sinus thrombosis, the predominant causes are the use of oral contraceptives[21,25,26] and pregnancy, especially in the third trimester and during the peripartum period.[22,27] Among persons with inherited thrombophilia, cerebral venous thrombosis is common in those with the prothrombin G20210A mutation[26] and in individuals with hyperhomocysteinemia due to the methylene tetrahydrofolate reductase C677T gene polymorphism[14,28,29] (although the evidence for the latter is weak). It is also seen in heparin-induced thrombocytopenia.[30]

In paroxysmal nocturnal hemoglobinuria (PNH), thrombosis is a major complication and cause of death.[31–33] Several factors account for the increased risk of thrombosis. In additional to the thrombogenic effects of massive hemolysis and platelet activation, the fibrinolytic balance is perturbed. Due to the absence of GPI-anchoring protein for the urokinase receptor (uPAR) in PNH clones of leukocytes, there is an excess

Table 1 Site-Specific Thrombosis in Veins, Microvasculature, and Arteries, Their Associated Anomalies and Contributing Factors

Sites of Thrombosis	Associated Anomaly	Contributing Factors
Veins		
Intracranial venous thrombosis (cerebral vein and sinus)	ALL in children[13–15]	L-asparaginase Central venous catheter[17]
	Pregnancy, last trimester; postpartum[14,27]	Cesarean delivery
	Young women on oral contraceptives[14,24,26]	
	Prothrombin G20210A mutation[26]	
	Hyperhomocysteinemia[28,29]	
	Paroxysmal nocturnal hemoglobinemia	
	Myeloproliferative disorder[36]	
Upper body venous thrombosis	Ovarian hyperstimulation syndrome[37–40]	
Lower limb vein thrombosis	Immobilization	Impaired blood flow
Splanchnic veins	JAK2V617F mutation (polycythemia vera, ET)	
	Paroxysmal nocturnal hemoglobinuria[24,25,27,68]	
Hepatic sinusoids	Sinusoidal obstruction syndrome	EC injury by drugs, toxins
Mesenteric veins	Multiple disorders	Lipids from gut
Ovarian vein	Pregnancy[74,75]	Sepsis
Microvasculature Dermal and adipose	Warfarin necrosis[76–80]	Protein C deficiency
	Heparin induced thrombocytopenia[82–84]	EC immune injury
Cerebral	Thrombotic thrombocytopenic purpura[85–90]	Immune targeting ADAMTS13
	Catastrophic antiphospholipid syndrome[100]	Rbc aggregation
	Malaria[102]	
Renal	TTP,[85–90] HUS[95,96]	Rbc aggregation
	Cold hemagglutinin disease[101]	
Extremities	Erythromelalgia[103]	Platelet aggregation
Arteries		
Coronary, carotid, femoral	Atherosclerosis	High PAI-1
Cerebral	Paroxysmal nocturnal hemoglobinuria	Complement injury
	Systemic lupus erythematosus	Immune targeting of EC
Muscular	Polyarteritis nodosa	Immune targeting of EC
Aortic arch	Takayasu's arteritis	
Carotid, temporal	Giant cell arteritis	Immune target of EC

ALL, acute lymphoblastic leukemia; ET, essential thrombocytosis; EC, endothelial cell; ADAMTS13, a disintegrin and metalloproteinase with a thrombospondin type 1 motif, Member 13; Rbc, red blood cell; TTP, thrombotic thrombocytopenic purpura; HUS, hemolytic uremic syndrome; PAI, plasminogen activator inhibitor.

of the soluble uPA receptor (suPAR) in the circulation.[34] The deficient uPAR on leukocytes acts synergistically with the circulating suPAR in reducing the cell-associated fibrinolytic activity. In a review of the thromboembolic events in 363 cases in published literature, it was found that cerebral vein and sinus thrombosis occurred in 20.9% of the events, second only to splanchnic vein thrombosis, which occurred in 63.9% of the events.[35] The explanation for this site specificity is unclear. One can speculate that in this disease, the lack of the regulatory proteins CD55 and CD59 causes a dysregulation of the complement system that can lead to endothelial injury. The preference of the injury site to the cerebral and splanchnic veins, however, cannot be explained. Cerebral vein and sinus thrombosis are also seen in myeloproliferative disorders,[36] which are discussed later.

Upper Body Venous Thrombosis

Venous thrombosis in the upper extremities and neck can be the result of injury, presence of a central venous catheter, obstruction by lymphoma, head and neck or mediastinal tumor, or the presence of congenital venous anomalies. However, venous thrombosis in this location is not commonly encountered in patients with hypercoagulability, with several exceptions. It is seen in women receiving hormone-assisted fertility treatment who develop iatrogenic endocrine excess in the ovarian hyperstimulation syndrome (OHSS).[37–40] These patients receive gonadotrophin-releasing hormone analogues and exogenous gonadotrophins for ovarian stimulation. The characteristic sites of thrombosis are the veins of the upper extremity, especially the internal jugular vein.[37,38,41–43] Thrombosis in this location also occurs during pregnancy and the postpartum period,

although with much less frequency. In OHSS, 70% of the thrombotic complications were venous with a preponderance of upper limb venous thrombosis.[43] Prothrombotic factors include the effect of high estrogen blood levels on vascular tone, blood flow, tissue factor released from monocytes-derived microparticles,[44] and an increase in coagulation factors.[45] Following the administration of gonadotrophic hormones, the stimulated ovaries produced more estrogen. The drainage of the abdominal lymphatics with high estrogen content into the thoracic duct, which ends in the right subclavian vein at the junction with the right jugular vein, has been suggested to be a contributory factor.[42,46] The prophylactic use of low molecular weight heparin is recommended, but on occasions, it was unable to prevent OHSS in some patients.[43]

Lower Extremity Deep Vein Thrombosis

Most major venous thrombotic events take place in the deep veins of the lower extremities. The causative factors are the nature of the anatomical structure and the characteristics of blood flow. Venous return requires the normal function of multiple valves. The formation of a thrombus usually starts in the valve pockets, where there is stagnant flow, anoxia, and deposits of platelets and leukocytes.[47–49] At the apex of the valve pocket, there is often a preexisting microthrombus.[50] In the event of immobilization, an ensuing stasis of blood flow enables thrombus extension into the main channel of the veins. This can occur without the presence of a hypercoagulable state. Prophylactic antithrombotic measures are thus needed, even in healthy subjects during periods of immobilization, and are especially essential in patients with an underlying prothrombotic risk.

Splanchnic Vein Thrombosis

Thrombosis in the splanchnic vasculature may occur in portal, mesenteric, and hepatic veins, the latter causing the Budd-Chiari syndrome. In the latest meta-analysis of published data involving 241 patients with splanchnic vein thrombosis (SVT), bcr/abl-negative myeloproliferative disorders (MPDs), as identified by the presence of the Janus kinase 2 (JAK2) V617F somatic mutation (JAK2V617F), were found in 45% of those with Budd-Chiari syndrome and in 34% of patients with portal vein thrombosis.[51] This supports earlier reports of the presence of the JAK2V617F mutation in 29.4% of portal vein thrombosis and 42.8% of SVT patients without overt manifestations of MPD.[52] JAK2 mediates the effects of hematopoietic cytokines such as erythropoietin and granulocyte colony-stimulating factor. The JAK2V617F mutation results in a gain of function and is believed to enhance proliferation of hematopoietic progenitor cells in MPDs.[53] The mutant is found in ~95% cases of polycythemia vera and at least 50% of patients with essential thrombocytosis. In both types of MPDs, both arterial and venous thrombosis are common complications.[54–56] In the recent European Collaboration on Low-Dose Aspirin in Polycythemia Vera (ECLAP) study,[55,57] low-dose aspirin was found to be effective in reducing thrombotic complications, suggesting a prothrombotic risk conferred by platelets. Numerous studies suggest that polycythemia vera and essential thrombocythemia are associated with a hypercoagulable state, as evidenced by platelet and endothelial activation.[58,59] In essential thrombocythemia, platelets were also found to be more sensitive to activation.[58,59] Leukocytes, polymorphonuclear neutrophils in particular, were also implied because they were found to be activated and to form aggregates with platelets in both essential thrombocythemia and polycythemia vera.[60] Lately, the concept that an elevated hematocrit and/or a high platelet count are thrombogenic has been challenged. For example, recent studies have failed to demonstrate a correlation between the thrombotic event and an increased hematocrit,[61] elevated platelet count,[61] or leukocytosis.[62] However, these data are at odds with a recent Norwegian study[63] and need to be confirmed in additional studies. In any case, one might consider another contributory factor to the thrombogenesis in MPD: endothelial dysfunction at the vascular sites affected. There is indirect evidence of endothelial dysfunction and activation, including changes in plasma levels of fibrinogen, von Willebrand factor (VWF), soluble thrombomodulin, and plasminogen activator inhibitor 1 (PAI-1).[64]

Although arterial thrombosis is more common than venous thrombosis in MPDs, it is unexplained why thrombosis at unusual sites such as splanchnic veins is frequently seen. Because the JAK2V716F mutation in hematopoietic cells is increased in SVT,[51] and this mutation is believed to cause endothelial dysfunction, one can postulate that this mutation may occur in the endothelial cells located in the splanchnic venous endothelium. As both the hematopoietic cells and endothelial cells can be sharing a common progenitor cell,[65] it is likely that the JAK2V617F mutation occurs in both lineages. In fact, some patients with JAK2V617F mutation may develop SVT before hematological manifestations take place.[51,66] In recent study, Sozer et al,[67] using laser capture micro-dissection followed by polymerase chain reaction (PCR) and real time (RT)-PCR, examined the endothelial cells lining the terminal hepatic venules. In two of three patients with polycythemia vera complicated by SVT, the hepatic venular endothelial cells showed homozygosity for the JAK2V617F mutation. In two other nonpolycythemic control patients with hepatoportal sclerosis complicated by portal vein thrombosis, their endothelial cells showed exclusively the wild-type JAK2. These findings open the way for more future

studies, exploring the extent of this mutation in the endothelial cells in other locations of the body.

Paroxysmal Nocturnal Hemoglobinuria

As previously mentioned, thrombosis is the major cause of morbidity and mortality in PNH. Among the thromboembolic events, splanchnic vein thrombosis accounts for 63.9%.[24,25,27,68] The thrombotic risk in this disorder was discussed earlier in the section on cerebral vein thrombosis.

Sinusoidal Obstruction Syndrome/Hepatic Veno-Occlusive Disease

In sinusoidal obstruction syndrome (SOS) or hepatic veno-occlusive disease (VOD), the hepatic venular and sinusoidal endothelium in zone 3 of the hepatic acinus is occluded by subendothelial edema, red cell extravasation, and fibrin deposition.[69–72] Originally termed hepatic veno-occlusive disease, the name was changed because central vein thrombosis occurs in only 55% of mild disease and in 75% of the severe cases. This is a remarkable example of site-specific endothelial damage leading to vascular occlusion and thrombosis. There are two major causes of the damage. One is associated with chemotherapy or radiation, used in myeloablative therapy. It is also seen in chemotherapy with agents such as gemtuzumab ozogamicin (Mylotarg), actinomycin D, dacarbazine, cytosine arabinoside, mitramycin, 6-thioguanine, and urethane. The other cause in non-Western countries is the ingestion of diet contaminated by pyrrolizidine alkaloids. SOS/VOD responds poorly to treatment with anticoagulants. However, defibrotide was found to be effective in about a third of the cases.[73] Defibrotide is a polydisperse oligonucleotide, which binds to the endothelium via adenosine receptors A1 and A2, has antithrombotic, anti-ischemic, anti-adhesive, anti-inflammatory, and thrombolytic properties, through binding to the liver endothelium via adenosine receptors A1 and A2.

Ovarian Vein Thrombosis

Ovarian vein thrombosis is an uncommon complication seen in delivery.[74,75] Sepsis is believed to play a key role.

Microvascular Thrombosis

In a hypercoagulable state, thrombosis often initiates in the microvasculature and then readily extends to larger venous channels. However, in two conditions, warfarin necrosis and heparin-induced thrombocytopenia, the microvascular occlusion is not only the presenting clinical feature but often remains as the only site of vascular involvement.

In warfarin necrosis, the site of thrombosis is in the skin and locations with abundant adipose tissues, commonly in the breast, abdomen, and thigh.[76–80] In the involved tissues, diffuse microthrombi in the capillaries and venules are present initially in the dermis and subcutaneous tissues. Subsequently, the thrombosis can extend to larger veins and can produce large areas of necrosis. This is an uncommon complication of oral anticoagulant therapy that occurs within 3 to 10 days of the initiation of therapy. It is believed to be due to inhibition of the hepatic synthesis of protein C, protein S, antithrombin, and the other clotting factors (II, VII, IX, X) by warfarin. Protein C and factor VII have a shorter half-life compared with that of the other vitamin K–dependent coagulation factors, especially factor X. As a result, a rapid fall in protein C takes place leading to a transient imbalance between the anticoagulant and the procoagulant proteins.[81] Such a hypercoagulable state is exacerbated in patients with a preexisting protein C deficiency.[79] This hypothesis is supported by our own observations that 11 of 13 patients who had recovered from warfarin necrosis had abnormally low levels of functional protein C in their plasma.[80] However, other mechanisms may be responsible for warfarin necrosis because it has been seen in patients without protein C deficiency.

Heparin-Induced Thrombocytopenia

The diagnosis of this condition is suspected when the platelet count falls significantly after initiation of heparin.[82] Thrombotic events are most commonly arterial with the finding of a platelet-rich "white clot." However, the condition starts with microvascular lesions caused by platelet aggregates, formed as the result of an immunoglobulin (Ig)G autoantibody/heparin-platelet factor 4 complex. The microthrombi then extend to form larger lesions. There is evidence suggesting that this IgG complex is not only present in the platelet thrombi but is also deposited on the endothelial surface at the site of thrombosis.[83] Endogenous glycosaminoglycans anchored to the endothelium may be an additional target of the anti-platelet factor-4 IgG. Endothelial cell hyperplasia is present as well, presumably as the result of the immune stimulus.[83] Microvascular endothelial cells have been shown to be more sensitive to this form of injury in vitro, whereas macrovascular endothelial cells require the presence of tumor necrosis factor α (TNFα).[84]

Acute Idiopathic Thrombotic Thrombocytopenic Purpura

The site-specific vascular lesion in thrombotic thrombocytopenic purpura (TTP) is a good example of endothelial heterogeneity.[85–88] First described by Moschcowitz in 1924[89] as "an acute febrile pleiochromic anemia with hyaline thrombi in terminal arterioles and capillaries,"

this disease is recognized today as a specific form of thrombotic microangiopathy associated with a congenital deficiency of, or an autoimmune disorder directed at, ADAMTS13, an integrin with a protease domain that breaks down large VWF multimers after the latter's release from endothelial cells. A severe deficiency of ADAMTS13 is characteristic of idiopathic TTP. The resulting accumulation of unusually large VWF multimers[90] leads to the formation of platelet aggregates and platelet microthrombi in arterioles and capillaries.[85] Although most organs are affected in advanced stages of the disease, the microvasculature in the brain, kidneys, skin, and myocardium is most commonly involved; that in the liver and lungs is least commonly affected. Among the various pathogenic factors for TTP is endothelial injury. In vitro studies have revealed that plasma from autoimmune TTP patients can cause apoptosis of microvascular endothelial cells (MVECs).[91] Furthermore, MVECs derived from the brain, kidneys, and skin are most sensitive to the TTP plasma, whereas those derived from lung and liver are not. More recently, some distinguishing features of the sensitive versus nonsensitive MVECs were found.[92,93] In the TTP-sensitive MVECs, apoptosis in the presence of TRAIL (tumor necrosis factor related apoptosis-inducing ligand), along with interferon-γ, was enhanced. This was due to a lack of inhibition of the caspase-8 pathway. Ubiquitination of an inhibitor of apoptosis, c-FLIP (a FLICE-like inhibitory protein), leads to its degradation of this proteosome. This is not the case with the nonsensitive MVECs derived from the lung. However, c-FLIP silencing by anti-FLIP siRNA rendered the lung MVECs susceptible to apoptosis. Such findings provide one explanation for the site specificity of TTP lesions. What is still an enigma is the link between the endothelial injury and the development of autoantibodies against ADAMTS 13. Because perturbation of the complement system occurs in the closely related HUS, it is postulated that complement-induced endothelial injury may also play a part in TTP.[94]

Hemolytic Uremic Syndrome

There are two forms of hemolytic uremic syndrome (HUS): the Shiga toxin induced form and the atypical/recurrent form.[95,96] In the Shiga toxin induced form, the toxin binds to a receptor (Gp3) in the endothelial cells, causing the injury.[97] The increased inflammatory cytokines caused by the Shiga toxin results in an upregulation of Gp3 expression the endothelial cells. However, in the atypical HUS, injury to the endothelial cells is induced by the terminal complement complex, C5b-9. In both forms of HUS, the renal endothelial cells are most susceptible to the respective injury, with clinical manifestation of acute impairment of renal function.[98] Involvement of the other organs is not common, although a small number of patients may have neurological changes. The human brain MVECs are also susceptible to apoptosis induction by Shiga toxin 1 and Shiga toxin 2.[99] The predilection of renal MVEC to this injury has not been adequately explained.

Miscellaneous

Disseminated intravascular coagulation (DIC) is usually associated with endothelial cell damage from bacterial toxins and physical and other chemical injuries, but extensive microthrombi formation is not common and requires additional comorbid disorders such as catastrophic antiphospholipid syndrome[100] or extensive microvascular occlusion by agglutinated red blood cells in cold hemagglutinin disease[101] and in malaria.[102] The renal arterioles are most susceptible to thrombosis, followed by small vessels in the brain and mesentery. In erythromelalgia, seen mostly in MPDs, platelet activation and thrombi formation are the cause of the painful manifestations, which are promptly relieved by antiplatelet drugs.[103]

Arterial Thrombosis

In contrast to venous thrombosis, most arterial thrombosis occurs in arteries with preexistent disease. Endothelial phenotypes differ between arteries and veins. Arterial thrombus is rich in platelets, whereas venous thrombus contains mostly fibrin and red cells. Arterial thrombi are formed under conditions of high or aberrant blood flow, but a venous thrombus begins with a stagnant flow. The site of arterial thrombosis is mostly determined by the distribution of atherosclerotic lesions, which is in turned influenced by the pattern of blood flow. Less commonly, arterial thrombosis occurs in the absence of atherosclerosis (e.g., in systemic lupus erythematosus, affecting cerebral arteries; in polyarteritis nodosa, affecting muscular arteries; in Takayasu's arteritis, affecting aortic arch; and in giant cell arteritis affecting the carotid, temporal, and vertebral arteritis) (Table 1). However, recent clinical observations indicate that both arterial and venous thrombosis share many common predisposing features[104] and the pattern of similarity between the two has emerged.[105,106] A common feature is the association with metabolic syndrome,[107,108] obesity,[109,110] smoking,[109,110] hypertension,[110] and hyperlipidemia.[111,112] In addition, the markers of arterial thrombosis such as increased levels of fibrinogen, VWF, PAI-1, and inflammatory cytokines can also be found in venous thrombosis.[113]

CLINICAL OBSERVATIONS ON SITE OF BLEEDING

Other than direct injury to a blood vessel, the site of bleeding in a patient with general bleeding diathesis can

Table 2 Site-Specific Bleeding and Their Associated Anomalies and Contributing Factors

Sites of Bleeding	Associated Anomaly	Contributing Factors
Intracranial	APL[117–120]	Increased annexin II
	ALL[127]	
	Hypertension	
	Thrombolytic therapy[129–134]	
Pulmonary	ALL, APL[135]	Hypoxia
	Neonatal diffuse alveolar hemorrhage	Immune targeting EC
	Goodpasture's syndrome	Immune targeting neutrophils
	Wegener's granulomatosis	Immune targeting EC
	Systemic lupus erythematosus	
Adrenals	Warfarin therapy	Structure of adrenal vein
	Multiorgan failure	Rich arterial supply[137]
		High catecholamines[8]
Retroperitoneal	Anticoagulant therapy	Old age,[139] atherosclerosis
Hemarthrosis	Hemophilia	Low procoagulants[141,142]
		High fibrinolytic activity[141,142]
Retina	Hyperviscosity	

APL, acute promyelocytic leukemia; ALL, acute lymphoblastic leukemia; EC, endothelial cell.

also show a higher incidence in certain locations. The site predilection is much less pronounced than in the case of thrombosis. Table 2 shows some of the locations.

Intracranial Hemorrhage

The major risk factors for intracerebral hemorrhage (ICH) are hypertension, high alcohol consumption, smoking, hypercholesterolemia, diabetes mellitus, and male gender.[114] Hemostatic risk factors have also been identified, including high tissue plasminogen activator (tPA)/PAI-1 complex and low VWF. The latter shows a synergistic interaction with hypertension.[115] Reduced platelet function portends a worse prognosis.[116] Several diseases with general bleeding diathesis but with a higher incidence of ICH are discussed next.

Acute Promyelocytic Leukemia

In acute promyelocytic leukemia (APL) (FAB classification: M3), there is a failure of differentiation of the myeloid lineage resulting in the arrest at the promyelocyte stage. On presentation, the patient is in a marked hypercoagulable state, due to an increased expression of tissue factor and of a cancer procoagulant by the leukemic promyelocytes.[117–120] This manifests as DIC with bleeding complications. In multiple clinical trials, the major cause of early deaths is bleeding. Among these, ICH is the most common form, accounting for 65 to 85% of early deaths.[121,122] The high risk of bleeding is due to multiple factors, including thrombocytopenia, hypofibrinogenemia from DIC, increased fibrinolysis with increased tPA and uPA, and decreased PAI-1 and α_2-antiplasmin.[120,123] In addition, there is an increase in annexin II on the leukemic promyelocytes. Annexin II is a co-receptor for plasminogen and for tPA, and it facilitates plasmin generation.[124] However, the high incidence of ICH requires added explanation. We studied the presence of annexin II in human endothelial cells in vitro and observed that it is constitutively expressed in much higher amounts in the cerebral microvascular endothelial cells than in endothelial cells derived from other parts of the body.[125] This finding offers one explanation why ICH is more common than bleeding at other sites. It also provides one reason for the higher incidence of ICH seen in thrombolytic therapy with tPA than with other fibrinolytic agents. In APL, in spite of these findings of increased fibrinolysis, the use of the antifibrinolytic agent tranexamic acid did not prevent this complication in one nonrandomized clinical trial.[126] In vitro treatment of the APL cell line, NB4, with all-trans retinoic acid (ATRA) resulted in prompt down-regulation of annexin II. Likewise, APL patients treated with ATRA also demonstrated reduced fibrinolytic activity in blood.[123] We recently observed that microparticles bearing annexin II are found in the blood of patients with APL. They were also reduced by ATRA treatment.

Acute Lymphoblastic Leukemia

Hemorrhagic complications is a major cause of death in childhood ALL.[127,128] As in the case of APL, two thirds of the bleeding events in ALL were also ICH.

Thrombolytic Therapy

ICH is an uncommon but important complication in thrombolytic therapy when used for pulmonary embolism,[129,130] myocardial infarction,[131] and ischemic

stroke.[132] In pulmonary embolism, the rate of ICH is 1.7% in clinical trials using tPA, but it is believed to be close to 3.0% in a large registry[110] and is lower with other thrombolytic agents. In myocardial infarction, the rate is <1%, but is higher (1.7%) with tPA in elderly patients.[114,131] In stroke, the incidence of ICH is much higher, ranging from 7.7% to 22.3% in different clinical trials,[132] with higher rates when treatment was begun later than 3 hours of onset of the stroke. An interesting additional risk factor is a low PAI-1 level.[133,134] In ICH complicating tPA therapy, the bleeding usually occurs in the absence of bleeding elsewhere in the body and takes place a day or so after the treatment, long after circulating tPA has been cleared. One explanation may be the fact that annexin II is higher in the brain microvasculature, as discussed earlier.[106]

Diffuse Alveolar Hemorrhage

In both APL and ALL, pulmonary hemorrhage was also common,[126,135] but second to ICH. Diffuse alveolar hemorrhage is also seen in other diseases as listed in Table 3. In neonates and in premature births, anoxia is a major risk factor. It is also seen in Goodpasture's syndrome, Wegener's granulomatosis, and systemic lupus erythematosus.

Table 3 Prothrombotic and Antithrombotic Factors in Endothelium

Prothrombotic Factors	Antithrombotic Factors
Coagulation	
Tissue factor (encrypted)	Tissue factor pathway inhibitor
von Willebrand factor	Protein C, Protein S
PAR-1, PAR-2 (thrombin receptors)	Thrombomodulin
	Endothelial protein C receptor
	Heparan
	Nitric oxide synthetase
Platelets	
Thromboxane A2	Prostacyclin
von Willebrand factor	ADAMTS13
Platelet activating factor	Ecto-ADPase
Plasminogen-plasmin system	
PAI-1	Tissue plasminogen activator
	Urokinase (uPA)
	uPA receptor
	Annexin A2
Adhesive molecules	
P-selectin; E-selectin	
PECAM-1; ICAM-1;VCAM-1	

PAR, protease-activated receptor; ADAMTS 13, a disintegrin and metalloproteinase with a thrombospondin type 1 motif, Member 13; PAI, plasminogen activator inhibitor; ADPase, adenosine diphosphatase; PECAM, platelet endothelial cell adhesion molecule; ICAM, intercellular adhesion molecule; VCAM, vascular cell adhesion molecule.

Adrenal Hemorrhage

This is a complication seen in long-term anticoagulant therapy, in the postoperative period, in the antiphospholipid-antibody syndrome, in heparin-induced thrombocytopenia, or in the setting of severe physical stress and multiorgan failure.[136] The hemorrhage can sometimes be the result of microvascular thrombosis. This organ is prone to these complications on account of its unique venous anatomy and rich arterial supply[137] and because it is high in catecholamine concentration.[8]

Retroperitoneal Hemorrhage

This complication is seen in patients receiving anticoagulation,[138] most often with warfarin but also with heparin. It also occurs after abdominal trauma, after rupture of aneurysm, and misadventure with transvascular catherization. Age is a risk factor, likely related to presence of more advanced atherosclerotic lesions in the elderly.[139] The bleeding usually occurs in multiple small arteries and can be readily demonstrated by computed tomography (CT) scan with contrast, and bleeding can be controlled by embolization.[138]

Hemarthrosis

In patients with hereditary coagulation defects, bleeding into the joints is perhaps the most frequent long-term complication. One explanation for a higher bleeding rate into this location is that the synovial membrane lacks procoagulants but is rich in fibrinolytic activity.[140,141]

THE BASIS FOR HETEROGENEITY IN THROMBOTIC AND BLEEDING SITES

Expression of Hemostatic and Fibrinolytic Factors

Endothelial expression of hemostatic and fibrinolytic factors is not consistent across the vasculature. For example, although most factor VIII is present in hepatocytes and in renal glomerular cells by mRNA analysis, its expression in the endothelium is limited to liver sinusoids.[142] In contrast, the mRNA of VWF is mostly expressed in veins, with the exception of the pulmonary veins, but is largely absent in arteries, with the exception of the distal aorta and pulmonary arteries, and has little expression in capillaries.[143–147] Another example is the case of thrombomodulin, which is virtually absent in brain.[148] Protein C is more highly expressed in the small compared with large blood vessels. A higher tissue factor expression is seen in the branches of arteries, where it is

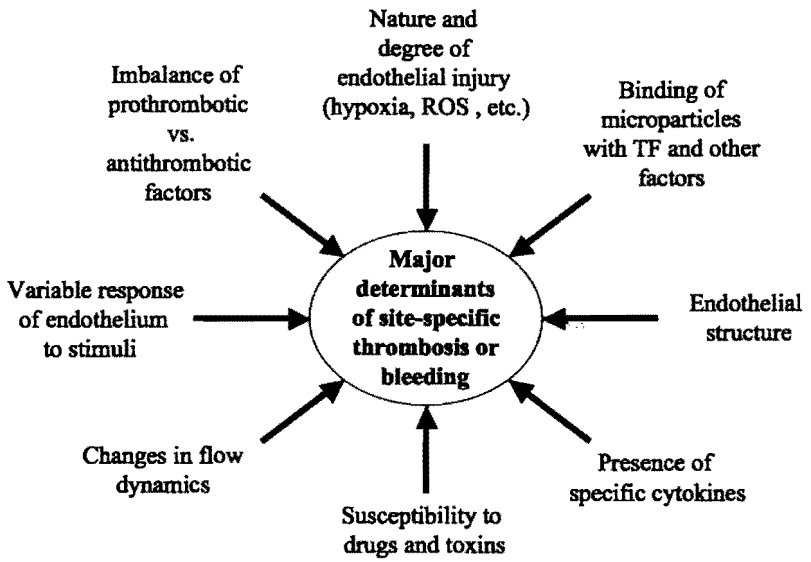

Figure 1 Major factors that determine the specific site of thrombosis or bleeding. TF, tissue factor; ROS, reactive oxygen species.

believed to play a role in early atherogenesis.[149] The components of the fibrinolytic system are also distributed unevenly.[150,151] Such heterogeneity may explain many clinical observations of the site of thrombosis in patients with a hypercoagulable state or of hemorrhage in bleeding diathesis.

Variable Response to Stimuli: Inflammatory Cytokines, Blood Flow, Drugs, and Toxins (Fig. 1)

Under physiological conditions, there is a hemostatic balance in the endothelium, maintained by both prothrombotic and antithrombotic factors (Table 3). The prothrombotic factors include tissue factor, encrypted on the cell membrane; VWF, stored in Weibel Palade bodies; protease-activated receptors (PAR-1 and PAR-4), serving as thrombin receptors; thromboxane A2 and platelet-activating factor, enhancing platelet activation; PAI-1, which inhibits fibrinolysis; and adhesive molecules, P-selectin (CD62P), E-selectin (CD62E), PECAM-1 (CD31), ICAM-1 (CD54), and VCAM-1 (CD106), which attract leukocytes to the endothelial surface, bringing with them reactive oxygen species (ROS) and procoagulants. In contrast, there are antithrombotic factors consisting of tissue factor pathway inhibitor, blocking activated tissue factor; protein C and protein S, inhibiting factors V and VIII; thrombomodulin that activates protein C and protein S; endothelial protein C receptor; heparan, inhibiting factors Xa and IIa (thrombin); nitric oxide synthetase, producing nitric oxide; prostacyclin, inhibiting platelet activation and promotes vasodilation; ADAMTS13, cleaving VWF; ecto-ADPase, inhibiting platelet function; and members of the plasminogen-plasmin system, which includes tPA, uPA, uPA receptor, and annexin A2.

Upon stimulation, this balance is switched to favor hemostasis, as nature's defense mechanism to conserve blood loss (Fig. 2). The most common stimulus is the inflammatory cytokines. TNFα and interleukin (IL)1 produce multiple changes in the endothelium, with upregulation of tissue factor and adhesive molecules. They upregulate many of the prothrombotic factors, such as tissue factor, but also the adhesive molecules. The latter in turn attract leukocytes to the endothelium, and along with them ROS and procoagulants. Other stimuli include altered blood flow with increased shear stress, drugs, and toxins. Depending on the nature and the degree of stimulation, a potential risk of thrombosis ensues. However, certain stimuli can produce a bleeding tendency. An example is increased fibrinolytic activity, which can result from physical stress such as exercise and surgical operations.[152–154] This is believed to account for severe hemorrhagic complications during surgery.[153,154]

The resulting response in the direction of the hemostatic balance, however, is not uniform throughout the vasculature. The expression of the prothrombotic and antithrombotic factors is also uneven in different locations.

Role of Altered Flow Dynamics

Under shear stress, the configuration of the molecules of both VWF and of ADAMTS13 are changed to expose their respective active reactive sites. Thus, in areas of high shear stress, VWF reacts maximally with the Gp1b on platelet membrane and produces the greatest platelet activation. This principle has been used to explain the

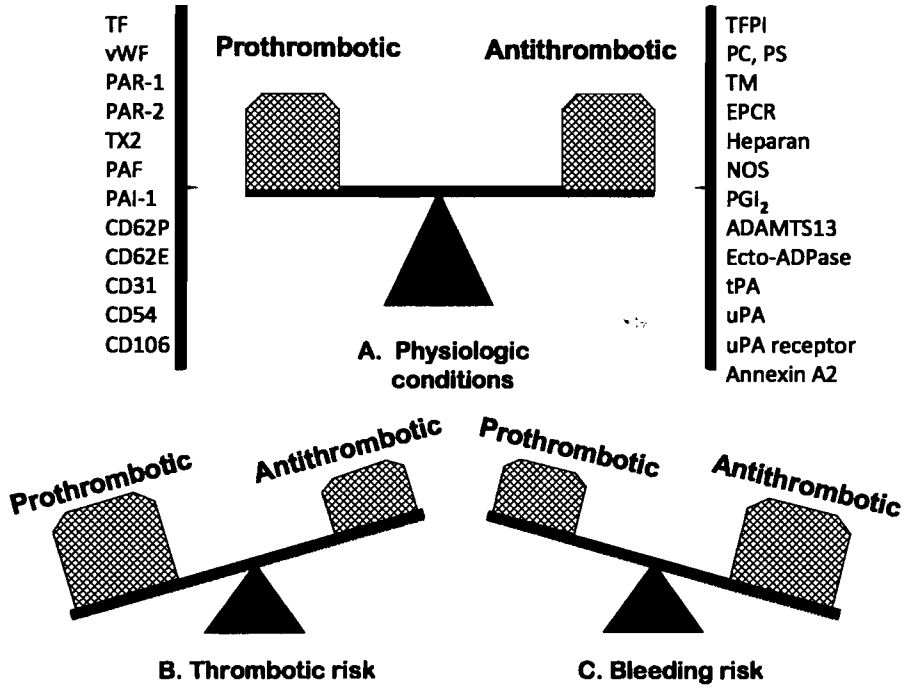

Figure 2 The prothrombotic and antithrombotic factors in the endothelium, shown in (A) the physiological state but can be shifted either in favor of (B) thrombosis or (C) to bleeding. TF, tissue factor; VWF, von Willebrand factor; PAR-1/PAR-2, protease-activated receptors 1 and 2; TX2, thromboxane A2; PAF, platelet-activating factor; PAI-1, plasminogen activator inhibitor-1; CD62P, P-selectin; CD62E, E-selectin; CD31, platelet endothelial cell adhesion molecule (PECAM-1); CD54, intercellular adhesion molecule (ICAM-1); CD106, vascular cell adhesion molecule (VCAM-1); TFPI, tissue factor pathway inhibitor; PC, Protein C; PS, Protein S; TM, thrombomodulin; EPCR, endothelial protein C receptor; NOS, nitric oxide synthetase; PGI_2, prostacyclin; ADAMTS13, a disintegrin and metalloproteinase with a thrombospondin type 1 motif, Member 13; Ecto-ADPase; tPA, tissue plasminogen activator; uPA, urokinase.

initial locations of atheroma. It is also applied in attempts to design drugs such as anti-Gp1b antibodies that inhibit platelet aggregation selectively in places with high shear stress, thus avoiding a generalized bleeding risk.

Role of Microparticles

Microparticles (MPs) are small fragments of cell membrane, measuring 0.1 to 1.0 μm in diameter, found in the circulation.[155–158] They carry both procoagulant and anticoagulant proteins and components of the plasminogen-plasmin system. They also carry adhesive molecules and cytokines. In healthy individuals, circulating MPs are mostly derived from platelets, but a small numbers are from monocytes, neutrophils, and endothelial cells. In disease, additional MPs are derived from tumor and leukemic cells and from atheromatous plaques. Increased tissue factor–bearing MPs have been associated with thromboembolic complications in many diseases, including various types of cancer, antiphospholipid syndrome, sickle cell disease, diabetes, and systemic inflammatory disorders. In inflammatory states, MPs derived from leukocytes contain P-selectin glycoprotein ligand (PSGL)-1, enabling them to attach to injured endothelial cells that express P-selectin.[159] Likewise, MPs derived from cancer cells also carry PSGL-1 and were observed to enhance thrombus formation in animals.[160] The thrombogenic role of the MPs is an active area of investigation because the procoagulant MPs may be another determinant in the selection of the site of thrombosis.

CONCLUSION

The acceptance of the concept of endothelial heterogeneity leads to a better understanding of the pathogenesis of thrombotic and bleeding disorders. In our current understanding of the pathogenesis of bleeding and thrombosis, there is a lack of adequate explanation for the predilection of bleeding or thrombosis to specific sites of the vasculature. Thus this topic provides a fertile field for further investigations. At present, the concept helps to improve diagnostic approaches to many diseases presenting with thrombosis or bleeding at specific sites. Examples include the high incidence of myeloproliferative disorders in splanchnic vein thrombosis. Another example is seen in the presentation of acute idiopathic TTP as neurological and renal manifestations. This concept can also provide directions for investigation into the cause of thrombosis or bleeding that are limited to specific sites. In addition, drug development for

treating thrombosis and bleeding can be better aimed at site-directed therapy. This topic is discussed in detail in another article in this issue of *Seminars in Thrombosis and Hemostasis* by Carnemolla et al.[161] It also dispels the notion of "one drug treats all."

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