Microvascular Thrombosis: A Serious and Deadly Pathologic Process in Multiple Diseases

Hau C. Kwaan, M.D., Ph.D.¹

ABSTRACT

Much of our understanding of the pathogenesis of thrombosis has long been based on observations made on large blood vessels. Nevertheless, there has been a recent shift in our attention to the microvasculature and to how microcirculatory occlusion affects function of various organs in diseases. This article provides an overview of microthrombosis in small blood vessels, with discussion of the progressive stages of its development. The initial event is triggered by a variety of diseases, followed by a second phase when multiple contributory factors amplify the process with the final phase of microvascular occlusion and microvascular thrombosis. The outcome is either recovery or injury to the affected organ. If the process is generalized, it is often associated with catastrophic or fatal outcomes. Our current knowledge of the major role of contributory factors leads to a new paradigm. A therapeutic approach limited to a single target of the underlying pathogenic factor, such as the use of anticoagulants, is insufficient and too often unsuccessful. Simultaneous management of all the contributory factors should therefore be considered.

KEYWORDS: Thrombosis, disseminated intravascular coagulation, inflammation, hypercoagulable state, sepsis

Until recently, most studies on the pathogenesis of thrombosis have been centered on veins or arteries, while the microvasculature has received relatively less attention. In the past decade, however, there has been a noticeable shift from macrovascular studies to those of microvascular changes following interruption of blood flow. The resulting microvascular occlusion has a great impact on the restoration of function of affected tissues. Microvascular thrombosis can also be the result of other forms of pathology, where it may serve as the initial locus of thrombosis that results in the extension of thrombosis to larger vessels, but more often is the result of a chain of events triggered by an underlying disease. The extent of microcirculatory obstruction often reflects the severity of the underlying illness.

This article is devoted to an overview of microthrombosis in arterioles, capillaries, and venules. Several clinical disorders with common characteristic features of the pathogenesis are presented.

THE MICROVASCULATURE

The microvasculature is composed of a vast network of arterioles, capillaries, and venules. It has a much larger

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capacity than that of both arteries and veins combined. Just considering the length of capillaries alone, it is 60,000 to 100,000 miles long. The total volume of capillaries is only 250 mL compared with an estimated 450 mL for arteries and 2800 mL for veins. The capillary wall is thin, comprising a layer of endothelium with pericytes and extracellular matrix. Because of their smaller diameter, the rheological characteristics of capillaries are also different than those of arteries and veins.

The blood flow and the hydrostatic pressure in the capillaries are controlled by vascular tone in the arterioles and venules, and are therefore slow, but the shear rate is high due to the smaller diameter. Accordingly, the vascular wall shear stress drops sharply with hematocrit. Both of these features are important in the development of microthrombosis. Our knowledge in this area is limited. Because of the frequent branching of the capillaries and the constant change in vascular tone, experimental results with rigid tubes when applied to the microvasculature should be interpreted with caution.

Within the microvasculature, there is a high degree of heterogeneity both in structure and function. The endothelial lining may be (1) continuous with tight junction between cells (e.g., in the brain to form the blood-brain barrier, in the lymph nodes to allow lymphocyte homing, and in muscles for metabolic exchange); (2) fenestrated (e.g., in endocrine glands to allow secretion, in the gastrointestinal tract for absorption); or (3) discontinuous with pores of 0.1 to 1.0 μm (e.g., in the liver sinusoids for particle exchange, in the bone marrow sinuses for passage of hematopoietic cells, and in the spleen for filtration). Under physiological conditions, these functions are organ-specific with exchange and transport of proteins in the respective organs. The endothelium has also an important role in hemostasis, maintaining the balance between prothrombotic and antithrombotic factors.

The major prothrombotic factors are (1) for coagulation activation: tissue factor, von Willebrand factor (VWF), and the protease-activated receptors (PARs); (2) for platelet activation: thromboxane A2, VWF, platelet activating factor; (3) for inhibition of fibrinolysis: plasminogen activator inhibitor-1 (PAI-1); and (4) for cell adhesion: various adhesive molecules such as P-selectin, E-selectin, platelet endothelial cell adhesion molecule-1 (ICAM-1), and vascular cell adhesion molecule-1 (VCAM-1). These factors are counteracted by (1) the anticoagulants: protein C, protein S, tissue factor pathway inhibitor (TFPI), thrombomodulin (TM), endothelial protein C receptor, heparan and nitric oxide synthetase; (2) profibrinolytic factors: tissue plasminogen activator (tPA), urokinase-type plasminogen activator (uPA), uPA receptor, annexin A2; and (3) antiplatelet factors: prostacyclin, a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13 (ADAMTS13) and ecto-ADPase. There is also heterogeneity in the expression of these factors. For example, the brain is poor in TM, and protein C is poorly expressed in capillaries. In various diseases, especially when there is an inflammatory response, the picture is altered with a predominance of prothrombotic factors, resulting in a hypercoagulable state.

**PATHOGENESIS**

The factors leading to the formation of microvascular thrombosis are multiple and parallel those implicated in the pathogenesis of macrovascular thrombosis. The traditional paradigm of Virchow triad is not only applicable but may be more relevant, because the smaller vessels are more susceptible to thrombosis. These factors are shown in Fig. 1. Though they appear to be similar to those found in large vessel diseases, their effects can be vastly different. Because of the size differences, both the volume and characteristics of blood flow are more sensitive to structural alterations in the microvasculature than in larger blood vessels. As discussed above, notable differences are found in their rheological properties, their expression of procoagulant and anticoagulant proteins, adhesive molecules, and of proinflammatory cytokines. The microvasculature is also more susceptible to the adverse effects of various abnormalities in blood. The smaller lumen is easily obstructed. There is a higher ratio of endothelial surface to luminal volume, thus providing greater exposure to the circulating blood and a higher concentration of locally released cytokines. Changes in small blood vessels rather than large vessels are the site of pathology in several disorders.

A review of recent observations on microthrombosis shows that several progressive stages can be recognized in its development. The primary occurrence can be a complication of a wide variety of disorders, including those with abnormality in the blood vessel, such as endothelial injury from severe sepsis, abnormal neovascularization in cancer tissues, or radiation injury. Microthrombosis can also develop as a consequence of abnormal blood flow, variably due to aggregated platelets, erythrocytes, or leukocytes. Other examples are seen in reperfusion after interruption of blood flow in organ infarction, embolism, or after reconstructive or transplant surgery. Aberrant blood flow can occur in tumor microvasculature or in vascular malformations, and in the hyperviscosity syndromes. The primary event can also be caused by abnormal blood components, as seen in hypercoagulable states with...
Pathogenesis of microthrombosis based on Virchow’s triad.

**Abnormal blood vessel**
e.g. Sepsis/severe trauma  
Tumor microvasculature  
Radiation injury

**Abnormal blood flow**
e.g. Aggregated platelets  
Aggregated erythrocytes  
Aggregated leukocytes  
Reperfusion after interruption of flow in organ infarction, embolism, reconstructive surgery  
Aberrant flow in vascular malformation, tumor microvasculature  
Hyperviscosity

**Abnormal blood components**
e.g. Increased procoagulants  
Hereditary thrombophilia  
Acquired prothrombotic states  
Antiphospholipid syndrome  
Traumatic brain injury  
Subarachnoid hemorrhage  
Decreased protein C, protein S  
Warfarin necrosis  
Post-varicella purpura fulminans  
Abnormal proteins  
Paraproteins (plasma cell dyscrasias)  
Abnormal fibrinogen  
Cryoglobulin  
Amyloid

Hypoxia  
Reactive Oxygen Species  
Inflammatory cytokines  
Leukocytes activation  
Generation of microparticles  

Vasoconstriction  
Endothelial activation  
Prothrombotic factors (tissue factor, PAI-1)  
Thrombin generation  
Platelet activation  
Adhesive molecules

**Microvascular Thrombosis/Occlusion**

Figure 1 Pathogenesis of microthrombosis based on Virchow triad. NO, nitric oxide; PAI-1, plasminogen activator type 1; PARs, protease activated receptors.

increased procoagulants, or with decreased protective anticoagulants. It can also occur when abnormal proteins initiate a process of microvascular occlusion and thrombosis.

A variety of factors contribute to the further development of the pathology. Their actions are often interdependent and, once activated, lead to further amplification of one another. Perhaps the most significant role is that of systemic inflammatory response. There is an increased expression of proinflammatory cytokines, including tissue necrosis factor (TNF)-α, interleukin (IL)-1β, IL-6, and IL-8. These cytokines induce the expression of prothrombotic factors with activation of the coagulation pathway. Thrombin generation leads to activation of the PARs present in endothelial cells, platelets, and leukocytes, resulting in the activation of these cells. The adhesive molecules P- and E-selectins are activated, leading to leukocyte migration and adhesion to the endothelium. Neutrophil proteases cause further injury to the endothelium. A combination of these effects results in blockage of the circulation as well as in microthrombi formation. With microcirculatory occlusion, hypoxia and generation of reactive oxygen species (ROS) further lead to vasculopathy. A vicious circle is thereby established, resulting in an unfavorable outcome. In the case of localized occlusion, there will be infarction of the affected organ. When the occlusion is generalized, multiorgan dysfunction syndrome will ensue. The major organs involved are kidneys, lungs, liver, and brain. The mortality rate varies with different clinical settings, but typically exceeds 50%.

The commonly encountered disorders with microvascular thrombosis, listed in Table 1, are reviewed in this article to illustrate different versions of the same pathogenic mechanisms.

### Disorders with Microthrombosis due to Endothelial Injury

**SEPSIS/SEVERE TRAUMA**

In the critically ill patient with severe sepsis or trauma, a series of events often lead to multiorgan dysfunction syndrome. A high mortality is associated with this complication. Studies exploring its pathogenesis have revealed a picture of endothelial injury with active systemic inflammatory response. Increased expression of cytokines and procoagulants are the major contributory factors in the development of severe disseminated intravascular coagulation (DIC) with a thrombotic phenotype. Widespread microthrombi have been
observed in autopsied specimens\textsuperscript{37,38} (Fig. 2). In severe sepsis, bacterial endotoxins such as lipopolysaccharides bind to endothelial receptors, triggering an inflammatory response. This amplifies the generation of cytokines, chemokines, adhesive molecules, and complement activation. As a result, the endothelial function is perturbed in several ways (Fig. 3). Multiple interactions between the activated inflammatory factors and the thrombotic factors occur, escalating the process until the vicious circle is broken.\textsuperscript{39} The inflammatory response results in an increased expression of the inflammatory cytokines TNF-\(\alpha\), IL-1\(\beta\), and IL-6.\textsuperscript{22,23} Tissue factor, normally not expressed at the surface of the endothelium, is induced by these cytokines.\textsuperscript{24} Upregulation of tissue factor is also found in monocytes and macrophages.\textsuperscript{40} Recent observations show that tissue factor is present in microparticles in the circulating blood with levels accentuated during sepsis.\textsuperscript{41,42} The inflammatory cytokines also induce increased expression of E- and P-selectins, resulting in adherence of neutrophils to the endothelial surface.\textsuperscript{56,43,44} The ensuing neutrophil-endothelial interaction results in release of proteases and ROS from the neutrophils, thus enhancing the endothelial injury. Complement activation leads to production of C3a and C5a and upregulation of C5a receptors in neutrophils, endothelial cells, lungs, cardiomyocytes, and neurons.\textsuperscript{45} The prothrombotic role of C5a has been verified in experimental sepsis, with anti-C5a antibodies shown to attenuate the coagulopathy.\textsuperscript{45,46}

Following endothelial injury, a coagulation response takes place, with increased expression of tissue factor in the endothelial cells, monocytes, and macrophages.\textsuperscript{40} In the normal endothelium, a receptor for the anticoagulant protein C is present. This is, however, poorly expressed in capillaries, thus rendering the capillaries more susceptible to thrombosis. The function of protein C is also impaired in sepsis,\textsuperscript{21,22,47} thus favoring the development of microthrombosis. Clinical trials of recombinant form of human activated protein C (rhAPC), drotrecogin alfa, in patients with sepsis showed mixed results.\textsuperscript{48} In the phase 3 PROWESS trial, a significantly reduced mortality was found in the rhAPC treated group.\textsuperscript{49} The patients who benefited most were those with severe sepsis\textsuperscript{50} and overt DIC.\textsuperscript{51} However, in a subsequent trial (ADDRESS),\textsuperscript{52} designed to test efficacy of rhAPC in patients with low APACHE score (<25), no difference in the 28-day mortality rate was found. The addition of heparin to rhAPC adds minimally to the survival benefit but significantly reduced stroke complications.\textsuperscript{53} Due to the high cost of rhAPC, justification for its use in low grade sepsis is questionable,\textsuperscript{54} while more clinical trials are evaluating its benefit in those with severe sepsis.\textsuperscript{48,55–57}

The functions of two other naturally occurring anticoagulants, antithrombin and TFPI, are also adversely affected in sepsis.\textsuperscript{39} Antithrombin levels are low, due to consumption by the increased thrombin generation in sepsis, decreased synthesis, and degradation by elastase released by activated neutrophils. The administration of recombinant antithrombin has been shown to reduce thrombin generation\textsuperscript{58} but did not lower the mortality in sepsis.\textsuperscript{59} TFPI is normally anchored to the endothelium by glycosyl phosphatidylinositol.\textsuperscript{60} In sepsis, TFPI is detached and thus has reduced function.\textsuperscript{61} A clinical trial of recombinant TFPI in severe sepsis showed reduction of the coagulopathy but did not lower mortality.\textsuperscript{62}

The fibrinolytic system is also perturbed in sepsis. Under various forms of stress, including the development of DIC, tPA is released from the endothelium. The resulting increased fibrinolytic activity is counterbalanced by an increase in circulating PAI-1, in part due

<table>
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<th>Diseases with Microvascular Thrombosis</th>
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<td>Radiation injury</td>
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<td><strong>Disorders with Abnormal Blood Flow</strong></td>
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<td>Aggregated erythrocytes: Malaria; sickle cell disease; cold agglutinin disease</td>
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<td>Aggregated leukocytes: Leukostasis</td>
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<tr>
<td>Reperfusion after interruption of blood flow: Postacute myocardial infarction; postsurgical reconstruction</td>
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<tr>
<td><strong>Disorders with Abnormal Blood Components:</strong></td>
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<tr>
<td>Hypercoagulable States</td>
</tr>
<tr>
<td>APS</td>
</tr>
<tr>
<td>TBI</td>
</tr>
<tr>
<td>SAH</td>
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<tr>
<td>Warfarin necrosis</td>
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<tr>
<td>Postvaricella purpura fulminans</td>
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TMA, thrombotic microangiopathy; APS, antiphospholipid syndrome; TBI, traumatic brain injury; SAH, subarachnoid hemorrhage.
to the action of inflammatory cytokines, such as TNF-α. In experimental sepsis, the increased PAI-1 was found to be the dominant effect. Fibrinolysis is also impaired by the activation of the thrombin activatable fibrinolytic inhibitor (TAFI). In addition, the activated TAFI displays carboxypeptidase activity, and, through this pathway, can modulate inflammation.

Thrombin and several coagulation proteases activate the PARs. Members of the PAR family, PAR-1 to PAR-4, are present in the endothelial cells, platelets, monocytes, and neutrophils. Activated PARs in turn activate these cells in a concerted effort to enhance the prothrombotic process.

Additional factors are frequently present in critical care unit patients, such as hypoxia, hypotension, and fever. These can magnify the progression of DIC and microthrombosis leading to multiorgan failure. Thus, in any given patient, the appropriate management should take into consideration the multiple and complex nature of the pathogenesis.

**TUMOR MICROVASCULATURE**

Microthrombi are frequently present in many types of tumors and are considered part of the pathology. A generalized microthrombi formation is, however, mostly seen in metastatic cancer, with findings similar to those seen in thrombotic microangiopathy (TMA), also known as microangiopathic hemolytic anemia. The features of cancer-associated TMA are distinct from those of acute idiopathic thrombotic thrombocytopenic purpura (TTP) and of hemolytic-uremic syndrome (HUS), in that there is no preferential localization of the microthrombotic lesions in the brain and kidneys. In contrast to TTP, there is systemic coagulopathy and the microthrombi are rich in fibrin.

The pathogenesis in cancer-associated thrombosis is also different. In the tumor microenvironment, in addition to invasion of tumor cells into the microvasculature, tumor angiogenesis results in malformed vessels with aberrant blood flow. These vessels are irregular in size, with increased permeability. There is hypoxia with increased vascular endothelial growth factor (VEGF) activity. Tumor cells themselves also produce proinflammatory cytokines such as TNF-α and IL-6, inducing increased tissue factor expression by the endothelial cells. In addition, tissue factor is also produced by many types of tumor cells. As tumor cells undergo apoptosis, exteriorization of phospholipids to the surface of cell
membrane leads to activation of tissue factor. Thus, apoptotic cells are more thrombogenic. Tissue factor bearing microparticles are also found in the blood of patients with cancer and acute leukemia. These are submicron cellular fragments that may contribute to the prothrombotic state. Yet, it is unclear why microthrombi are not seen as fragments as expected.

Microthrombi are more frequently seen in two organs, the renal glomeruli being involved in 26% of autopsied specimens of cancer patients, while the lung is also a common site for microthrombi. These microthrombi do not contain tumor cells. Whether they form the basis for metastasis is not still unknown. On the other hand, microthrombi in low grade brain tumors (astrocytoma) may signal progression toward glioblastoma.

RADIATION INJURY
Vascular injury due to ionizing radiation is well recognized, with the capillaries being most sensitive. The effects of ionizing radiation on the endothelial cells include apoptosis, increased endothelial permeability, expression of chemokines, and adhesion molecules. The natural antithrombotic state of the endothelium is impaired with increased VWF, tissue factor, impaired protein C function, and reduced fibrinolytic activity. Thrombotic occlusion of small vessels is a common feature of focal radiation necrosis following radiation to the brain. As local radiation is the major therapeutic modality for head and neck cancer, a common complication is osteoradionecrosis of bone. Endothelial cell damage with necrosis of microvasculature along with an inflammatory response is believed to be leading pathogenic factors.

Disorders with Microthrombosis due to Abnormal Blood Flow
The microvasculature can be blocked by circulating cellular elements, including erythrocytes, leukocytes, and platelets (Table 2). The consequences are dependent on the nature of the occluding elements.

AGGREGATED PLATELETS: THROMBOTIC MICROANGIOPATHY (TMA)
The characteristic microvascular lesion in TMA, consists of a microthrombus rich in platelets with variable amounts of fibrin and erythrocytes. The clinical manifestation is one of microangiopathic hemolytic anemia and thrombocytopenia. TMA is present in a wide spectrum of diseases. The classical TMA is acute idiopathic TTP, while the other forms of TMA are HUS, and TM secondary to cancer, pregnancy, connective tissue disorders, tissue transplantation, infection, and drug toxicity. Acute idiopathic TTP is an autoimmune disorder with an immunoglobulin G (IgG) autoantibody directed against ADAMTS13, an enzyme that cleaves VWF. The lack of ADAMTS13 results in the accumulation of ultralarge multimers of VWF at locations with high shear stress, causing extensive platelet aggregation with platelet thrombi formation in arterioles, capillaries, and rarely in venules. The characteristic lesion is a platelet-rich thrombus partially occluding a small blood vessel (Fig. 4). Impaired endothelial function such as decreased prostacyclin synthesis and impaired fibrinolytic activity also contributes to the pathology. Microvascular endothelial cells derived from the brain, kidneys, and heart have been found in vitro to be most susceptible to this form of injury, while those from lungs and liver are not. This observation is supported by the clinical picture in which neurological abnormalities and renal failure are the most common presenting features. The mortality rate is over 95% when left untreated. In contrast, survival with more than 90% remission can be achieved with early diagnosis and appropriate management with therapeutic plasma exchange and immunosuppression. The acute idiopathic TTP should be distinguished from HUS, where microthrombi are localized to the renal glomeruli. Two forms of HUS are present. The diarrhea associated form

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Table 2 Microthrombosis as Result of Blockage of Microvasculature

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Occluding Elements</th>
<th>Mechanism</th>
<th>Local/General</th>
</tr>
</thead>
<tbody>
<tr>
<td>TTP/HUS</td>
<td>Platelets</td>
<td>Severe deficiency of ADAMTS13</td>
<td>Brain, heart, kidney</td>
</tr>
<tr>
<td>HIT</td>
<td>Platelets</td>
<td>Antibody against PF4/heparin</td>
<td>Skin, adipose tissue</td>
</tr>
<tr>
<td>Malaria</td>
<td>Erythrocytes</td>
<td>Changes in infected red cells</td>
<td>Brain</td>
</tr>
<tr>
<td>Sickle cell</td>
<td>Erythrocytes</td>
<td>Sickling</td>
<td>Brain, lungs/generalized</td>
</tr>
<tr>
<td>Cold agglutinin disease</td>
<td>Erythrocytes</td>
<td>Agglutination from antibodies</td>
<td>Generalized</td>
</tr>
<tr>
<td>Leukostasis</td>
<td>Leukemic cells</td>
<td>Increased adhesive molecules</td>
<td>Brain, heart, lungs</td>
</tr>
<tr>
<td>DIC</td>
<td>Fibrin</td>
<td>Endothelial damage</td>
<td>Kidney/generalized</td>
</tr>
<tr>
<td>Warfarin necrosis</td>
<td>Fibrin</td>
<td>Low protein C</td>
<td>Skin, adipose tissue</td>
</tr>
<tr>
<td>Miscellaneous</td>
<td>Cryoglobulin</td>
<td>Erythrocyte aggregation</td>
<td>Generalized</td>
</tr>
<tr>
<td>Miscellaneous</td>
<td>Paraproteins</td>
<td>Erythrocyte aggregation</td>
<td>Generalized</td>
</tr>
</tbody>
</table>

TTP, thrombotic thrombocytopenic purpura; HUS, hemolytic uremic syndrome; ADAMTS13, a disintegrin and metalloproteinase with a thrombospondin type1 motif, member 13; HIT, heparin-induced thrombocytopenia; DIC, disseminated intravascular coagulation.
(D⁺HUS) is seen in hemorrhagic enterocolitis caused by Shiga toxin from Escherichia coli infection. The renal glomerular microvascular endothelium is most sensitive to the toxin, resulting in renal failure. The illness is usually self-limited after clearing the Shiga toxin. On the other hand, in the atypical form (D⁻HUS or aHUS) severe renal disease is the rule. It is associated with genetic defects of the complement system including factor H, factor I, membrane cofactor protein (MCP/CD46), and defect of the CFHR1 gene that regulates C5 convertase. Autoantibodies against factor H are also found in aHUS.

In the other forms of TMA, the formation of microthrombi is secondary to an underlying disease. Some of them are associated with endothelial damage, such as in connective tissue disorders, infection, and drug toxicity. The concomitant presence of hypercoagulability, such as in cancer, further increases the thrombotic risk.

**AGGREGATED PLATELETS: HEPARIN-INDUCED THROMBOCYTOPENIA**

Platelet-rich microthrombi are commonplace in heparin-induced thrombocytopenia. IgG immune complexes of antiplatelet factor 4/heparin activate platelets, causing thrombocytopenia and hypercoagulability. The immune complexes also interact with endothelial cells, amplifying the endothelial injury. The platelet thrombi initially start in the microvasculature of skin and adipose tissues (Fig. 5A, B), but often extend to larger vessels causing serious arterial and venous thrombotic complications.

**AGGREGATED ERYTHROCYTES**

Under physiological conditions, a smooth passage of erythrocytes through the microcirculation requires specific rheological characteristics. Erythrocytes are highly deformable and orient their shape with the stream of blood flow. They also aggregate to form linear arrays like a stack of coins known as rouleaux. Aggregate formation is dependent on the deformability of erythrocytes, adhesiveness of the erythrocyte membrane as well as plasma proteins, especially fibrinogen, and flow characteristics. Erythrocyte aggregation is encountered in several disorders with microthrombosis as a common complication. Some examples are reviewed here.

**Malaria** In Plasmodium falciparum infection, impaired microcirculation has been observed in brain, retina, and spleen. The brain abnormalities account for much of the neurological symptoms. Because of the clinical
severity of cerebral malaria, most investigations are centered on *P. falciparum*. In severe cases, microthrombi are also found in sites other than the brain (Fig. 6). The older concept that altered properties of the infected erythrocyte such as decreased plasticity and increased adhesiveness cause the vascular obstruction, has now given way to viewing the microvascular lesion as the combined result of multiple events. In addition to changes in the erythrocytes, platelet activation occurs. A membrane protein, *P. falciparum* erythrocyte membrane protein, present on *P. falciparum* infected erythrocytes, is believed to mediate a series of interactions including adhesion between the parasite and endothelium, binding of platelets and erythrocytes via CD36 (an adhesive molecule on microvascular endothelial cells). Other adhesive molecules, including ICAM-1, VCAM-1, P-selectin, E-selectin, are also involved. Evidence of endothelium activation has also been observed. In addition, the *P. falciparum*-infected erythrocytes induce tissue factor expression in the endothelium and lead to activation of the coagulation pathway. Thrombin generation results in PAR activation and release of inflammatory cytokines, amplifying many of the above interactions.

Microparticles derived from endothelial cells have also been detected in patients with severe malaria and their number correlate with plasma TNF levels. As malaria is still an important worldwide disease, and cerebral malaria carries a high mortality, it is hoped that some of these findings may be translated to a better therapeutic approach.

**Sickle Cell Disease** Although erythrocyte sickling can offer a rheological blockage to the microcirculation, events that lead to the vasculopathy are much more complex. Hemolysis releases hemoglobin and impairs the arginine-nitric oxide (NO) pathway by scavenging NO. Arginase is also released from hemolyzed erythrocytes, reduces the NO synthetase activity, and further decreases the availability of NO. It also shifts the metabolism of L-arginine to L-ornithine, producing polyamines and L-proline, substances that promote smooth muscle cell growth and fibrosis. There are also changes in the coagulation profile, toward a prothrombotic state enhanced by thrombin generation, tissue factor activity, decreased anticoagulants, abnormal fibrinolysis, and platelet activation. However, it is unclear to what extent these factors contribute directly to the microvascular occlusion. The roles played by factors such as endothelial activation and inflammatory cytokines are considered more important. Many of these changes correlate with the hemoglobin levels, reflecting the degree of hemolysis. These findings led to the concept that vascular complications are dependent on the level of hemolysis and the resulting NO scavenging. Those patients with a high degree of hemolysis would be expected to have pulmonary hypertension, leg ulceration, priapism, and stroke, while others with less hemolysis would be expected to have vaso-occlusion (presumably from rheological obstruction of the sickled erythrocytes) with pain crisis, acute chest syndrome, and osteonecrosis. However, this concept of NO scavenging, while attractive, has recently been questioned as the vasculopathy is not seen in other severe hemolytic disorders with higher levels of free plasma hemoglobin, such as paroxysmal nocturnal hemoglobinuria. Furthermore, clinical trials designed to abrogate the deleterious effects of NO did not show benefits. An example is seen in the clinical trial of hydroxyurea. Although the degree of hemolysis was reduced in patients treated with this compound, the pulmonary hypertension was not affected. Another example was the clinical trial of the phosphodiesterase (PDE) inhibitor, sildenafil. Inhibition of PDE was expected to enhance the effect of NO on guanylate cyclase. However, the trial was terminated due to excessive rate of pain crises. It is clear that the underlying mechanism for microvascular occlusion in sickle cell disease is complex and that the temporal (massive hemolysis, crisis) and spatial (difference with different vessels, e.g., pulmonary vs. bone) changes have to be taken into consideration.

**Cold Agglutinin Disease** In contrast to malaria, microcirculatory blockade by agglutinated erythrocytes is less common in autoimmune hemolytic anemia. However, widespread vascular occlusion can occur and the profound effects of agglutinated erythrocytes are frequently not recognized. This is mostly seen in cold agglutinin disease. As erythrocytes aggregate at low temperature, the skin microvasculature is more prone...
to occlusion, with livedo reticularis as the leading clinical manifestation. The severity can be intensified by additional risk factors. This was illustrated in a patient with cold autoimmune hemolytic anemia refractory to immunosuppressive therapy. Following the intravenous administration of immunoglobulin, which increased the blood viscosity and the erythrocyte aggregation, severe generalized microvascular occlusion abruptly took place with profound livedo reticularis, the patient succumbed to coma (Fig. 7). Another example is seen when autoimmune hemolytic anemia developed in a patient with cryoglobulinemia, resulting in catastrophic mesenteric thrombosis and cerebral infarction. At postmortem extensive mesenteric thrombi were found in the small arterioles, capillaries, and venules, and consisted mostly of agglutinated erythrocytes (Fig. 8).

AGGREGATED LEUKOCYTES: LEUKOSTASIS

The microcirculatory obstruction can be severe and life-threatening. It may impair blood flow to the brain, eyes, myocardium, lungs, and kidneys, and progress to multi-organ failure (Fig. 9A, B). Initially, the microvasculature is obstructed by the leukocytes without significant activation of hemostasis, but as the obstruction progresses, thrombosis will develop and can lead to extensive arterial and venous thromboembolism. Involvement of both the brain and lungs is associated with a high mortality rate. In acute leukemia, the risk of leukostasis is increased when the peripheral blast count exceeds 100,000/L. The frequency of hyperleukocytosis ranges from 5 to 13% in adult acute myelogenous leukemia (AML) and from 12 to 25% in pediatric AML. Acute leukemia with monocytic differentiation (FAB classification: myelomonoblastic [M4], monoblastic [M5]) often presents with hyperleukocytosis as a common complication. Nearly 40% of patients presenting with poorly differentiated monoblastic leukemia have white blood cell counts greater than 100,000/L at diagnosis. Myelo-monoblasts, especially in the M4, M5 subtypes, are larger and less deformable, and thus

Figure 7 Extensive livedo reticularis developed in a patient with cold autoimmune hemolytic anemia right after administration of intravenous immunoglobulin.

Figure 8 Microthrombi in the submucosa of small intestines in a patient with catastrophic mesenteric thrombosis from combined cryoglobulinemia and cold autoimmune hemolytic anemia.

Figure 9 Pulmonary alveolar capillaries blocked by myelomonoblasts in a patient with acute monoblastic leukemia. (A) Wright stain; (B) Positive CD 33 immunostain.
more frequently cause leukostasis. This picture is often seen in AML in infants as the leukemia is often M4 or M5 and shows high blast counts.141

The cause of leukostasis was previously thought to be due to hyperleukocytosis. However, this concept was recently challenged. It is now generally believed that adhesive molecules in both endothelium and leukemic blasts play an essential role. In vitro studies showed an increased expression of adhesive molecules by endothelial cells and corresponding receptors in the myeloblasts.142 Myeloblasts, through their production of cytokines including TNFα and IL-1β, were found to upregulate the expression of ICAM-1, VCAM-1, P-, and E-selectins by endothelial cells. At the same time, their corresponding receptors (e.g., CD11b) are present in the myeloblasts. In such a microenvironment, the endothelial cells are able to recruit additional myeloblasts, forming a vicious cycle where more leukemic cells are trapped leading to more leukostasis. In the M4 and M5 subtypes of AML, CD11b, a receptor of ICAM-1, ICAM-2, is highly expressed in the myeloblasts, whereas in M0, M1, M2, and M3 subtypes there is less expression of this receptor, thus providing another reason for the higher frequency of leukostasis in the M4 and M5 subtypes. This new concept has implications on the management of the leukostasis. Cytoreduction by leukapheresis, though popular, may not be sufficient to change the outcome.90

REPERFUSION AFTER INTERRUPTION OF BLOOD FLOW

Interruption of Blood Flow as Seen in Acute Myocardial Infarction The degree of restoration of myocardial function following acute myocardial infarction is dependent on early reperfusion.143 Microvascular occlusion, also known as the "no-reflow" phenomenon, follows the reperfusion of the ischemic area. The microvascular endothelial cells show explosive swelling while neutrophils, erythrocytes, and platelets filled the vascular lumen.17 There is loss of vasomotor tone. Hemorrhage and edema in the surrounding tissues are also present.144 Metabolic injury to the surrounding myocytes results in loss of myocardial function and fibrosis. Increased understanding of the pathogenesis and the consequences of the microvascular occlusion has led to clinical approaches that require better assessment of the abnormal blood flow and to use of therapeutic agents to prevent the ischemic injury and salvage myocardial function.145 This subject has been extensively studied both clinically and in experimental myocardial infarction models and reviewed elsewhere.144,146–148

Reperfusion after Surgical Reconstruction The current technique employing microvascular free-tissue transfer (free-flap reconstruction) has a remarkably successful rate, up to 90 to 99%.149 However, thrombosis still occurs in 8 to 14% cases.18 The risk factors for thrombosis have been shown to be preexisting focal arterial thickening, prior radiation, hypercoagulable state such as that seen in cancer, smoking, diabetes, hypertension, and hypercholesterolemia.150 Antiplatelet agents (aspirin, dextran) and anticoagulants are standard prophylactic measures in this procedure. The role of NO has also been investigated. It activates guanylate cyclase thus inhibiting platelet aggregation.151 The NO inhibitor L-NAME has been found in experimental animal models to promote microvascular thrombosis in the setting of microvascular anastomosis.152

Disorders with Microthrombosis due to Abnormal Blood Components: Hypercoagulable States

ANTIPHOSPHOLIPID SYNDROME (APS) Among the hypercoagulable states, thrombosis in APS153–156 is a good example of how microthrombi can form in multiple organs, including the liver, intestines, brain, heart adrenals, and kidneys as well as in skin, retina, and nail fold. The hypercoagulable state in APS is associated with the presence of autoantibodies against phospholipid-binding plasma proteins (antiphospholipid antibodies; aPL) and β2-glycoprotein I. These autoantibodies, in particular the anti-β2-glycoprotein I, bind to endothelial cells. They induce endothelial and platelet activation as well as expression of tissue factor, and adhesion molecules. The C3 and C5 components of the complement system are also activated. Although most individuals harboring aPL show little clinical manifestations, severe thrombotic complications can occur, with a major risk for morbidity and mortality. In less than 1% of patients, massive generalized thrombosis can develop over a short period of time, leading to the term “catastrophic APS.”157–159 In a review of 97 patients with intestinal involvement in the Catastrophic Antiphospholipid Syndrome Registry, microthrombosis had been observed in 4% of the classic APS in contrast with 75% in the catastrophic APS. A correspondingly higher mortality of 55% was seen in the catastrophic APS. In another prospective 5-year study of 1000 patients with APS,160 the mortality was 5.3% with main cause of death being cerebral involvement in 27.2%, cardiac involvement in 19.8%, and infection in 19.8% cases. In catastrophic APS, rigorous antithrombotic measures along with intravenous immunoglobulin (IVIG), corticosteroids, and therapeutic plasma exchange have been shown to reduce the overall mortality.161 For the noncatastrophic APS, the long-term use of anticoagulants is recommended for those with thrombotic complications while recognition of the autoimmune nature of aPL leads to new approach with immune suppression.
TRAUMATIC BRAIN INJURY (TBI)
Thrombotic complications may occur in the microvasculature as a result of the hypercoagulability observed in patients with TBI. Abnormalities of coagulation parameters are present in the vast majority of patients following TBI. The severity of these changes also predicts the clinical outcome. Brain is rich in procoagulants, especially tissue factor, while devoid of thrombomodulin, the cofactor of the anticoagulant protein C. Thrombogenesis in TBI involves the release of tissue factor and the activation of the extrinsic coagulation pathway. Platelet activation takes place, the severity of which is also correlated with the outcome. Following brain trauma, increase in inflammatory cytokines such as TNF-α and IL-6 further upregulates tissue factor expression in the endothelium and increase the procoagulant effect. Locally, this can lead to formation of microthrombi and systemically to the development of DIC. Postmortem studies demonstrated microthrombi affecting arterioles, capillaries, and venules with diameter of 6 to 100 μm. The extent of the microthrombosis has been shown to correlate with the degree of cerebral ischemia and selective neuronal necrosis. The fibrinolytic pathway is also activated, secondary to the local microthrombosis and to the systemic DIC. Evidence of increased fibrinolysis, such as increased fibrinogenolysis and decrease in α2-plasmin inhibitor had been shown to have correlation with the incidence of intracranial bleeding and with poor outcome in TBI. The above findings have been applied to the current therapeutic approach for TBI. Antithrombin had been tried, but this approach did not substantially influence the clinical outcome. Low molecular weight heparin had been found to be of some benefit in rats, but there are no conclusive data in humans. When used for thromboprophylaxis, low molecular weight heparin was shown to have low bleeding risk. In a recent clinical trial, only 1 in 287 patients had expansion of the intracranial hemorrhage. Guidelines for reducing the morbidity of brain injury must await results of other clinical trials on a range of anticoagulant, antiplatelet, and anti-inflammatory approaches.

SUBARACHNOID HEMORRHAGE (SAH)
A similar pattern of coagulopathy is also present following SAH. For up to 10 days after SAH, there is an increased risk of delayed cerebral ischemia, which can progress to infarction. This has generally been attributed to cerebral vasospasm. However, recent observations showing the lack of benefit from therapeutic use of endothelin antagonists, suggest that vasospasm alone may not account for all of the ischemia. The additional pathogenic factor is believed to be microthrombosis. Transcranial Doppler studies have demonstrated microembolic signals in as many as 70% of patients with SAH. Markers of platelet activation, elevated procoagulant activity in blood, and increased tissue factor level in cerebrospinal fluid have been observed in the acute phase, before vasospasm and delayed cerebral ischemia set in. Increased markers of procoagulant activity are an early predictor of infarction. Postmortem studies also support the concept that cerebral vasospasm cannot account for most of the infarction and that microthrombosis plays a significant role. In an angiographic study, vasospasm was identified in only 37% of patients with infarction. A recent observation confirmed the correlation between the microthrombi burden and evidence of ischemia based on clinical, radiological, and histological criteria. As our concept shifts from vasospasm to microthrombosis, the current approach to management of SAH needs to be reexamined. Measures that can reduce thrombosis have been advocated. In a recent clinical trial of enoxaparin given for 3 weeks following SAH, there was a reduction of the incidence of delayed ischemic deficit and cerebral infarction 1 year later, without an increased risk of recurrent SAH or secondary bleeding. On the other hand, the use of a prothrombotic approach, such as inhibitors of fibrinolysis or recombinant factor VII, has been found to reduce recurrent SAH, although long-term use may increase risk of cerebral ischemia. Hopefully, clinical trials with larger number of patients will clarify the roles of these therapeutic agents.

WARFARIN NECROSIS
This occurs at the start of warfarin therapy in patients with heterozygous deficiency of protein C. Warfarin produces a more rapid inhibition in the synthesis of protein C than that of factors VII, IX, X, and prothrombin. A hiatus of relative deficiency of protein C occurs in the first 24 to 72 hours following the initiation of warfarin. Patients with low baseline levels of protein C are at risk of developing local ischemic changes with microthrombosis and resulting in tissue necrosis. This is usually found in skin and adipose tissues (Fig. 10). In a study measuring protein C in patients who had recovered from warfarin necrosis, 11 of the 13 patients had abnormally low levels ranging from 23 to 69%. This

Figure 10 Fibrin thrombus in a small vessel in the breast in warfarin necrosis. Hematoxylin and eosin stain.
finding supports the role of protein C deficiency in the pathogenesis of warfarin necrosis. A similar association has also been reported in patients with deficiency of protein S, factor V Leiden, or antithrombin.  

POST-VARICELLA PURPURA FULMINANS

Although chicken pox is almost always a benign childhood illness, rare occurrences of purpura fulminans with microvascular thrombosis have been observed. This complication has also been seen after streptococcal infections. In the case of varicella, an autoimmune response may occur, with formation of antibodies against protein C and protein S leading to severe acquired protein C and protein S deficiency. aPL have also been found. The recognition of the autoimmune nature of this complication has led to successful use of immunosuppressive modalities such as corticosteroids, IVIG, or therapeutic plasma exchange.

CONCLUSION

Microvascular thrombosis is the outcome of a series of events, triggered by many underlying disorders. Several factors, especially inflammatory response and coagulation activation, contribute to further progression, often with a poor outcome. Both the initiating disorder and the contributory factors play equally important roles. Without vigorous efforts to treat all of these factors concurrently, attempts to deal only with the primary triggering disorder have all too often been unsuccessful. With a new understanding of the mechanisms of action of many of the contributory factors, it is now possible to address them simultaneously. The appropriate management should be analogous to that of cancer, in which multiple modalities of combination chemotherapy, radiation, hormonal, immunologic, and targeted receptors inhibition are concurrently applied. This new paradigm is increasingly being addressed in ongoing clinical trials.

REFERENCES

33. Stegmayr BG. Apheresis as therapy for patients with severe sepsis and multiorgan dysfunction syndrome. Ther Apher 2001;5(2):123–127
78. Key NS, Kwaan HC. Microparticles in thrombosis and hemostasis. Semin Thromb Hemost 2010;36(8):805–806


134. Creutzig U, Ritter J, Buddle M, Sutor A, Schellong G. Early deaths due to hemorrhage and leukostasis in childhood acute myelogenous leukemia. Associations with hyperleukocytosis...
and acute monocytic leukemia. Cancer 1987; 60(12):3071–3079


180. Crompton MR. The pathogenesis of cerebral infarction following the rupture of cerebral berry aneurysms. Brain 1964;87:491–510


