REVIEW ARTICLE

Microvascular thrombosis: experimental and clinical implications

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A significant amount of clinical and research interest in thrombosis is focused on large vessels (eg, stroke, myocardial infarction, deep venous thrombosis, etc.); however, thrombosis is often present in the microcirculation in a variety of significant human diseases, such as disseminated intravascular coagulation, thrombotic microangiopathy, sickle cell disease, and others. Further, microvascular thrombosis has recently been demonstrated in patients with COVID-19, and has been proposed to mediate the pathogenesis of organ injury in this disease. In many of these conditions, microvascular thrombosis is accompanied by inflammation, an association referred to as thromboinflammation. In this review, we discuss endogenous regulatory mechanisms that prevent thrombosis in the microcirculation, experimental approaches to induce microvascular thrombi, and clinical conditions associated with microvascular thrombosis. A greater understanding of the links between inflammation and thrombosis in the microcirculation is anticipated to provide optimal therapeutic targets for patients with diseases accompanied by microvascular thrombosis. (Translational Research 2020; 225:105−130)

Abbreviations: ADAMTS13 = A disintegrin-like and metalloproteinase with thrombospondin type 1 motif 13; AP = Alternate pathway; APC = Activated Protein C; APS = Antiphospholipid syndrome; CAPS = Catastrophic APS; ASFA = American Society for Apheresis; ATP = adenosine triphosphate; CFH = Complement factor H; Con A = concavalin A; COX = cyclooxygenase; DAMP = Damage-associated molecular pattern; DIC = Disseminated intravascular coagulation; GBM = Glomerular basement membrane; HELLP = Hemolysis, elevated liver enzymes, low platelets; HITT = Heparin-induced thrombocytopenia and thrombosis; HLH = Hemophagocytic lymphohistiocytosis; HUS = Hemolytic-uremic syndrome; ISTH = International Society for Thrombosis and Haemostasis; IVIG = Intravenous immunoglobulin; LDH = Lactate NOS, Nitric Oxide Synthase; NET = Neutrophil extracellular trap; PAI-1 = Plasminogen activator inhibitor 1; PF4 = Platelet factor 4; PRR = Pattern recognition receptor; RBC = Red blood cell; SCD = Sickle cell disease; SLE = Systemic lupus erythematosus; TLR = Toll-like receptor; TF = Tissue factor; TFPI = Tissue factor pathway inhibitor; TMA = Thrombotic microangiopathy; TNF-α = Tumor necrosis factor-α; TPE = Therapeutic plasma exchange; ULC = ultra large heparin-PF4 complexes; ULVWF = Ultra-large von Willebrand factor; VWF = von Willebrand factor

INTRODUCTION

Thrombosis, or the pathologic formation of blood clots, is associated with a significant health and economic impact world-wide. A great deal of clinical and research attention on thrombosis focuses on arteries in common conditions such as stroke or myocardial infarction, or in veins such as in deep venous thrombosis. Thrombosis also affects the microcirculation with significant consequences; this review will focus on the connection between microvascular thrombosis pathogenesis and clinical implications. Microvascular thrombosis often occurs in diseases characterized not only by disordered clot formation but also by disordered inflammation. In fact, many of the diseases discussed in this review are characterized by a "loss of focality" or escape from regulation of the physiologic pathways involved in hemostasis and innate immunity. There is increasing awareness of the close association between inflammation and thrombosis, also referred to as "thromboinflammation.[1]" The complex pathways of inflammation and coagulation appear to have a common evolutionary origin to defend against deadly insults. This is strikingly illustrated by the horseshoe crab, whose innate immune and coagulation systems are so intertwined that scientists consider them to be the same system.[2,3] Going forward, a greater understanding of the mechanisms responsible in the links between thrombosis and inflammation is expected to aide in the design and development of more effective therapies for these conditions.

OVERVIEW OF THE MICROCIRCULATION

In a simplified manner, the microcirculation may be defined as those blood vessels that cannot be observed clearly by the human eye without assistance.[4] There are also some suggestions of defining the microvasculature based on topography and hemodynamic responses, corresponding to a upper threshold of arteriole diameter of $\sim$100 μm[5]; however, in reality there is no abrupt transition between macro- and microvessels but rather a gradual transition, thus each definition will have limitations. However, particularly when comparing the precapillary arterioles and postcapillary venules to large arteries and veins, there are clear structural and functional differences, discussed below.

The microvasculature is subdivided into 3 main categories of blood vessels: arterioles, capillaries and venules distinguished by location, function and structure. Sequentially the arterioles deliver oxygenated blood to the capillaries, which deliver oxygen to the parenchyma, then the venules collect deoxygenated blood from the capillaries. The arteriolar endothelium is surrounded by dense, circumferential smooth muscle cells; the capillary endothelium is surrounded by sparse pericytes and the venule endothelium is surrounded by sparse smooth muscle cells.[6] These cellular configurations are distinct from the 3 defined layers found in large vessels (intima, media, and adventitia). Functionally, arterioles have a primary role of regulating distribution of blood flow, while the capillaries represent the primary site of fluid and solute exchange and the venules are the primary site of interaction with immune cells. The capillary beds do not hold a significant portion of the blood volume − estimated at less than 10% of the total volume − but have an enormous surface area for exchange. For example, morphometric analyses of human lungs estimate a capillary surface area of 126 m^2[27] and total body skeletal muscle capillary surface area is estimated to far exceed 180 m^2.[8,9] To place these estimates in perspective, the sum of capillary surface area of lungs and skeletal muscle is larger than the area of a tennis court for doubles matches (nearly 261 m^2). There are densely packed networks of capillaries throughout most organs, facilitating delivery of nutrients to surrounding tissues. Capillaries are in a unique position to directly exchange solutes and fluid with the parenchyma and also have the most organ-specific phenotypes within the vasculature.[10] The structure of microvasculature is influenced by biomechanical forces including shear stress and biochemical signals including hormones, growth factors, cytokines, chemokines, complement, and nitric oxide (NO) contribute to the unique highly adaptive microvascular endothelial cell phenotype.[11] While some features of endothelial cells are maintained in vitro, a significant amount are dependent on temporal and location specific signals.[11-13]

Microvessels are subject to significantly increased wall shear stress, a tangential force exerted on vascular walls as a result of blood flow, as their size decreases.[14] Measures of mean wall shear stress in arterioles greatly exceed values in arteries within the same species.[14,15] For example, within the same microvascular bed, mean wall shear stress measured in arterioles (>100 dyn/cm^2 for the smallest arterioles) exceeded that of venules of comparable diameter by about one order of magnitude.[16] Higher shear stress is deemed to contribute to significant morphologic differences of endothelial cells between arterioles and venules, with arteriolar endothelium appearing elongated in the direction of flow as compared to a cuboidal appearance of venular endothelium[17]; this phenomenon is also well described in cultured endothelial cells.[18] Of note, blood rheology in the microcirculation is complex and does not follow the assumptions of traditional Newtonian flow; further, measures of microvessel hematocrit (an important determinant of viscosity and thus shear stress) by microscopy often yield values $\leq$50% than that of systemic hematocrit.[19,20] These

properties impact the validity of wall shear stress measurements based on centerline blood flow velocity (a common experimental technique), although mathematical models have been developed to account for the unique environment.[21] In addition to the shear stress-induced differences in endothelial cell morphology between arterioles and venules, endothelium in these microvessels exhibit a variety of functional differences, including those exemplified in Fig 1: leukocytes interact primarily with venular endothelium, and in some experimental models, a dramatic difference in von Willebrand factor (VWF) expression.

Endothelial cells demonstrate wide heterogeneity in structure and function between microvessels and large blood vessels, between organs, within individual tissues, within regions of individual vascular networks, and as shown by single-cell analyses, also between cells of individual vessels; these differences are also obvious in the comparison of endothelial cells between healthy and diseased subjects.[22-24] In contrast to thrombosis in large arteries, thrombosis in microvessels can result in a more diffuse impairment of perfusion and widespread dysfunction of the affected organs.

REGULATORY MECHANISMS OF THE MICROVASCULAR ENDOTHELIUM THAT PREVENT THROMBOSIS

Under resting conditions, the healthy microvascular endothelium is uniquely positioned to provide a powerful regulatory balance against similarly powerful stimuli predisposing to thrombosis. The resting endothelium provides an antiadhesive, anti-inflammatory, and antithrombotic barrier vital to maintaining homeostasis. Endothelial cells possess a broad range of endogenous regulatory mechanisms which can inhibit platelets, their adhesion to vascular walls, leukocyte-endothelial interactions, and/or the coagulation system. These are discussed briefly below with an emphasis on the microcirculation; these concepts are reviewed in greater detail in other publications.[1,25,26]

Endothelial glycocalyx. This is a thin, flexible boundary layer between the phospholipid membrane of the endothelial cell and the cellular and macromolecule components of the blood. This endothelial surface layer provides the interface between flowing blood and endothelial cell membranes and represents a hydrodynamically significant layer. The glycocalyx's constituents include proteoglycans, glycoproteins, and glycosaminoglycans containing heparan sulfate, chondroitin sulfate, hyaluronan and various core proteins (eg, glypicans, syndecans, etc.).[27] These components, organized in a mesh-like array, provide a steric- and charge-dependent semipermeable barrier to fluid and solute transport and prevents blood cell adhesion to the endothelium. Measurement of the precise thickness of the endothelial glycocalyx is limited by its physicochemical characteristics which may alter its dimensions during preparation for ultrastructural studies. Using a variety of techniques, reported values of glycocalyx thickness in microvascular endothelium in vivo range from 0.1 to > 1 μm.[28-31] Although the endothelial glycocalyx is also present in large blood vessels,
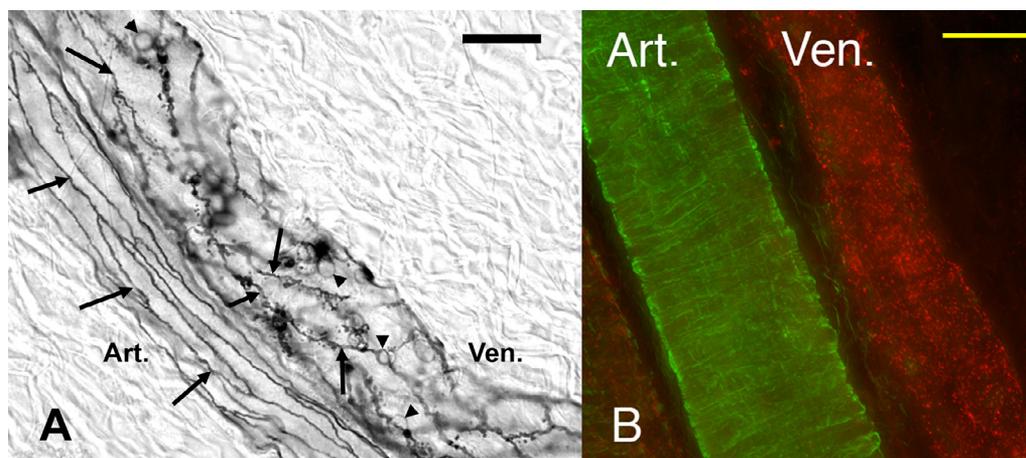
Fig 1. Examples of striking differences between arterioles and venules in vivo. (**A**) Silver-stained microvessels of rat mesentery outlining endothelial cell borders (arrows) showing long spindle-shaped cells in arterioles (Art.) and polygonal cells in venules (Ven.). Adherent leukocytes (arrowheads) are evident only on venules. (**B**) Immunofluorescence of mouse cremaster microvessels demonstrating vascular smooth muscle limited to the arteriole (stained with alpha smooth muscle alpha actin, green) and considerably greater von Willebrand factor expression (stained red) in the venule. Bar = 30 μm. From reference,[28] with permission.

the hemodynamic implications of the glycocalyx are considerably greater in the microcirculation since it occupies a significant proportion of the vascular volume in microvessels with diameters as small as 5 μm. While platelets are known to be activated by shear stress through several mechanisms,[32] shear stress on the endothelium induces a balancing effect. The endothelial glycocalyx is well recognized as a key mechanotransducer, mediating shear stress-dependent responses in endothelial cells, including release of nitric oxide and prostacyclin,[33-35] which are endothelial-derived inhibitors of platelets mentioned below. Experimental degradation of the endothelial glycocalyx has been shown to promote adhesion of platelets to microvascular endothelium.[36,37] Preclinical models of conditions associated with thromboinflammation such as sepsis and ischemia/reperfusion injury have been shown to result in degradation of the endothelial glycocalyx.[38,39] There is increasing interest in measurement of circulating and/or urinary glycocalyx components including syndecans, hyaluronan, heparan sulfate[40-44] as biomarkers in septic humans. Further, a recent study reported an association between syndecan-1 levels and disseminated intravascular coagulation (DIC) in patients with sepsis.[45] Overall, these studies suggest that degradation of the endothelial glycocalyx may contribute to the pathogenesis of certain thromboinflammatory conditions such as sepsis, and strategies aimed at preservation of the glycocalyx might represent future therapeutic targets in these conditions.[46]

Nitric oxide. Is a gaseous signaling molecule initially discovered as an endothelial-derived relaxing factor,[47,48] but is now known to mediate a myriad of responses on a broad variety of cells. Endothelial cell nitric oxide synthase (eNOS) is one of 3 isoforms of NOS; while it is constitutively expressed, its activity may be regulated by various mechanisms including phosphorylation, protein−protein interactions, and subcellular localization, among others.[49] Microvascular endothelial cells release nitric oxide in response to hemodynamic forces mechanotransduced by the glycocalyx and several agonists.[50,51] Preclinical models suggest that endothelial release of nitric oxide provides endogenous protection against thrombosis and regulates inflammation through various mechanisms, including inhibition of endothelial adhesion molecule expression, release of P-selectin and VWF, and inhibition of platelet activation.[52-54] Of interest to this review, mice with targeted deficiency of endothelial nitric oxide were reported to develop microvascular thrombosis in the kidney during aging[55], comparable to thrombotic microangiopathy (TMA) discussed below. Modulating the regulatory functions of the microvascular endothelium, including nitric oxide,

has been suggested as potential future therapeutic strategies for TMA.[56]

Prostacyclin. This product of arachidonic acid metabolism exerts several physiological responses comparable to those of nitric oxide. As in the case of nitic oxide, prostacyclin is released by microvascular endothelium in response to shear stress, resulting in vasodilatation as well as inhibition of platelet activation.[57-60] Prostacyclin synthesis is dependent on cyclooxygenase-1 (COX-1, expressed constitutively) as well as an inducible form, COX-2.[61] Endothelial cell-derived prostacyclin is presumed to be primarily dependent on cyclooxygenase-1 (COX-1); however, COX-1 is also expressed on platelets and mediates release of the prothrombotic molecule thromboxane A2.[62] Recent data generated from mice with cell-specific deletion of COX-1 and COX-2 have clarified the relative contribution of these enzymes in regulation of thrombotic tone in endothelial cells: endothelial cell COX-1 and COX-2 both prevent thrombosis, albeit via distinct and complementary pathways.[63] From a clinical standpoint, the antithrombotic protection induced by low-dose aspirin (an inhibitor of COX-1 and COX-2) is presumed to reflect a balance favoring prostacyclin over thromboxane A2.[64] Prostacyclin has been approved for clinical use for pulmonary hypertension in the US since 1995, and isolated case reports of its off-label use for thrombotic microangiopathies describe conflicting findings.[65-67] A greater understanding of the role of cell-specific regulation of COX isoforms may provide insight into therapies targeting prostacyclin for microvascular thrombosis.

Other endothelial antithrombotic mechanisms. Microvascular endothelial cells possess a variety of additional mechanisms that have been proposed to contribute to its endogenous antithrombotic properties. These include CD39/ectoADPase, a membrane bound enzyme that hydrolyzes adenosine triphosphate and adenosine diphosphate to adenosine monophosphate and thus inhibits platelet activation.[68] Other antithrombotic molecules expressed in microvascular endothelium include tissue factor pathway inhibitor,[69] activated protein C, thrombomodulin,[70] and antithrombin.[71] Of interest, these molecules failed to improve outcomes in large clinical trials in patients with sepsis,[72-75] including a trial focused on sepsis-induced coagulopathy in which thrombomodulin failed to demonstrate a clinical benefit.[75] Despite the failure of past large clinical trials performed on heterogenous groups of patients with sepsis, there is increased interest in targeting these pathways in selected subsets of patients with microvascular thrombosis in sepsis and related thromboinflammatory disorders.[76]

EXPERIMENTAL MODELS OF MICROVASCULAR THROMBOSIS IN VIVO

Much of our understanding of the molecular mechanisms responsible for microvascular thrombosis is derived from preclinical studies utilizing intravital microscopy, or microscopy-based observation of biological responses in vivo. Under physiologic conditions, the endogenous antithrombotic mechanisms described above prevent interactions between platelets and microvascular endothelium and prevent activation of coagulation on microvascular walls. A variety of experimental models have been used to induce focal microvascular thrombosis (Fig 2), with a general common feature being the disruption of one or more of the normal endogenous antithrombotic mechanisms outlined above. While microvascular thrombosis in human diseases tends to be diffuse and several models discussed here are focal, single vessel models allow for real-time observation of the kinetics of microvascular thrombus formation in vivo. The study of focal insults can provide insight into molecular mechanisms involved in microvascular thrombosis, which can be used for targeted manipulation of these pathways (eg, with genetically modified animal models, monoclonal antibodies, pharmacological agents, etc.) in preclinical models relevant to human diseases. Selected models are outlined very briefly below; for more in-depth discussion of this category of model, readers are referred to other reviews.[77,78]

Physical and electrical models of microvascular injury. Mechanical injury models are used commonly in preclinical studies in large vessels focusing on hemostasis (physiologic cessation of blood flow following vascular injury), particularly transection of the tail to quantify bleeding time.[79-81] Physical injury models have also been used in the microcirculation, mostly decades ago, with techniques including micropuncture or vessel transection.[82-84] Thrombosis in these models occurs as a result of focal damage to microvascular endothelium and exposure of flowing blood to the subendothelium. While these physical models are relevant to hemostasis, challenges involved in standardizing the degree of vascular injury might contribute to their infrequent use in current studies in the microcirculation. As in the case of physical stimulation, electrical injury to microvessels to induce thrombosis was more prevalent in studies performed many decades ago.[85-87] The infrequent use of direct electrical stimulation as a model of microvascular thrombosis may relate not only to the artificial nature of the injury but to the reported variability in responses in vivo.[87,88]

Photochemical and laser injury. The photochemical injury technique involves systemic administration of a fluorescent dye (or dye-labeled macromolecule) and exposure of a segment of the microcirculation to a carefully controlled light dose. The laser-induced model of injury involves laser injury to a carefully defined segment of the microcirculation in vivo. Both photochemical and laser models result in focal damage to microvascular endothelium and formation of platelet-rich thrombi at the site of injury. Electron microscopy studies have demonstrated focal areas of endothelial cell denudation in some models of laser and photochemical injury, though in others, platelet thrombi are evident in the presence of injured, though not denuded, endothelium.[78,89,90] Both methods are used in contemporary studies by various authors to define the kinetics of microvascular thrombosis in both venules and arterioles in vivo.[91-96] Although admittedly photochemical and laser stimulation represent artificial methods of microvascular injury, a significant
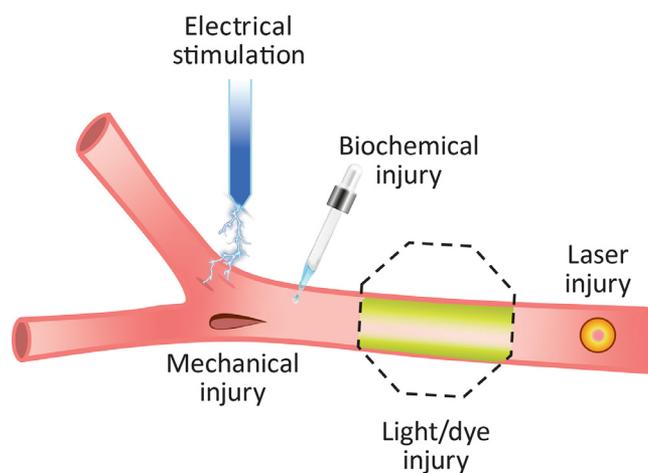

Fig 2. Schematic of experimental models of microvascular thrombosis induced by focal stimulation of individual microvessels, adapted from a schematic from reference.[78]

advantage of both approaches is the ability to standardize the degree of microvascular injury yielding highly reproducible results. Both models depend on dose of stimulation (ie, power and duration of exposure to laser or light) and the photochemical model also depends on dye concentration. Both techniques enable authors to define the influence of various biological conditions on the kinetics of microvascular thrombosis in vivo. Since these techniques are well established in various vascular beds in mice (eg, cremaster, mesentery, and pial), their use in genetically modified mice has greatly expanded our understanding of the molecular mechanisms involved in microvascular thrombosis in normal conditions and in experimental models of disease. It is important to note that mice and humans differ with regards to various parameters relevant to thrombosis, including platelet count (about 3-fold higher in mice), protease-activated receptors, responsiveness to certain agonists, a host of platelet transcriptomes, leukocyte subset number and expression of certain molecules, among others.[97-99] Recognizing these species-specific differences is important in the design and interpretation of microvascular thrombosis experiments in mice. Despite these differences, mice are used commonly for thrombosis studies in vivo and have provided observations relevant to human conditions.[97,98] Further, preclinical studies in mice enable sophisticated high-resolution multicolor intravital confocal microscopy imaging not feasible in humans, as exemplified by the studies by Kubes et al, which have revealed many novel observations of the links between thrombosis and inflammation in vivo. Two examples include defining the role of platelets in bacterial clearance in the liver during blood-borne infection and characterizing formation of neutrophil extracellular traps in vivo during infection.[100,101]

Biochemical stimulation. A variety of biochemical agents have been applied topically to macro- and microvessels to induce thrombosis in vivo 1 common method involves topical application of ferric chloride, used commonly in microvessels like the carotid artery[102-104] as well as microvessels as in the mesentery[104-106] and quantifying thrombus formation by reduction in arterial flow and/or recruitment of platelets by intravital microscopy. The mechanism of thrombus formation is proposed to involve oxidant injury to endothelium with denudation,[102,103] although recent findings suggest a more complex mechanism including participation of red cells.[107,108] Despite the relative uncertainty of the mechanisms resulting in injury, ferric chloride remains a commonly used method to study thrombosis in vivo. Additionally, targeted agonists can be applied topically, such as histamine, calcium ionophore, adenosine diphosphate, among others.[109,110]

However, ferric chloride remains the most commonly utilized biochemical approach to study thrombosis and/or platelet recruitment in single vessels in vivo. In addition to agonists applied topically to microvessels, targeted agonists can also be administered systemically resulting in experimental models of disease associated with microvascular thrombi, which can be utilized in preclinical evaluation of therapeutics. Some examples of these systemic agonists include lipopolysaccharide,[111] soluble VWF,[112] lectin concanavalin A (Con A) followed by anti-Con A,[113] among others.

An intriguing observation derived from several of the models described above is that the ultrastructural organization of microvascular thrombi is highly heterogenous. Several reports have demonstrated that microvascular thrombi in vivo and ex vivo contain a core area of tightly packed platelets with extensive shape change and frequent degranulation and a distal area containing loosely packed platelets with less evidence of activation.[84,114,115] This heterogeneity is deemed to be functionally significant, by influencing transport of biologically active substances across thrombi as well as thrombus stability,[114,115] likely to have clinical implications. Fig 3 demonstrates the 3-dimensional ultrastructure of a microvascular thrombus generated by our group using the photochemical injury model in a mouse cremaster venule (procedures approved by the Institutional Animal Care and Use Committee of Baylor College of Medicine), using the relatively new imaging technique of serial block-face scanning electron microscopy.[116]

Many of the experimental models of microvascular thrombosis described above result in platelet-rich thrombi, often with little evidence of fibrin by electron microscopy.[78] As described below, the structure of thrombi in clinical conditions associated with microvascular thrombosis varies considerably; for example, platelet-rich thrombi predominate in thrombotic thrombocytopenic purpura and fibrin-rich thrombi in hemolytic-uremic syndrome and DIC.[117,118] Similarly, the composition of thrombi in large vessels also varies according to vessel type and underlying disease[119]; of interest, the well-defined fibrin mesh often demonstrated in large vessel thrombi is not characteristic of the experimental microvascular thrombi described above.

CLINICAL OVERVIEW OF MICROVASCULAR THROMBOSIS

Imaging of the microvasculature. A key obstacle in effectively treating dysfunctional microvasculature is correctly identifying that there is an issue in that physiologic compartment. There have been significant advances in
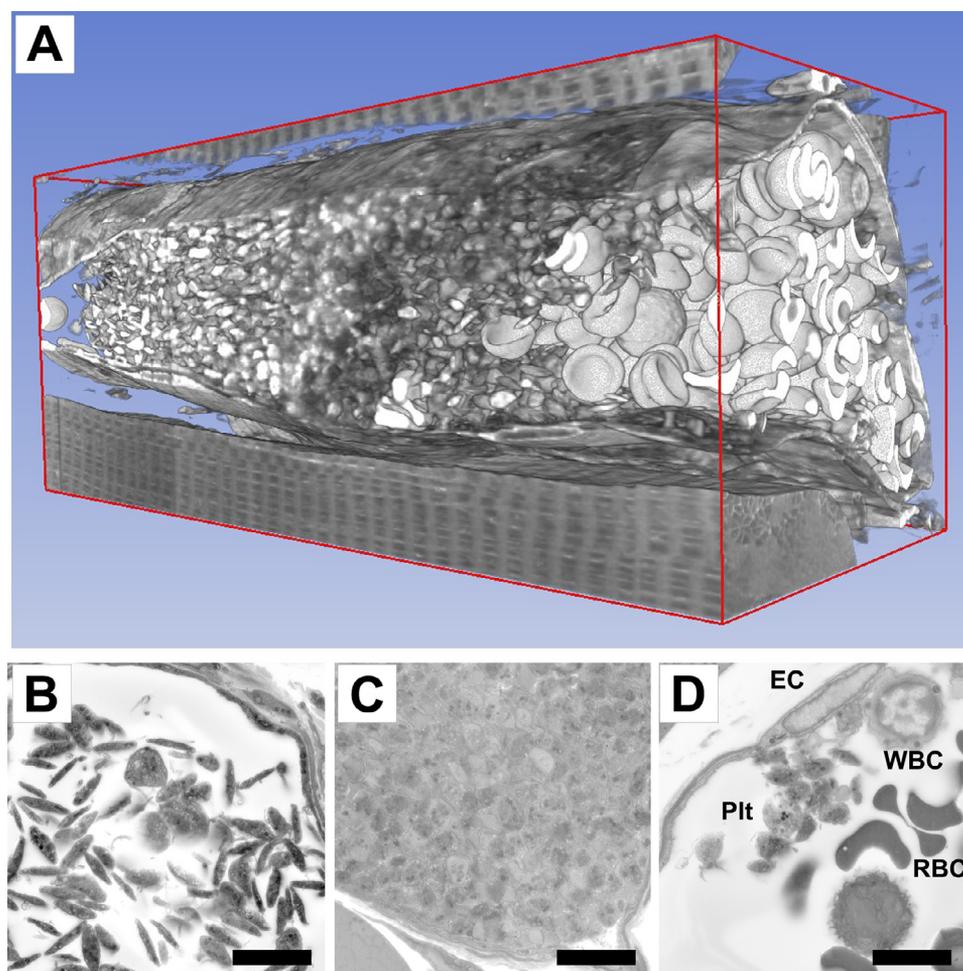
Fig 3. Three-dimensional ultrastructure of a photochemical injury-induced thrombus in a mouse cremaster venule (45 μm in diameter) by serial block-face scanning electron microscopy. (**A**) Longitudinal section demonstrates cellular heterogeneity within the thrombus; the dimensions of the bounding box are $43 \times 43 \times 107$ μm. (**B**) Cross section of the distal end of the thrombus shows loosely packed, discoid (unactivated) platelets. (**C**) Cross-section of the center of the thrombus shows densely packed platelets with evidence of significant degranulation. (**D**) Cross-section of the proximal end of the thrombus shows platelet (Plt)-endothelial cell (EC) adhesion, leukocytes (WBC) and erythrocytes (RBC). Scale bar = 5 μm. Images courtesy of Drs. Alan Burns and Samuel Hanlon, University of Houston College of Optometry.

aids to the human eye, but the microvasculature continues to elude observation by many common clinical imaging techniques. The resolution of a computed tomography angiogram or magnetic resonance angiogram typically does not provide information about the structure of these microscopic vessels. Even a conventional angiogram of the brain does not resolve vessels smaller than 500 μm.[120] The nailfold capillaries are grossly visible using commonly available clinical tools such an otoscope, but this does not reveal the internal contents of the capillaries. Dedicated nailfold video capillaroscopy has been utilized in a variety of conditions, particularly rheumatologic; for example, it has been reported to help identify microthrombi and microhemorrhages in some patients with systemic sclerosis or antiphospholipid syndrome.[121,122]

While these approaches may have utility, particularly for screening in a subset of the conditions outlined below, in the vast majority of these conditions defined by and/or accompanied by microvascular thrombosis, the diagnosis is typically made clinically and sometimes confirmed histologically if possible. Early recognition is typically an important factor in delivering effective therapy.

Diseases accompanied by microvascular thrombosis. Many of the diseases characterized by microvascular thrombi were initially classified solely based on clinical phenotype; however, advances in science have broadened understanding of the mechanisms of dysregulation in many diagnoses and subsequent reclassification based on pathogenesis. In some cases, microvascular thrombosis may develop in a previously

healthy individual following exposure to a devastating stimulus (eg, Shiga toxin-producing *Escherichia coli* [*E. coli*]). In other cases, microvascular thrombosis may develop in individuals with significant medical conditions (eg, cancer, severe systemic lupus erythematous) following a relatively less severe stimulus. In this review, we discuss a variety of clinical entities associated with microvascular thrombosis, although this is not intended to be an exhaustive all-encompassing discussion of such conditions. Similarly, we discuss common therapeutic interventions for various conditions, noting that in many cases of microvascular thrombosis associated with an underlying disease, the standard of care in therapy consists of treatment of the underlying disease.

Thrombotic microangiopathies (TMAs). TMAs are defined as having microangiopathic intravascular hemolytic anemia, thrombocytopenia, and organ dysfunction (renal or neurological) in the absence of evidence of diffuse intravascular coagulation (DIC). A hallmark of TMA is the presence of mechanically damaged red blood cells (RBCs) in circulating blood, termed schistocytes, deemed to result during passage of highly deformable RBC through porous microthrombi and/or interactions with fibrin strands in the microthrombi.[123,124] The primary underlying etiology and specific therapy for the TMA subtypes differs. For example, although therapeutic plasma exchange (TPE) has been used for various TMA subtypes, it may be reasonable to bypass TPE for eculizumab for definite cases of TMA-complement mediated, also known as atypical hemolytic-uremic syndrome (aHUS).[125,126] In TMA characterized by direct endothelial damage such as TMA-infection associated (also HUS) and some types of TMA-drug induced, the endothelium may often spontaneously recover and not require TPE.[125] This logic may be less applicable in severe cases of endothelial damage that have pushed other fluid-phase pathways into a dysregulated state.[127,128] Often, the specific etiology of TMA is not immediately clear and patients require treatment prior to the results of specific laboratory testing. The British Committee for Standards in Haematology and the Mayo Clinic have developed consensus algorithms to guide treatment decision-making while diagnostic evaluation is pending.[123,129] The specific etiology of the TMA is particularly helpful in long-term management and prognosis.

Thrombotic thrombocytopenia purpura (TTP). TTP was distinguished as a unique TMA with the discovery of a disintegrin-like and metalloproteinase with thrombospondin type 1 motif 13 (ADAMTS13).[130] Endothelial cells are one source of VWF, a large plasma glycoprotein, which serves multiple functions in hemostasis. ADAMTS13 cleaves a specific bond in the VWF-A2 domain—releasing the VWF from endothelial cell surface and reducing the size. The ultra-large VWF multimers are unfolded under high levels of shear stress in the arterioles and capillaries, which induces platelet aggregation specific to the microcirculation.[131-133] The brain is commonly involved clinically, although autopsy studies have revealed diffuse organ involvement.[134] The mechanisms behind this organ-specific predominance are unclear, although endothelial heterogeneity is a plausible explanation since a wide range of molecular and functional differences in endothelial cells among organs have been described, including VWF.[22,135-137] Deficiency of ADAMTS13 can be congenital or acquired, and the acquired form can occur spontaneously or secondary to an existing inflammatory condition. The standard of care for initial therapy for most forms TTP is TPE. The American Society for Apheresis (ASFA) strongly recommends TPE in TTP with grade 1A evidence. TPE removes potential antibodies targeting ADAMTS13 and replaces ADAMTS13 from healthy plasma.[126]

Primary TTP-Acquired. Acquired primary TTP is characterized by anti-ADAMTS13 antibodies that results in severe deficiency of ADAMTS13.[130] There are 2 types of anti-ADAMTS13 antibodies: neutralizing and clearing, which require different assays for their measurement. Many hospital laboratories do not perform ADAMTS13 panels, requiring that physicians send patient specimens to outside laboratories, delaying diagnosis. The PLASMIC score was developed to rapidly assess TTP risk using rapidly available laboratory results including: platelet count, reticulocyte count, haptoglobin, indirect bilirubin, mean corpuscular volume, international normalized ratio, creatine and clinical characteristics.[138] Patients with elevated PLASMIC scores should generally start TPE therapy while awaiting the results of the ADAMTS13 panel. While TPE removes circulating pathogenic anti-ADAMTS13 antibodies, it does not halt their production. Therefore, immunosuppression should be initiated in conjunction with TPE. Corticosteroids are the mainstay of treatment, although there are no comparative trials definitively demonstrating their efficacy.[126] While TPE can remove circulating pathogenic anti-ADAMTS13 antibodies, it does not halt their production. There are multiple prospective studies demonstrating the efficacy of B-cell depletion with the anti-CD20 chimeric monoclonal antibody rituximab in acquired primary TTP in shortening the initial episodes.[139,140] Additionally a retrospective comparison found that patients who received rituximab experienced fewer relapses compared to historical controls who did not receive this therapy.[140] There has been a recent phase 3 trial of caplacizumab, a humanized immunoglobulin fragment

which targets the A1 domain of VWF to prevent its interaction with the platelet glycoprotein Ib-IX-V receptor, demonstrating efficacy over TPE and steroids alone. Severe ADAMTS13 deficiency was not an inclusion criteria for this trial, although 85% of patients were found to have ADAMTS13 activity of less than 10% of baseline.[141] Other potential therapies reported include cyclosporine, azathioprine, vincristine, bortezomib, and other immunosuppressive agents.[126] Additionally these patients should be carefully monitored for development of systemic autoimmune conditions such as systemic lupus erythematous, particularly when acquired TTP occurs in childhood.[142]

Primary TTP - Congenital. If there is no antibody detectable and ADAMTS13 activity is less than 10%, then genetic defects should be considered.[123] Congenital TTP comprises approximately 5% of cases of primary TTP and typically presents early, with 50% of cases presenting in infancy. Of interest, it can present at any age, including adulthood, particularly in the setting of common inflammatory stimuli such as trauma and infection or pro-thrombotic stimuli such as pregnancy. The clinical spectrum of disease varies significantly from asymptomatic thrombocytopenia to life threatening organ dysfunction. Depending on the severity of presentation and clinical course, some patients may benefit from prophylactic plasma infusion to minimize relapses.[143] Additionally, recombinant ADAMTS13 or BAX 930 is currently being assessed in clinical trials for this indication.[144]

Secondary TTP. Acquired ADAMTS13 deficiency is known to complicate existing inflammatory diseases, including systemic lupus erythematosus (SLE), systemic sclerosis, polymyositis/dermatomyositis, rheumatoid arthritis (RA), sepsis, and pancreatitis.[130,145,146] A prospective study compared the level of ADAMTS13 in connective tissue disease (CTD)-TMA to primary TTP and found that severe deficiency is more common in primary TTP.[130] The subset of patients with severe deficiency of ADAMTS13 in CTD-TMA also had a higher incidence of detectable ADAMTS13 auto-antibodies. These CTD-TMA patients with a phenotype similar to primary TTP were more likely to have a diagnosis of rheumatoid arthritis or SLE. Interestingly, severe deficiency of ADAMTS13 was associated with better outcomes in CTD-TMA compared to mild-moderate deficiency.[130] Acute inflammation, chronic inflammation, antiphospholipid antibodies, and immunosuppressive medications are all potential contributors to microvascular endothelial dysfunction that may be additive to a moderate ADAMTS13 deficiency.[147] Acquired deficiency of ADAMTS13 has also been described in patients with sepsis and is associated with severity of

disease, DIC, and mortality risk.[145,146] Secondary TTP without anti-ADAMTS13 antibodies typically does not result in undetectable levels of ADAMTS13 or less than 10% of baseline activity[130,145,146] but may be a sign of an extremely dysregulated microvascular environment. The treatment of secondary TTP with severe deficiency consistent with anti-ADAMTS13 autoantibodies is comparable to that of primary TTP with additional attention to treating the underlying condition. Without clinical presentation consistent with anti-ADAMTS13 autoantibody, the treatment should be tailored to triggering factors.[148]

HUS. This subtype of TMA is unified by the frequent involvement of the microvasculature of the kidney, as indicated by the term "uremic" in its name. To distinguish HUS from TTP, the PLASMIC score includes creatinine of less than 2.0 mg/dL as a point in favor of the diagnosis of TTP.[138] Relative to TTP, there is a decreased incidence of cerebral injury in HUS. An in vitro study revealed that human brain microvascular EC displayed a stronger regulatory response when alternative pathway (AP)-related gene expression was increased compared to renal microvascular EC.[149] Additionally renal microvascular EC possess a higher baseline level of AP activation compared to human umbilical vein ECs. AP activation was significantly increased in both cell types by the addition of tumor necrosis factor-α.[150] HUS is composed of subcategories that share a similar clinical phenotype but with different pathogenesis and initial treatment.

TMA-infection associated (also typical HUS). In the majority of patients with HUS (90%), the TMA is triggered by Shiga-like toxin (Stx) produced by bacteria, predominately *E. coli* O157:H7.[151] The Stx directly damages the renovascular endothelium by binding to the glycosphingolipid globotriaosylceramide present on renal glomerular endothelial, mesangial and tubular epithelial cells.[152,153] In contrast to TTP, the dysfunctional component of the microcirculation is not the plasma but the endothelium thus routine use of TPE is not recommended by the ASFA.[126] The treatment is primarily supportive care with adequate hydration to account for diarrheal losses and microvascular leakage. In cases of severe diarrhea or neurologic involvement, some retrospective studies suggest a benefit for use of eculizumab.[154] The ASFA advises that physicians may consider the use of TPE in patients with severe bloody diarrhea or neurologic involvement as some case reports suggest benefit in this subset (grade III recommendation with grade 2C evidence).[126,128] The use of antibiotics in OH157:H7 infections was found by a retrospective study to be associated with a higher risk of development of TMA-infection.[153,155] Another less

common cause of TMA-infection is triggered by invasive disease with *Streptococcus pneumoniae*. The mechanism of TMA development is less well understood in HUS triggered by *S. pneumoniae*. It has been proposed that *S. pneumoniae* produce neuramidase that cleaves sialic acid from cell membrane surfaces exposing the Thomsen−Friedenreich antigen (T antigen) which then reacts with naturally occurring anti-T antibodies causing hemolysis.[156,157] However, another study found that this antigen exposure did not correlate well with HUS development.[158] Additionally this mechanism describes the process of an autoimmune hemolytic anemia, which is classically extravascular, in contrast to intravascular TMA.[159] The ASFA recommends TPE based on clinician discretion (grade 2C evidence). In this case, treatment of the underlying infection is recommended. Triggering infections tend to be severe and include sepsis, pneumonia and meningitis.

TMA-complement mediated (also atypical HUS). TMA-complement mediated disorders compromise a group of conditions associated with abnormalities in the complement system, which can either be inherited or acquired. The complement system is a powerful part of the innate immune system and accompanied by a complex regulatory system to prevent inappropriate activation. There are 3 main pathways in which complement activation occurs: the Classical Pathway, Lectin Pathway, and Alternative Pathway, which converge on activation of C3. There are membrane-bound and fluid-phase complement regulators that prevent inappropriate deposition of C3 onto endothelial cells.[160] The inherited complement disorders include mutations in complement regulators and activators and often present after an inflammatory trigger.[161] The cascade of events typically leads to fibrin-rich thrombi, compared to the more platelet-rich thrombi of TTP.[162] The glomerulus of the kidney is especially reliant on soluble complement regulators (such as factor H) due to the unique properties of the glomerular basement membrane. The glomerular basement membrane is directly exposed to blood in the gaps in the fenestrated glomerular endothelium—and therefore lacks endothelial membrane and all regulatory proteins bound by endothelial membrane at these locations.[160] Greater than 70% of genetic mutations have been identified in complement factor H (CFH), a soluble regulator of the alternative pathway. Additionally, anti-CFH antibodies have been described in a form of acquired TMA-complement mediated.[160,163] Other commonly mutated complement regulatory proteins include complement factor I, membrane-cofactor protein (MCP/CD46), and thrombomodulin (discussed below under TMA-coagulation mediated).[164] The diagnosis may be suspected based on TMA features with prominent renal findings and the absence of bloody diarrhea or positive stool test for Stx producing bacteria. Measured levels of complement, including C3 and C4, are often low due to consumption but these findings lack specificity to aHUS.[165] In some cases it is clinically unclear whether the TMA is due to underlying TTP or HUS—the Mayo clinic consensus guidelines recommend initial TPE in these cases.[123] Additionally TMA-complement-mediated with a positive anti-CFH autoantibody can benefit from TPE.[126] Eculizumab has been confirmed by phase 3 trials in children and adults to be superior to TPE alone and has significantly reduced the mortality and morbidity in this disease. Earlier initiation (within 1 month of presentation) is associated with improved outcomes compared to months or years after presentation.[166,167] TMA-complement mediated is a prime example of thromboinflammation, as immune overactivation directly leads to thrombosis.[1] The key therapeutic for this class, eculizumab, was actually developed for use in classic rheumatologic/inflammatory conditions such as SLE, rheumatoid arthritis and dermatomyositis.[168]

TMA-coagulation mediated (also atypical HUS). A smaller subset of patients within the classification of atypical HUS have been found to have activating defects in the coagulation cascade. During physiologic hemostasis, the coagulation cascade is activated through the tissue factor pathway after the formation of platelet plug at sites of endothelial injury. This process, also known as secondary hemostasis, results in the generation of thrombin, which cleaves fibrinogen into fibrin, which then polymerizes into a stable fibrin plug. One study identified thrombomodulin mutations in 5% of aHUS patients who were sequenced.[151] Thrombomodulin is a thrombin cofactor that negatively regulates coagulation activation and fibrinolysis. This study found that thrombomodulin also has regulatory roles in complement activation and binds to C3b and factor H in vitro.[151] Additionally thrombomodulin negatively regulates leukocyte trafficking.[169] Thrombomodulin provides one of many links between coagulation, complement and innate immunity. An additional coagulation-based mutation identified in aHUS is diacylglycerol kinase epsilon which inhibits protein kinase C and plasminogen.[164,170] Eculizumab is also used in this category but may be less effective than use in TMA-complement mediated.[170] Currently, there is no specific treatment for this subtype of aHUS, but potential future directions could include pharmaceuticals that have been developed for DIC including recombinant thrombomodulin for patients with pathogenic thrombomodulin mutations.[75,151]

TMA-transplantation associated. TMA has been observed after both solid organ and hematopoietic stem cell transplantation (HSCT).[171,172] TMA after HSCT can affect any organ but typically involves the kidney, resulting in a similar phenotype to aHUS.[173] A prospective cohort identified proteinuria and hypertension as the earliest signs of TMA-TA; creatinine was also elevated but not specific in comparison to HSCT recipients without TMA.[174] The diagnosis after HSCT is particularly challenging as many of the laboratory hallmarks of TMA are present at baseline, including schistocytes,[175] elevated lactate dehydrogenase (LDH), and thrombocytopenia. Severe ADAMTS13 deficiency is not a hallmark of TMA-TA; the etiology is thought be related to endothelial damage from chemotherapy and the post-transplant inflammatory state leading to classical and alternative complement activation.[173,176,177] Alterative pathway dysfunction is supported by complement regulatory mutations and anti-factor H antibodies in a subset of patients.[178] TMA-TA also frequently co-occurs with other insults including infection and graft versus host disease so it can be challenging to assess the relative severity of TMA-TA.[172] Regarding treatment, retrospective studies on the effectiveness of TPE in TMA-TA have not suggested efficacy.[126,179] Calcineurin inhibitors are frequently implicated in the development of TMA-TA and it is recommended to reduce the dose or discontinue the medication on an individualized basis.[172] Given the prominent role of complement, eculizumab has been used with some success,[180] although it may carry increased risk of infection in this vulnerable group.[181]

TMA-drug associated, or drug-induced (DI)-TMA. This category includes the diagnoses of DI-HUS and DI-TTP. Drug-induced TMA occurs by a variety of mechanisms, dependent on the properties of the individual drug. Two main mechanistic categories have been proposed: immune-mediated reaction and dose- and duration-related toxic reactions. Immune-mediated reactions tend to occur acutely within weeks after drug exposure whereas toxicity-related reactions which could be either acute (with a supra-therapeutic dose or known toxic substance) or gradual (with therapeutic dosing). A literature review in 2015 identified 78 drugs with published evidence of TMA association and found that 22 met their criteria for definite causal association. The top 3 drugs implicated in the literature were quinine, cyclosporine and tacrolimus.[182] TPE's efficacy varies with the triggering agent; the mainstay of treatment is avoidance of re-exposure to the triggering agent.[126]

Hemolysis, elevated liver enzymes and low platelet count. Hemolysis, elevated liver enzymes, and low platelet (HELLP) syndrome is a TMA syndrome typically presenting in the second or third trimester of pregnancy and occasionally postpartum.[183] HELLP is a clinical diagnosis which can be aided by the Tennessee classification. The classification requires hemolysis (evidenced by schistocytes or burr cells, elevated serum bilirubin, low serum haptoglobin or elevated LDH) with anemia, liver damage and thrombocytopenia.[184] HELLP is characterized by endothelial injury, fibrin deposition, platelet activation and consumption.[185] The exact pathogenesis is not well elucidated, however it is thought to be similar to pre-eclampsia, as about 80% of cases with HELLP are preceded by pre-eclampsia.[186] At a cellular level, effectors of this syndrome include abnormal placental factors, endothelium, and immune system. Defective spiral artery modeling and abnormal extra villous trophoblast cell proliferation results in hypoxia and shedding of debris.[187] The resultant tissue necrosis elicits a strong inflammatory response with cytokine production,[188] activation of the complement system,[189] and leukocytosis.[190] Indeed, germline mutations in the alternative complement pathway (Factor H, MCP/CD46, and Factor I) have been shown to predispose women to HELLP.[191,192] There is literature suggesting that placental derived antiangiogenic factors contribute to endothelial dysfunction and decreased renewal which results in platelet activation, hemolysis, and microvascular thrombosis.[193] The mainstay of treatment of HELLP is prompt delivery.[194] In addition, other therapies such as betamethasone for promoting fetal lung maturity, antihypertensives, magnesium sulfate for neuroprotection and supportive care with RBC or platelet transfusions are used prior to delivery.[195] Initially, corticosteroids were considered for treatment of HELLP based on the role of leukocytes in its pathophysiology,[190,196] however, their effect appears to be modest, with improved platelet recovery in those treated with corticosteroids and no effect on maternal-fetal outcomes.[197] Despite a role for complement activation, to date no major trials have been conducted using eculizumab, however, case report exists for its efficacy.[198] As we gain better understanding of pathogenesis of disease, more targeted treatments are expected to become available.

DIC. DIC is a different, but not mutually exclusive, category of microvascular occlusion than TMA. The fibrin-rich microvascular occlusions in DIC[111] are presumed to not allow for a significant percentage of RBCs to extrude through the occlusions resulting in anemia from characteristic mechanical damage.[124] Although schistocytes may be present in DIC, they tend to occur at a much lower frequency than in TMA.[199] Additionally, DIC is differentiated from TMA by widespread fibrin deposition resulting in low fibrinogen and coagulation cascade factor consumption

leading to coagulopathy.[200,201] Like TMA, DIC can be incited by a variety of insults which result in unique phenotypes of DIC; we will discuss 4 common triggers of DIC including sepsis, trauma, malignancy and hemophagocytic lymphohistiocytosis (HLH). The International Society on Thrombosis and Haemostasis (ISTH) defines DIC as "An acquired syndrome characterized by the intravascular activation of coagulation with loss of localization arising from different causes. It can originate from and cause damage to the microvasculature, which if sufficiently severe, can produce organ dysfunction." Additionally the ISTH developed scoring criteria for DIC comprised of the platelet count, d-dimer (fibrin degradation marker), prothrombin time and fibrinogen level.[202] DIC is associated with both excessive coagulation and enhanced fibrinolysis; the predominance of these pathways can result in a primary phenotype of bleeding, of microvascular thrombosis with organ failure, or both bleeding and microvascular thrombosis simultaneously.[203] Although bleeding clearly has significant clinical implications in DIC, we will focus our discussion on microvascular thrombosis, the topic of this review.

DIC-Sepsis. The Society of Critical Care Medicine and the European Society of Intensive Care Medicine third consensus definition of sepsis is life-threatening organ dysfunction caused by a dysregulated response to infection. Organ dysfunction is defined according to the Sequential Organ Failure Assessment score of 2 points or more; one of the parameters being platelet count.[204] DIC in sepsis provides a clear connection between the innate immune system and the coagulation cascade. During healthy physiology, the innate immune system and the coagulation cascade cooperate to control infections at the source of inoculation. The innate immune system primarily attacks the invading organism, while the coagulation cascade helps contain the infected area with a fibrin mesh — potentially contributing to an abscess capsule.[205,206] The crosstalk between inflammation and thrombosis is extensive and covered by several reviews.[1,207,208] During sepsis, interactions between pathogen-associated patterns (PAMPs) and pattern recognition receptors (PRRs), including toll-like receptors (TLRs), on sentinel immune cells result in increased production of a broad array of cytokines via transcription factor nuclear factor-kappa B (NK-kB) and mitogen activated protein (MAP) kinases.[209] Key cytokines released include tumor necrosis factor alpha, interleukin-1 (IL-1), interleukin-2 (IL-2), interleukin-6 (IL-6), and interleukin-8 (IL-8).[210] Sepsis is a highly heterogeneous syndrome[211] and therefore the path to DIC within sepsis may also be variable. A broad range of mechanisms have been proposed in the pathogenesis of DIC in

sepsis. Due to the volume of research invested in understanding DIC, it is not possible to briefly summarize this body of work. We selected 2 from a multitude of interesting pathways with evidence of relevance to DIC: tissue factor and neutrophil extracellular traps (NETs), and do not wish to construe that these are the most relevant. The innate defense against invaders is complex with multiple, redundant pathways to counteract the rapid evolutionary potential of pathogenic microorganisms. Likely numerous different pathways may escape regulation and lead to a clinical phenotype that is currently described as sepsis with DIC. For more in-depth overview of the pathogenesis of DIC, including sepsis, readers are referred to recent reviews.[212,213]

Tissue factor. The coagulation cascade has 2 primary pathways to activation: the contact activation pathway (intrinsic) and the tissue factor pathway (extrinsic). Tissue factor is expressed at a high density on stationary cells protected from exposure to the soluble factor VII.[214,215] In states of injury, tissue factor is exposed to the circulating components of the blood and aids in physiologic hemostasis. Tissue factor can also be expressed by stimulated monocytes, which has been demonstrated in animal and human models.[216,217] Whether human platelets express tissue factor remains controversial.[216,218] Similarly, while tissue factor expression by endothelial cells is well characterized in vitro, there are few reports of its expression endothelial cells in vivo, possibly as a result of lower levels of expression.[219] Despite promising preclinical studies, a large phase 3 clinical trial with recombinant tissue factor pathway inhibitor, tifacogin, did not show a decrease in all-cause mortality and was associated with a higher risk of hemorrhagic events.[72] As in the case of countless other clinical trials that have failed in sepsis, including several other agents targeting coagulation (with promising preclinical data), it is conceivable that additional insight into phenotyping subsets of patients may identify patients to benefit from this and other therapies in a personalized manner.[220]

NETs. Neutrophils are the most abundant circulating nucleated cell and respond to cytokines released from initial PAMP−PRR interactions. Neutrophils' classic roles in pathogen offense is through generation of endogenous reactive oxygen species via nicotinamide adenine dinucleotide phosphate and phagocytosis of pathogens. Neutrophils are also capable of undergoing a unique version of programed cell death called NETosis in which they release a DNA meshwork with attached histones, granular enzymes and cytoplasmic proteins including neutrophil elastase,

myeloperoxidase, cathepsin G, proteinase 3, gelatinase, LL-37, lactoferrin and calprotectin. A number of components of NETs, including extracellular DNA and histones, can predispose to thrombosis through multiple potential pathways.[221-223] Extracellular DNA functions as a damage-associated molecular pattern (DAMP) which interacts with the coagulation cascade directly through multiple potential pathways.[224,225] NETs can function as a scaffold for fibrin deposition analogous to the platelet plug scaffold.[226] Additionally, VWF has been shown ex-vivo to interact with extracellular DNA, providing a possible link between NETs and platelet interaction.[227] Further, serine proteases attached to the DNA meshwork including neutrophil elastase and cathepsin G have been demonstrated in vitro to promote fibrin deposition by degrading regulators of blood coagulation.[228,229] NETs have also been shown to be associated with deep vein thrombosis in mouse models.[230,231] The imbalance of NET formation and NET degradation may contribute to the DIC phenotype in sepsis in humans.[225] In mice, DNase1 and DNase1-like 3 (DNase1L3) mediate clearance of NETs. DNase1-/- or DNaDNase1L3-/- mice showed a hemolytic anemia, increased concentrations of LDH, and schistocytes after receiving lipopolysaccharide (LPS) to mimic sepsis—consistent with a TMA or DIC-like clinical phenotype.[232]

DIC-trauma. While the immediate mortality from severe trauma or "polytrauma" often arises from direct damage, such as hemorrhagic shock or primary brain injuries, delayed mortality is associated with secondary damage from the body's dysregulated response to injury and infection. Trauma patients can develop a systemic inflammatory response that can be complicated by DIC.[233] The excessive response to injury may also be followed by an excessive opposing response termed compensatory anti-inflammatory response syndrome.[234] In contrast to DIC-Sepsis with an initial PAMP−PRR interaction that can lead to self-induced DAMP−PRR interactions such as NETs, DIC-Trauma is characterized by initial DAMP−PRR interactions. One suggested contributory interaction in DIC-trauma is between mitochondrial DNA and TLR9, which also recognizes viral or bacterial DNA.[235] Interestingly, mitochondrial DNA shares similar features to pathogen DNA and may contribute to the similar phenotypes of DIC-trauma and DIC-sepsis.[236,237] The tissue factor pathway has also been implicated in the pathogenesis, as evidenced by the significant activation of tissue factor in patients with DIC-trauma.[238-240] Additionally, the balance of clot formation and clot breakdown may be different than DIC-Sepsis. In experimental models, TF-induced DIC resulted a compensatory response in fibrinolysis while LPS-induced DIC resulted in suppressed fibrinolysis.[241] Plasminogen activator inhibitor 1 (PAI-1) is a potent inhibitor of fibrinolysis secreted by endothelial cells and also present in the alpha granules of platelets.[242] While PAI-1 expression increases in LPS models of sepsis,[243] a relative reduction in activity during DIC-Trauma can result in DIC with enhanced fibrinolysis or a bleeding predominant phenotype.[244] The general management of DIC-trauma includes avoidance of additional DAMP production, for example, avoiding immediate operations on fractures that can be temporarily addressed noninvasively.[234,245]

DIC-cancer. There has been a longstanding association between various types of malignancies and an increased incidence of thrombosis including arterial, venous and microvascular in the form of DIC. In some regards, malignancy may be viewed as even more heterogeneous than sepsis and trauma, so it is difficult to discuss pathogenesis under this broad category of disease. In infection, fibrin deposition functions to defend the body from an invader, but certain cancers may coop this physiologic function to defend themselves from detection by the immune system. One study that analyzed cell lines from patients with pancreatic cancer found that some patients' cancer expressed TF microparticles into culture medium.[246] Another study in human breast cancer cells connected the upregulation of TF expression by nearby vascular endothelial cells as a marker for angiogenesis.[247] One study identified the presence of DIC in 6.8% of patients with solid tumors in 3 center cohort with independent risk factors of DIC including older age, male, advanced stage, breast cancer and the presence of necrosis on biopsy.[248] Fragmented RBCs were also identified in approximately two-thirds of the patients. Additionally, this study identified an increased prevalence of bleeding manifestations compared to thrombotic manifestations in their cohort.[248] For additional insight into the association between cancer and thrombosis, readers are referred to a recent review.[249]

DIC-HLH. Primary/familial HLH is a form of genetic immunodeficiency, often a malfunction of the cytotoxic machinery of natural killer cells and cytotoxic lymphocytes that results in ineffective removal of intracellular pathogens including viruses.[250] This can drive a compensatory over-reaction of other pathways of innate immunity resulting in high fevers, cytokine storm, and overactivation of macrophages. Secondary HLH (also known as macrophage activation syndrome) can be triggered by a variety of conditions including rheumatic disease and malignancy.[250] The 2004 classification criteria for HLH include low fibrinogen and thrombocytopenia as 2 of the 8 possible criteria, with a cutoff of 5 criteria being required for inclusion in HLH

clinical trials.[251] The ISTH guidelines for the clinical diagnosis of DIC including low platelet count, elevated d-dimer, prolonged PT and low fibrinogen have also been described in patients with HLH. One study reported that 50% of a 117 patient HLH cohort met the ISTH diagnostic cutoff for DIC.[252] The microvascular histopathologic findings on liver biopsies and autopsy studies in HLH include a cellular lymphohistiocytic infiltrate of sinusoids[253,254] as opposed to the classic fibrin-rich microvascular occlusion in Sepsis-DIC. Measurement of serum ferritin can help distinguish sepsis-DIC from HLH: the HLH-2004 classification criteria include ferritin levels > 500[251] and levels > 10,000 have been demonstrated to have specificity for HLH in isolation.[255] Diagnosis may be suspected with hyperferritinemia, but is made clinically, ideally by a specialist or team with experience in identifying HLH and MAS. In contrast to DIC-sepsis, patients with primary HLH and MAS often require immunosuppression for therapy, in many cases prior to clearly meeting published classification criteria. Even with treatment, the mortality rate is high in both conditions: the 5-year survival in the HLH-2004 study was 66%[256] while a large multicenter, multinational trial revealed an initial mortality of 8% in MAS complicated systemic juvenile idiopathic arthritis.[257] The treatment of HLH can include steroids and chemotherapy per the HLH-2004 protocol and/or newer anti-interferon gamma monoclonal antibody emapalumab, typically followed by bone marrow transplant after remission is achieved.[251,258] Secondary HLH/MAS is treated with a variety of immunosuppressive agents including steroids, cyclosporine and IL-1 cytokine blockade with anakinra.[259,260]

Treatment of DIC. While DIC may be initiated by various conditions, the mainstay of treatment primarily involves treating the underlying disease (ie, infection, cancer, etc.). A number of specific treatments have been suggested for patients based on the distinct clinical presentations (eg, thrombotic, bleeding, and asymptomatic); however, in many cases the quality of the evidence is not high, resulting in distinct treatment recommendations by various societies.[203] A variety of treatments have been examined in clinical trials of patients with DIC, including heparin, antithrombin, activated protein C, and thrombomodulin[261-263] without firm evidence of therapeutic benefit. In patients with DIC and significant thrombosis, therapeutic doses of heparin anticoagulation may be considered, yet this is recommended with a low level of evidence by various society guidelines.[203] In contrast, the use of low molecular-weight heparin for prevention of deep venous thrombosis in patients with DIC in the absence of bleeding or thrombocytopenia is generally recommended.[203,264,265] The management of DIC in

patients with a predominant bleeding phenotype is beyond the scope of this review and is discussed elsewhere.[203]

Catastrophic antiphospholipid syndrome. Antiphospholipid syndrome (APS) is typically characterized by macrovascular thrombosis in arteries or veins or focal placental thrombosis resulting in miscarriage. Catastrophic antiphospholipid syndrome (CAPS) is a unique subset of APS that primarily targets the microvasculature resulting in multiorgan dysfunction. The revised Sapporo classification can assist in the clinical diagnosis of APS. The revised Sapporo classification does account for microvascular involvement in APS but does not delineate CAPS as a specific subtype of APS.[266] Merrill et al proposed a classification criteria for definite CAPS including (1) 3 or more organ systems involved, (2) development of manifestations in less than one week, (3) histopathological confirmation of small-vessel occlusion in at least 1 organ, (4) laboratory confirmation of lupus anticoagulant or anticardiolipin antibodies twice at least 6 weeks apart.[267] These criteria emphasize the fulminant, rapidly progressive or "catastrophic" nature of CAPS as distinct from microvascular thrombi that may be observed in classic APS. The microvascular preference of CAPS and lack of significant coagulopathy (in contrast to DIC) can also create a clinical picture overlapping with TMA.[267] CAPS commonly involves the kidneys, skin, brain, cardiovascular system, lung, and liver.[268] In classic APS the antibodies are considered pathogenic but not sufficient for disease. However, "triple positivity" with positive lupus anticoagulant, anti-cardiolipin and anti-beta-2-glycoprotein confers increased risk of developing thrombosis.[269] A "second hit" hypothesis has been proposed where existing antiphospholipid antibodies become pathogenic under inflammatory conditions leading to thrombus formation.[270] In vivo models of APS have used either mechanical, chemical or photochemical trauma or LPS to demonstrate increase in thrombosis in APL-infused animals over the secondary trigger alone.[271,272] APS antibodies have also been shown to directly interact with endothelial cells in animal models causing endothelial dysfunction.[273] It is not clear why a small subset of APS patients (<1%) develop widespread microvascular thrombi as opposed to more focal insults. A recent study identified evidence of increased complement activation in patients with CAPS compared to APS and SLE, with clear statistical significance despite a low number of 7 total CAPS patients.[274] Additionally, in this CAPS cohort, there was a high incidence of rare germline variants in complement regulatory genes. This study assessed the anti-B2-glycoprotein-I aPL from 2 of the CAPSs patients and found that these aPLs induced

complement activation through the classical complement pathway.[274] Due to the rarity of CAPS, an international registry was established and has published demographic, clinical and serologic data from 500 CAPS patients.[275] The main identified triggers of CAPS are also inflammatory in nature: infection (47%), malignancy (18%), and surgery (17%). Forty percent of patients had an underlying rheumatic disease, 75% of those having the diagnosis of systemic lupus erythematous. These younger patients with underlying SLE experienced the highest mortality approaching 50%.[275] Especially in SLE, the classification criteria of CAPS may overlap with features of secondary TTP.[130] Recommended treatment for primary CAPS includes "triple therapy" with heparin, high-dose steroids and TPE or intravenous immunoglobulin (IVIG)[275] and typically also includes aggressive treatment of triggering conditions such as rheumatic disease.[268] The ASFA strongly recommends TPE with a level I endorsement that is supported by grade 2C evidence.[126] Additionally, rituximab has shown potential benefit in a subgroup analysis of the CAPS registry.[276]

Heparin-induced thrombocytopenia and thrombosis. HITT (Type 2 HIT) is an immune-mediated, medication-induced prothrombotic disorder characterized by development of auto-antibodies against platelet factor 4 (PF4) and heparin complexes. While HITT is associated with large vessel thrombosis, microvascular thrombosis can occur in addition or isolation. Microvascular thrombosis in HITT has been implicated in skin lesions and surgical flap failure.[277,278] These lesions may occur without thrombocytopenia.[278] It is important for clinicians to be aware of microvascular sequalae for timely diagnosis of HITT. If intermediate or high probability of the clinically based 4T score[279] or clinical concern for microvascular complication, further testing with enzyme linked immunosorbent assay for PF4-heparin complex and/or a functional assay such as serotonin release assay is recommended for further confirmation.[280] The HIT antibody binds to platelets via FcγRIIa receptors with resultant activation of tyrosine kinases including spleen tyrosine kinase (Syk) which results in platelet aggregation, release of procoagulant microparticles and granules.[281-285] In addition, HIT antibodies can induce expression of TF in monocytes,[284] activate neutrophils and promote formation of NETs which independently adds to the prothrombotic milieu.[286,287] Treatment is primarily focused on avoidance of all heparin products and preventing and/or treating thrombosis with pharmaceuticals including direct thrombin inhibitors (such as bivalirudin and argatroban), danaparoids, fondaparinux, or rivaroxaban.[280] Anticoagulation with warfarin is usually not recommended prior to platelet recovery. The alternative anticoagulation is often continued at least until platelet recovery (in some cases up to 4 weeks) in those without thrombosis and up to 3−6 months for those with thrombosis, although optimal duration is unknown.[280,288] With growing insight into the pathogenesis of disease, adjunctive treatments are being studied and considered. IVIG has been reported to be effective in selected refractory cases of HIT[289] and for prophylaxis in patients planned for heparin re-exposure.[290] It is believed that IVIG, through competition, prevents binding of HIT antibodies to FcγRIIa receptors,[289] a necessary step for platelet activation. TPE can be an adjunctive treatment in patients not responsive to initial therapy or need to undergo emergent surgery.[126] TPE removes the activating alloantibodies against PF4-heparin complex.[291] Studies inhibiting the Syk pathway have shown promise with decreased platelet aggregation and thrombosis, but to date this finding has not been confirmed in clinical trials.[283,284] PAD4 inhibition with GSK484 to prevent NETosis have also demonstrated reduction in thrombosis, however, have not been studied in major clinical trials.[286] For a broader discussion of HITT, readers are referred to the accompanying review on this topic in this journal.[292]

Sickle cell disease. Sickle cell disease (SCD) is a group of inherited hemolytic anemias arising from mutation in β-globin gene resulting in an abnormal hemoglobin tetramer HbS.[293,294] The most frequent cause of hospitalization in SCD are vaso-occlusive crises (VOCs),[295] which result from a complex interplay of red cells, endothelial cells (ECs), platelets and leukocytes in the microcirculation that cause characteristic bone pain.[294] Other common microvascular events includes acute chest syndrome, pulmonary hypertension and chronic kidney disease.[294] Under hypoxia, intraerythrocytic Hb S polymerize to form elongated fibers, which results in changes in cell rigidity, membrane distortion, and hemolysis.[296] Sickling in RBCs is not enough to initiate vaso-occlusion and stasis. The sentinel event is thought to be a multi-cellular adhesion between sickle RBCs, endothelial cells, and leukocytes.[297] Sickle RBCs have increased cell rigidity and viscosity due to hemoglobin polymerization as well as cytoskeleton remodeling which increases resistance to blood flow.[298] This slows the transit time of the sickle RBCs in the micro-circulation such as postcapillary venules and allows them to adhere to endothelium and leukocytes.[299] The time for contact between sickle RBCs and the endothelium seems more important than high-affinity receptor−ligand interactions.[300] Ex vivo data reveal that the nondeformable (more rigid, not irreversibly sickled RBCs) sickle RBCs tend to adhere more readily to fibronectin (a mimic for vascular

endothelium) due to an increase in adhesion molecules from RBC damage resulting from hemoglobin polymerization.[301]

Sickle RBCs can contribute to coagulation activation in several ways. Sickling-related membrane damage exposes phosphatidylserine and other adhesion molecules such as intercellular-adhesion-molecule-4,[302] which contribute to thrombogenesis. Free heme and hemoglobin released from the damaged RBCs can activate platelets, activate neutrophils induce NETs and endothelium dysfunction which promote hypercoagulability.[303] In addition, intravascular hemolysis can directly injure the vessel wall, leading to progressive vessel wall thickness, and anemia from hemolysis can raise systolic blood pressure and increase the risk of organ dysfunction such as stroke.[304] Subsequently, ECs upregulate expression of adhesion molecules such as E- and P-selection which increases recruitment of leukocytes to the site of hemolysis.[297,305-308] A cascade of reactions ultimately result in activation of platelets,[309] leukocytes,[310] and coagulation cascade.[311] Free heme also promotes NETosis,[312] IL-8 production,[313] and inflammasome activation.[314] The activated neutrophils and monocytes can form heterocellular aggregates with platelets and RBCs thus furthering ischemia, vessel occlusion and VOC.[306] In SCD, the composition of large and small vessel thrombosis differs by location. Autopsy studies in patients with pulmonary emboli show that fresh large and small arterial vessel thrombosis are mostly composed of sickle RBCs, fibrin, and CD45+ inflammatory cells.[315] However, in patients with stroke, thrombus was mostly composed of platelets, fibrin, and sickle RBCs.[316] The composition of microthrombi also differ by location. Microthrombi are thought to form from stasis induced by microvascular occlusion and coagulation activation. In one murine model of SCD, vaso-occlusion in the cremaster microcirculation was dependent on sickle RBCs, neutrophils, and endothelium interactions.[297] In another study, vaso-occlusion in precapillary pulmonary arterioles was mediated by platelet-neutrophil-rich microthrombi.[317] Last, in mucosal-intestinal microcirculation, sickle RBCS directly adhere to endothelium resulting in micro-occlusions in postcapillary venules.[307] The difference in contribution of various cellular components may be dependent on local environment such as the presence of high oxygen concentration in lungs. There is no biomarker for VOC in SCD, so the diagnosis relies on patient-reported pain.[295] Based on data from surveys, there are often misunderstandings in physicians of the risks of opioid addiction in SCD, which may lead to undertreatment of severe pain.[318] Hydroxyurea is helpful in preventing crises; the proposed mechanism is via an increase in fetal hemoglobin which decreases HbS's tendency to polymerize. In addition, hydroxyurea influences neutrophil count, adhesiveness and also promotes NO balance.[319] Other FDA approved drugs include L-glutamine, which reduces oxidative stress, ROS and inflammasome activation[320], crizanlizumab, a P-selectin inhibitor[320], and voxelotor, which reduces HbS polymerization.[321] Other emerging therapies such as an E-selectin inhibitor (rivipansel), and inhibitors of RBC-endothelial adhesion (MEK inhibitors) have shown promise.[322,323] Although drugs targeting IL-1β and IL-1R are already approved for rheumatoid arthritis, their role in SCD is yet to be studied.[294] HSCT is currently the only curative option for select group of patients,[324] however, preliminary results are promising for gene therapy.[325]

Degos disease. Systemic malignant atrophic papulosis, also known as Degos disease, is an extremely rare acquired condition, which can present with an intriguing level of microvascular occlusion relative to the level of systemic response, and thus we selected to include it in this review. It is characterized by amyloid-rich microvascular thromboses that accumulate in the brain, skin, and gastro-intestinal tract.[326] The age of onset is typically in adulthood but there are several cases described in children as young as 4 years old.[327] The etiology is unknown but histological analysis have found complement deposition and increased interferon alpha signal in the surrounding microvasculature.[328] However, it is unclear if complement and interferon are present in response to an unidentified insult or are causing the microvasculature damage. There may not be a significant systemic inflammatory response to these severe insults. This condition is also associated with the loss of endothelial progenitor cells, although the method of depletion (lack of production verses destruction) is unknown.[328] It does not appear to be clear autoimmune destruction since patients with Degos disease do not respond to broad immunosuppression including steroids. Based primarily on individual case reports, treatment strategies that have been reported include eculizumab to target the complement deposition and treprostinil to increase the supply of endothelial progenitor cells.[329] However, the role of these therapeutic approaches on disease progression is unclear. The study of rare diseases such as Degos may lead to a new understanding of relevant pathways in common diseases that are typically obscured by other features.

COVID-19. There has been a great deal of interest in understanding the unique pathophysiology of COVID-19, the disease caused by the novel coronavirus SARS-CoV-2. While some patients present with minimal or no symptoms, others develop a devastating acute
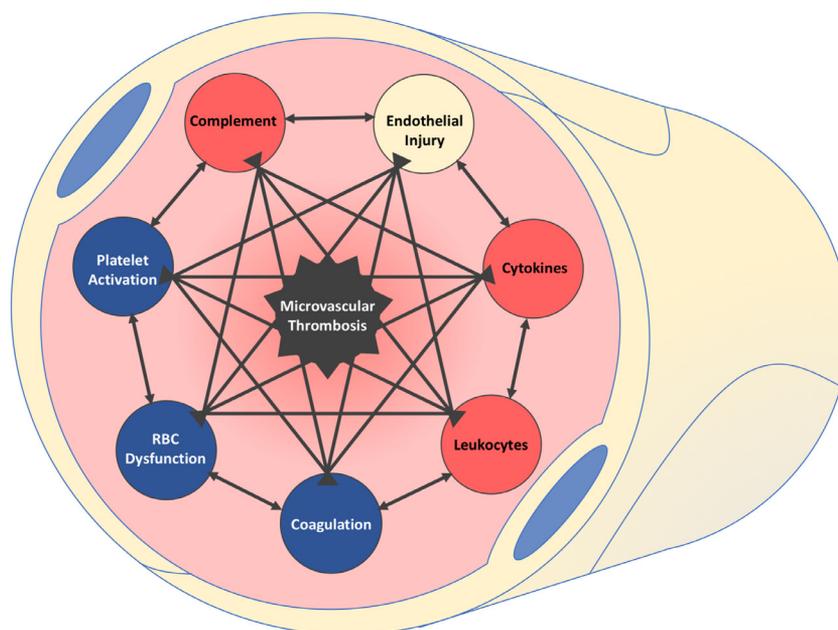
Fig 4. Schematic of inter-related pathways that contribute to the clinical conditions associated with microvascular thrombosis discussed in this review; please see the body of the manuscript for details of each condition. The pathways depicted in red circles are traditionally viewed in the context of inflammation, while those depicted in blue circles are traditionally viewed in the context of coagulation/thrombosis.

respiratory distress syndrome and death. Initial observations of high levels of inflammation led to investigations of immunomodulatory medications for potential treatments.[330] More recent observations of an increased frequency of large vessel thrombosis has led to concern that the classically thrombotic pathways are also highly activated.[331] Histological examination of postmortem tissue of 5 patients revealed evidence of microvascular thrombosis in skin and lung. Samples from 2 patients with acute respiratory distress syndrome in that study showed severe capillary damage with intra-alveolar fibrin deposition and relative absence of diffuse alveolar damage consistent with pulmonary microvascular thrombosis. These findings were accompanied by terminal complement deposition evidenced by C5b-9 in the pulmonary microvasculature. Skin biopsies of 3 different patients with vasculopathic-appearing rashes revealed a similar pattern of microvascular occlusion without frank vasculitis.[332] Another autopsy study revealed a more classic appearance of diffuse alveolar damage and hyaline membrane formation without striking microvascular involvement.[333] COVID-19 manifestations further demonstrate the connection between inflammation and thrombosis in the microvasculature. The mechanisms responsible for microvascular thrombosis in COVID-19 are unclear; one intriguing possibility involves NETs. A recent study on 50 patients with COVID-19 demonstrated significant increases in serum of markers of NET release, including cell-free DNA, myeloperoxidase, and citrullinated histone H3.[334] Further studies in patients with COVID-19 are needed to define the nature of microvascular dysfunction and thrombosis in this condition.

CONCLUSION

The diseases characterized by microvascular thrombosis span a wide variety of medical specialties. In many conditions outlined in this review, microvascular thrombosis results from uncontrolled activation of endogenous pathways aimed at protecting the host (Fig 4). These powerful pathways require rigorous regulation to ensure they are unharnessed at the appropriate time, site, and duration; otherwise they may become extremely dangerous to the organism they are meant to protect. Despite the close links and common evolutionary origin of inflammation and thrombosis (as exemplified by the horseshoe crab), these pathways are often viewed separately in medical education and also through the historical separation of clinical disciplines (ie, hematology, immunology, rheumatology, infectious disease, etc.). It is helpful to conceptualize inflammation and thrombosis as interconnected not only from a basic science perspective but also from a clinical perspective. A greater understanding of the nature of dysregulation of these inter-related pathways in disease

is expected to help identify optimal targets for therapeutic intervention. Historically, the pattern of medicine involves describing a broad category of disease based on clinical similarities, which is then further subdivided into categories as different pathogeneses are discovered. This trend towards increased clinical resolution of pathogeneses could continue to an extreme where pathway dysregulation replaces the concept of diagnosis. As the future continues to trend toward personalized medicine, the ability to precisely define the nature of the dysregulation of these pathways in individual patients will help determine specific treatment dosing and duration to push the microvascular environment back into a self-regulating state.

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