Genesis of Endotheliopathy

Subjects: Health Care Sciences & Services | Hematology

Contributor: Jae C. Chang

Endotheliopathy, according to the "two-activation theory of the endothelium", is triggered by the activated complement system in critical illnesses, such as sepsis, diabetes and polytrauma, leading to two distinctly different molecular dysfunctions: (1) the activation of the inflammatory pathway due to the release of inflammatory cytokines, such as interleukins, interferons and tumor necrosis factors, and (2) the activation of the microthrombotic pathway due to the exocytosis of hemostatic factors, including ultra-large von Willebrand factor (ULVWF) multimers and FVIII. These lead to inflammation and microthrombogenesis. The former produces inflammatory diseases, and the latter produces endotheliopathy-associated vascular microthrombotic disease (EA-VMTD), which orchestrates not only TTP-like syndrome characterized by the triad of consumptive thrombocytopenia, microangiopathic hemolytic anemia and multiorgan dysfunction syndrome, but also many other endotheliopathic syndromes. The diagnostic features of EA-VMTD are well established now and therapeutic strategies are being formulated.

microthrombogenesis   endotheliopathy   inflammation   microthrombosis   hemostasis

consumptive thrombocytopeenia   endotheliopathy-associated vascular microthrombotic diseasew

TTP-like syndrome   ITP-like syndrome   ADAMTS13

1. Introduction

Endotheliopathy is a very common vascular disorder that develops due to functional and/or anatomical changes in the endothelium, which results in serious molecular responses that produce a variety of human diseases. This condition is triggered by the activated complement system secondary to external insults, such as the toxin of pathogen and polytrauma, or to internal physiologic changes, such as pregnancy and autoimmune disease [1][2][3]. It promotes two endothelial molecular pathways: inflammatory and microthrombotic [4][5][6][7][8]. Both inflammation and microthrombosis occur commonly in sepsis and critical illnesses [4][5][6][7][8][9][10] due to the released molecules from endotheliopathy caused by the toxin of pathogen, drug, poison, chemical, vaccine, or venom, and from functional changes after surgery, transplant, or polytrauma [5][6]. Inflammation and disseminated vascular microthrombotic disease (VMTD) often simultaneously occur, and they cause a variety of phenotypes of clinical pathology. The endothelial damage provokes major molecular and biological events, and it produces a spectrum of clinical disorders, from focal, multifocal, localized, and disseminated disease to multisystem inflammatory syndrome (MOIS) and multiorgan dysfunction syndrome (MODS), not only due to the cellular response and hemostatic activation, but also due to the delayed autoimmune response [11][12][13][14].

Until recently, both the pathophysiological mechanisms of inflammation and microthrombosis had not been clearly understood. Because of the concurrent occurrence of inflammation and thrombosis [15][16], the hypothesis of immunothrombosis, which supports the cross-talk between inflammation and thrombosis, was introduced as a newly proposed hemostatic mechanism to expand the concept of macrothrombosis based on the molecular event of neutrophil extracellular traps (NETs) [17][18][19]. Immunothrombosis was thought to be the host defense mechanism to limit the systemic spreading of the pathogen in sepsis [18]. However, despite extensive research efforts to find an interaction between inflammation and thrombosis, no persuasive evidence supporting the "cross-talk theory" has been materialized. Furthermore, during the current COVID-19 pandemic, the concept of the previously defined disseminated intravascular coagulation ("DIC"), which was thought to play a critical role in coagulation dysfunction, was proven to be incorrect because disseminated intravascular "microthrombosis" is confirmed to be the true pathology of sepsis-associated coagulopathy [6][8][10][20].

According to recently proposed "two-activation theory of the endothelium", depicted in **Figure 1**, endotheliopathy leads to the activation of two molecular pathways, "inflammatory" and "microthrombotic", promoting inflammation and microthrombosis, respectively [4][5][6]. This theory delineates that two pathways are completely independent, but are concurrent events that promote two different molecular pathogeneses. Additionally, the "two-path unifying theory" of hemostasis in vivo (**Figure 2**), the genesis of microthrombosis (i.e., VMTD), was established to be the result of partial hemostasis triggered by ultra-large von Willebrand factor (ULVWF) path of hemostasis. In disseminated endotheliopathy, inflammation and microthrombogenesis often coexist, but they each promote separate pathogeneses, producing many distinctly different and combined clinical phenotypes [6][10].
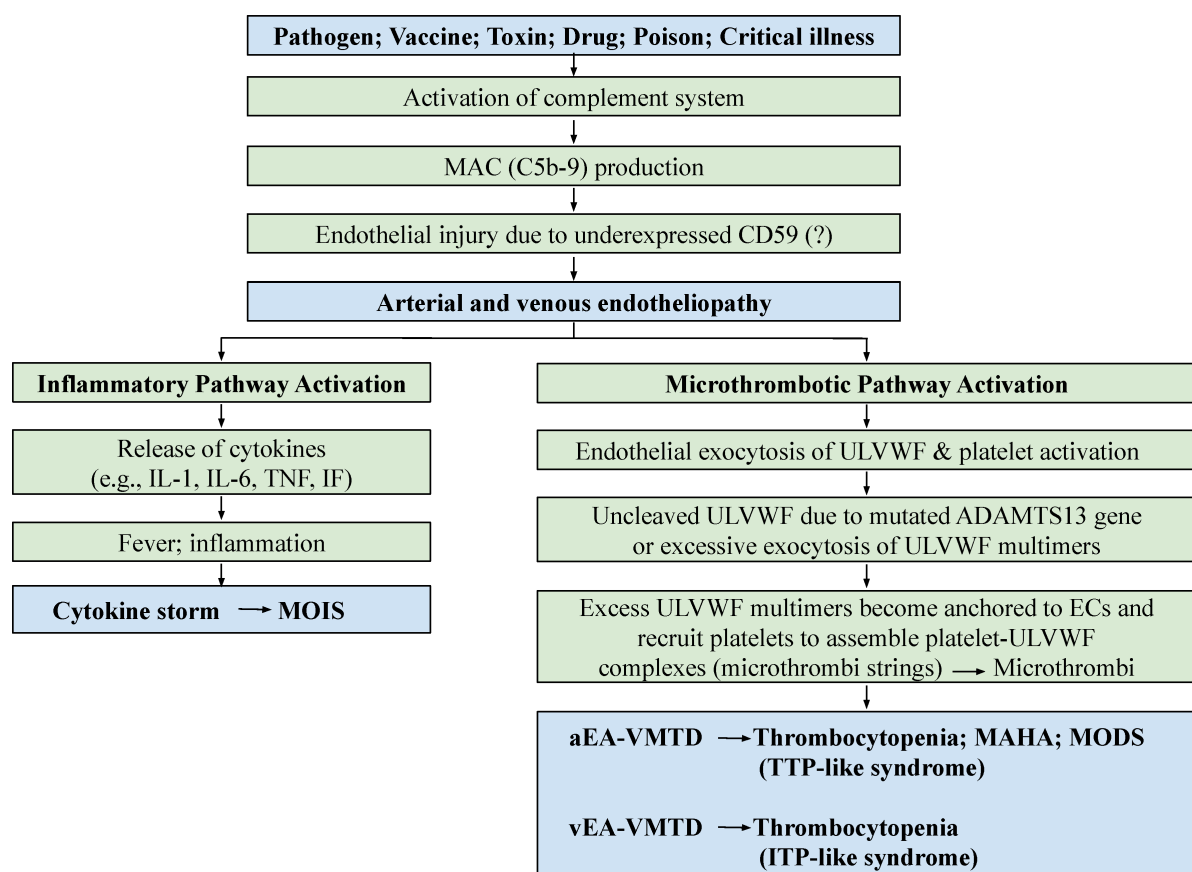
Figure 1. Pathogenesis of endotheliopathy based on "two-activation theory of the endothelium". Endothelial molecular pathogenesis is initiated by the activated complement system following exposure to a pathogen, toxin, drug, poison, venom, vaccine, polytrauma, hyperglycemia, severe hypertension, and others. Endotheliopathy releases inflammatory cytokines and hemostatic factors, and activates two clinically important pathways: inflammatory and microthrombotic. Both arterial endotheliopathy and venous endotheliopathy provoke inflammation via the inflammatory pathway, leading to inflammatory syndrome, such as MOIS due to cytokines, but arterial endotheliopathy promotes microthrombosis via the microthrombotic pathway and produces aEA-VMTD due to the activation of the ULVWF path of hemostasis. Arterial endotheliopathy is characterized by the triad of thrombocytopenia, MAHA, and MODS, which is called "TTP-like syndrome", but venous endotheliopathy is characterized by ITP/"ITP-like syndrome" due to silent microthrombi with consumptive thrombocytopenia, as explained in the text. The distinguishing features are caused by the different anatomies, physiological functions, and hemodynamic characteristics between arterial system and venous system. These are very important pathophysiological features in the understanding of the complexity in variable phenotypes amongst the endotheliopathic syndromes. Abbreviations: EA-VMTD: endotheliopathy-associated vascular microthrombotic disease; ITP: immune thrombocytopenic purpura; MAC: membrane attack complex; IF: interferon; IL: interleukin; MAHA: microangiopathic hemolytic anemia; MODS: multiorgan dysfunction syndrome; MOIS: multiorgan inflammatory syndrome; TNF: tumor necrosis factor; TTP: thrombotic thrombocytopenic purpura; aEA-VMTD: arterial EA-VMTD; vEA-VMTD: venous EA-VMTD; ULVWF: ultra-large von Willebrand factor.
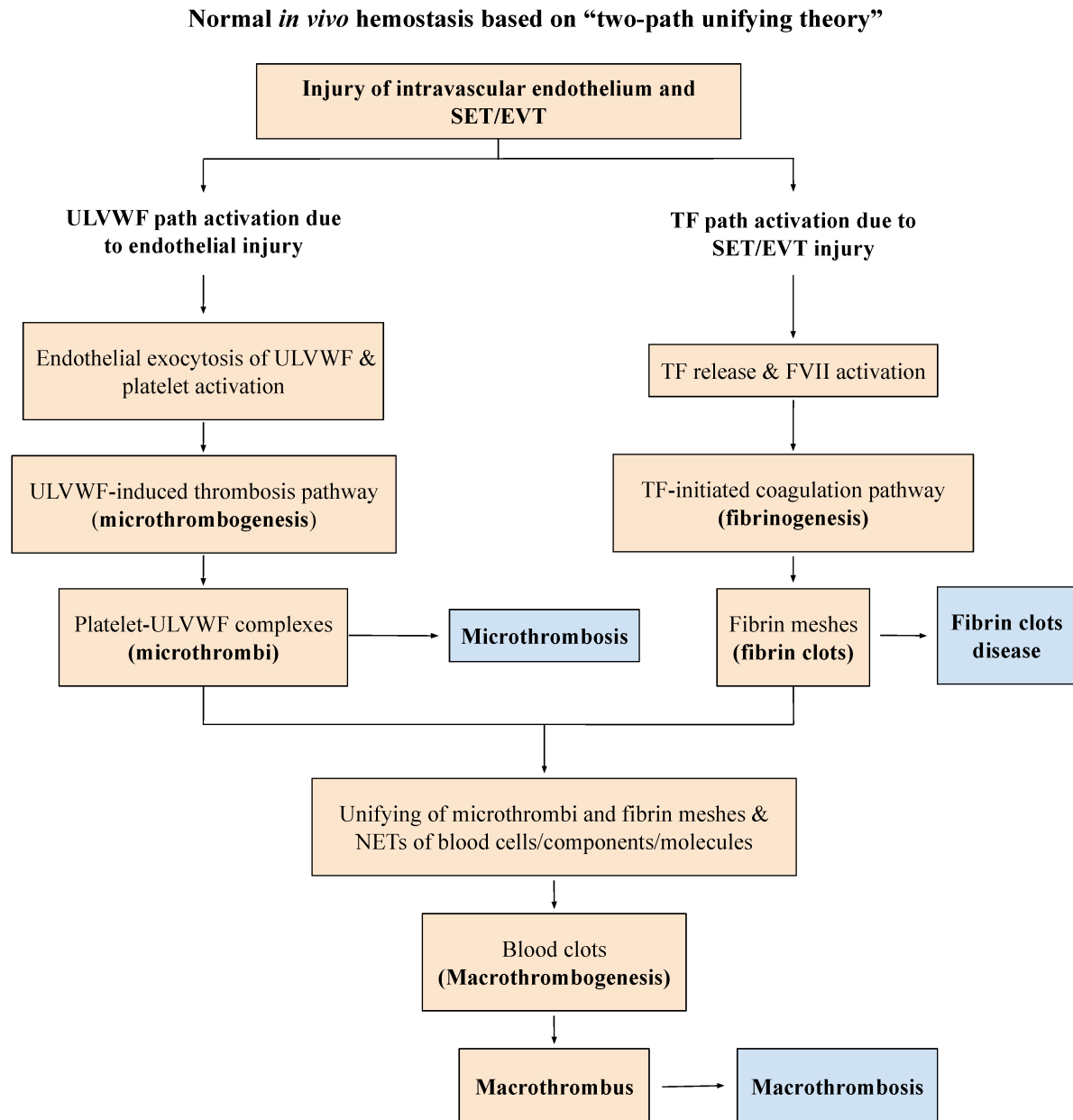
Normal *in vivo* hemostasis based on "two-path unifying theory"

Figure 2. Normal in hemostasis in vivo based on "two-path unifying theory" (reproduced and modified with permission from the Chang JC. Thrombosis Journal. 2019;17:10). Following a vascular injury, the hemostatic system in vivo activates two independent sub-hemostatic paths: microthrombotic (ULVWF path) and fibrinogenetic (TF path). Both are initiated by the damage of ECs and SET/EVT due to external bodily injury and traumatic intravascular injury. In activated ULVWF path from EC damage, ULVWF multimers are released and recruit platelets, which produce microthrombi strings via microthrombogenesis, but in activated TF path from SET/EVT damage, released TF activates FVII. The TF–FVIIa complexes produce fibrin meshes/fibrin clots via fibrinogenesis of the extrinsic coagulation cascade. The final path of in vivo hemostasis is macrothrombogenesis, in which microthrombi strings and fibrin meshes become unified together with incorporation of NETs, including red blood cells, neutrophils, DNAs, and histones. This unifying event, "macrothrombogenesis", promotes the hemostatic plug and wound healing in external bodily injury, and produces macrothrombosis in intravascular injury. Abbreviations: EA-VMTD: endotheliopathy-associated vascular microthrombotic disease: ECs: endothelial cells; EVT:

extravascular tissue; NETs: neutrophil extracellular traps; SET: subendothelial tissue; TF: tissue factor; ULVWF: ultra-large von Willebrand factor multimers.

2. Genesis of Endotheliopathy

The endothelium is a unique single layer of cells lining between the blood in circulation and the subendothelial tissue of every organ to protect the body from the intrusion of harmful external toxic molecules and microorganisms. It also provides the direct and indirect contact of nutrients and metabolic components to the subendothelial and extravascular tissues to sustain life, and it is the barrier to and partner with the blood cells to maintain hemostasis and physiologic homeostasis. Therefore, an insult causing endothelial damage disrupts the normal anatomy and physiologic function. The endothelium may lead to diversified paths of the pathogenesis that trigger the dysfunction of the cells, tissues, organs, and multisystem. Until now, clinical medicine has designated most human diseases with terms that define and appropriate the dysfunction of the cell (e.g., microangiopathic hemolytic anemia), tissue (e.g., rhabdomyolysis), organ (e.g., acute kidney failure), and multisystem (e.g., systemic lupus erythematosus). However, clinical medicine has not been able to put together the etiologic identities and pathogeneses of many clinical syndromes in relation to molecular, metabolic, and structural alteration. This paucity of knowledge in endotheliopathy has also entailed the utilization of imprecise terms for diagnosis, such as Kawasaki disease, acute respiratory distress syndrome (ARDS), multisystem inflammatory syndrome in child (MIS-C), thromboangiitis obliterans (e.g., Buerger's disease), cerebral venous sinus thrombosis (CVST), DIC-like coagulopathy, limb gangrene, and others, to define the clinical syndromes that have been occurring during the COVID-19 pandemic and in other diseases [10].

2.1. Causes of Endotheliopathy

Endotheliopathy is not a distinctive disease that delineates the dysfunction of a specific cell type, tissue, organ, or system, but an intermediary pathology that leads to almost every human disease involving the vascular-tree system. To date, the accepted medical tenet has been that endothelial damage results in a transient endothelial intravascular injury, typically due to localized vascular pathology, and often with limited clinical consequence. However, now it is abundantly clear that "endotheliopathy" could become a major pathology that produces life-threatening cellular, tissue, and organ damage, leading to their dysfunction, and even to multisystem disease [4][5][6][7][8][9][10]. The endothelium is the most important structure protecting lives, in which the pathology of many human diseases could originate or be influenced. Its integrity often determines the clinical outcomes of critical illnesses.

2.1.1. Complement System

Endotheliopathy is triggered by the activated complement system in response to pathogens and other insults [3][6][21]. Complement activation can occur through one of three different pathways (i.e., classical, alternate, and lectin). The complement system can not only be activated by an external insult, such as a pathogen, toxin or drug, vaccine, venom, surgery, transplant, or polytrauma, but also by internal physiologic changes, such as pregnancy, hypertension, hyperglycemia, as well as the autoimmune process [5][6][22][23].

The lytic function of the complement membrane attack complex (MAC: C5b-9) serves as an important defense against bacteria, viruses, and parasites, and the genetic deficiency of the terminal complement proteins C5 through C9 predisposes the host to recurrent infections [24]. The innate immune response of complement activation is not only the first step in protecting the host against a variety of pathogens, but it may also play a detrimental role in innocent bystander endothelial cells (ECs). Should the unchecked production of MAC be deposited to the endothelial membrane, channel formation could be formed and lead to endothelial dysfunction in the host [3]. When MAC provokes either structural or functional endothelial damage in the host, it activates at least two unique molecular mechanisms, which promote clinically significant endotheliopathic syndromes [4][5][6][7].

2.1.2. Endothelial Molecular Pathogenesis

The molecular pathogenesis of endotheliopathy is elaborated in the "two-activation theory of the endothelium", which shows complement-induced endothelial molecular events (**Figure 1**), leading to the activation of: (1) the inflammatory pathway, and (2) the microthrombotic pathway [4]. MAC-induced endotheliopathy is suspected to occur if the endothelium is "unprotected" due to downregulated CD59 [25][26].

Both functional and structural injuries of the endothelium release several mediators altering homeostasis and activate a host of biomolecules expressed in ECs, which can promote, directly and indirectly, the creation of different pathologic and clinical phenotypes. The numerous biomolecules that are released from ECs include inflammatory cytokines, hemostatic factors, and adhesive molecules, which are interleukins (IL), tumor necrosis factors (TNF), interferons (IFN), coagulation factors, tissue plasminogen activator, prostacyclin, nitric oxide, and others [6][7][27]. Although their roles in vascular disease have not been well defined yet, the pathogenesis of inflammation is postulated to be due to released cytokines and chemokines, such as interleukins IL-1, IL-2, IL-6, TNF, IFN, and others, and microthrombogenesis is promoted by ULVWF multimers and hemostatic factors, resulting from endotheliopathy [5][6][7][8][9][10][28]. These molecular pathogeneses were predicted before the onset of the COVID-19 pandemic [6][7], and important molecular events have been unequivocally confirmed during the pandemic [29][30].

The mechanism of molecular pathogenesis in disseminated arterial and venous endotheliopathy based on the "two-activation theory of the endothelium" is shown in **Figure 1**. On the one hand, the activated inflammatory pathway provokes inflammation due to released inflammatory cytokines in sepsis, or other critical illnesses. On the other hand, the activated microthrombotic pathway triggers the excessive release of ULVWF/FVIII from endothelial exocytosis [5][6]. Should a relative insufficiency of ADAMTS13 be present due to the heterozygous gene mutation or polymorphism of the gene, and/or due to the excess release of ULVWF multimers, partial hemostasis of lone ULVWF path becomes activated and ULVWF attract platelets, which leads to the formation of "microthrombi strings" composed of platelet–ULVWF complexes [5][6]. This process is called microthrombogenesis, and leads to endotheliopathy-associated disseminated vascular microthrombotic disease (EA-VMTD) [5][6][11].

This partial hemostasis due to lone activation of the ULVWF path is characterized by consumptive thrombocytopenia, overexpressed ULVWF/VWF/VWF antigen and increased activity of FVIII due to their

endothelial release, resulting in relative ADAMTS13 insufficiency. Endotheliopathy is systemic in sepsis and disease due to certain drugs or toxins, and it is clinically disseminated in the microvasculature, promoting "microvascular microthrombosis" (i.e., EA-VMTD). In the past, the "coagulopathy" occurring in the septic patient was thought to be due to the overactivation of the tissue factor (TF) path leading to systemic "fibrin clots" formation, and had been called "DIC". However, its pathogenesis has never been clearly established. Based on the two novel hemostatic theories, "DIC" has been identified as a form of thrombosis, which is exclusively due to "microthrombosis" caused by the activated ULVWF path that develops from the injury of ECs [5][6][8][20]. The conflict in the terms between "coagulation" and "thrombosis" related to "hemostasis" has contributed to the misunderstanding of the pathogenesis of "DIC", and of the identification of the pathophysiological mechanism of hemostasis in vivo. Thus, in the past, the true meaning and genesis of "fibrin clots" and "microthrombi" could not been conceptualized, which contributed to uncomfortable debates between coagulopathy and thrombosis (thrombopathy) in the coagulation community. To clarify the different meanings of their conceptual terms, I have briefly summarized the discriminating interpretations of the terms in **Table 1**.

Table 1. Conceptual understanding of the terms: hemostasis, coagulation, and thrombosis.

	Hemostasis	**Coagulation**	**Thrombosis**
Term concept	Philosophical	Physiological	Structural
Implied meaning	Natural process in vivo	Artificial process in vitro Physiologic process at bleeding site	Pathological process in vivo
Involved site	Blood-vessel wall	Test tube Extravascular trauma to vessel wall	Intravascular lumen
Products	Hemostatic plug	Fibrin mesh/fibrin clot *	Microthrombi/macrothrombi
Critical role	Vascular-wall injury	Coagulation test Hemorrhage	Physiologic thrombogenesis
Components	Endothelium SET EVT Blood in circulation	TF/thromboplastin Coagulation proteins/serine proteases	ULVWF/FVIII from ECs Platelets$^+$ from circulation Coagulation proteins/serine proteases TF from SET/EVT
Phenotypes	Determined by the level of vascular damage	Determined by participating coagulation factors	Determined by ULVWF, platelets, TF, hemostatic factors, and unifying mechanism
Inciting example	Endotheliopathy Vascular injury	Coagulation tests for PT and aPTT	VMTD/MODS due to microthrombosis EA-VMTD: TTP-like syndrome: "DIC"

Hemostasis	Coagulation	Thrombosis
		Arterial macrothrombosis
		VTE
		Combined micro–macrothrombosis

Abbreviations: "DIC": false disseminated intravascular coagulation; DVT: deep vein thrombosis; ECs: endothelial cells; EVT: extravascular tissue; EA-VMTD: endotheliopathy-associated vascular microthrombotic disease; MODS: multiorgan dysfunction syndrome; PT: prothrombin time; aPTT: activated partial thromboplastin time; SET: subendothelial tissue; TF: tissue factor; TTP: thrombotic thrombocytopenic purpura; ULVWF: ultra-large von Willebrand factor multimers; VMTD: vascular microthrombotic disease; VTE: venous thromboembolism. * The only coagulation disorder in classic hemostasis due to EVT damage in which fibrin clots are formed without platelets.

...esses are reactive changes in response to inflammation promoting the "hypercoagulable state" of "DIC". This interpretation has led to a conceptually and structurally wrong conclusion on the nature of microthrombi because the increased expressions of FVIII and VWF are always the result of endothelial exocytosis from endotheliopathy, which is perfectly consistent with vascular injury limited to ECs producing "microthrombi strings", as shown in the "two-path unifying theory" of hemostasis (**Figure 2**) [11][12].

2.2. Genesis of Clinical Phenotypes in Endotheliopathy

Endotheliopathy, even generalized, only activates lone ULVWF path of hemostasis because the complement-activated endothelial damage that releases biomolecules due to the pathogen toxin is limited to ECs, without affecting the subendothelial tissue (SET) and extravascular tissue (EVT) [6]. Disseminated microthrombosis is made of microthrombi strings in the microvasculature because SET/EVT damage that releases TF to the intravascular space does not occur, and fibrin clots are not produced to form complete blood clots (i.e., macrothrombosis), as presented in **Figure 2**.

In contrast, a bleeding intravascular injury that causes both local EC and SET/EVT damage typically occurs in local trauma following local vascular damage. An in-hospital vascular access/device, or indwelling central vascular catheter, which releases not only a small amount of ULVWF/FVIII from local ECs, but also releases sufficient TF from SET and EVT, produces macrothrombosis. The relationship between ULVWF release from ECs and TF release from ECs and SET/EVT from their respective damage is illustrated in **Figure 3**. In sepsis, endotheliopathy is disseminated even though its extent and phenotype expression are dependent upon endothelial heterogeneity and the tropism of the pathogen. When microthrombogenesis is triggered, disseminated microthrombosis causes clinical phenotypes by affecting different organs and producing organ dysfunction syndrome, including MODS. Furthermore, more complex phenotypes of microthrombosis can occur depending on: (1) the extent of the vascular-tree involvement (e.g., the multiplicity of the tissues and organs), (2) the localization of different vascular functional characters (e.g., arterial vs. venous system), and (3) an additional interacting mechanism with an underlying gene mutation or environmental factor (e.g., thrombophilia, such as FV Leiden, or protein C deficiency, and von Willebrand disease, pathogens, and toxins) [13]. All of these different phenotypes that occur in endotheliopathy-associated "focal", "multifocal", "localized", "regionalized", and "disseminated" microthrombosis are inclusively called EA-VMTD [5].
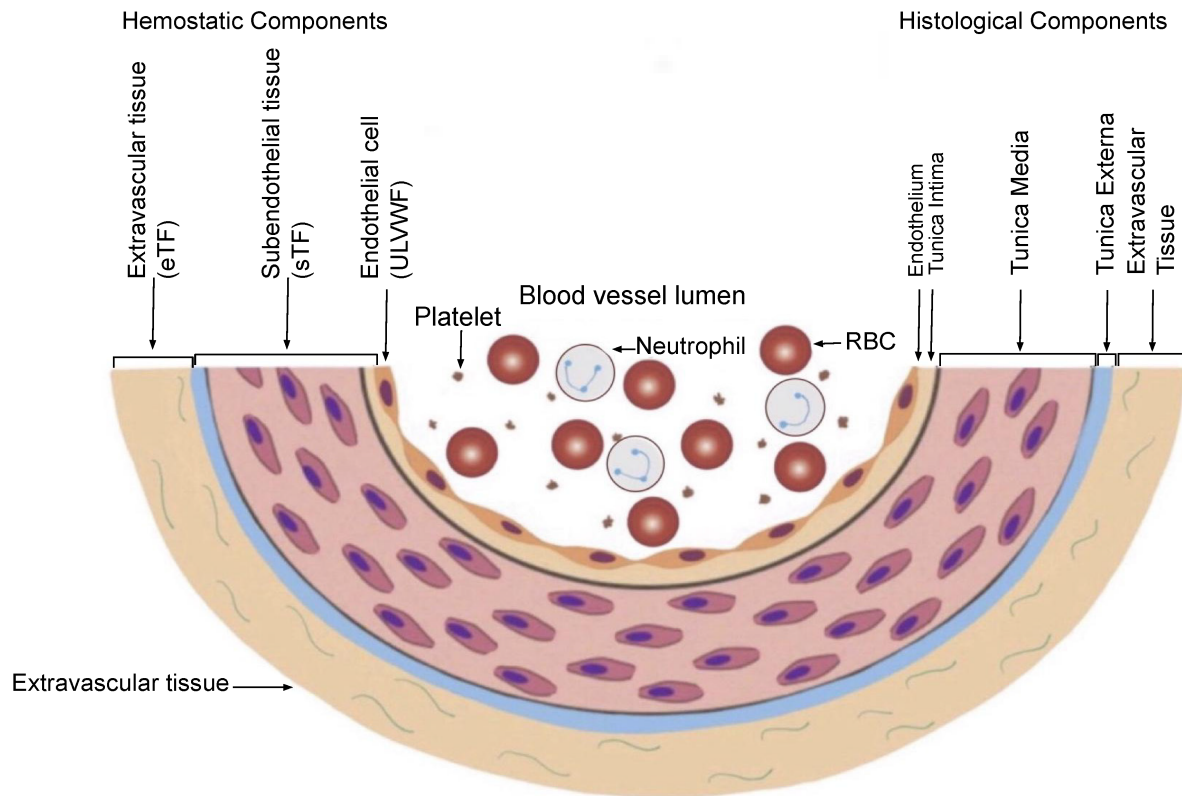
Figure 3. Schematic illustration of cross section of blood-vessel histology and hemostatic components (reproduced and modified with permission from Chang JC. *Clin Appl Thromb Hemost* 2019 January–December; 25:1076029619887437). The blood-vessel wall is the site of hemostasis (coagulation) in the external bodily injury at which blood clots (hemostatic plug) are produced and hemorrhage is stopped. It is also the site of hemostasis (thrombogenesis) in the intravascular injury in which intravascular blood clots (thrombus) are produced to cause thrombosis. Its histologic components are divided into the endothelium, tunica intima, tunica media, and tunica externa, and each component has its function that contributes to molecular hemostasis. As illustrated, EC damage triggers the exocytosis of ULVWF and SET damage, and it promotes the release of sTF from the tunica intima, tunica media, and tunica externa. EVT damage releases eTF from the outside of the blood-vessel wall. This depth of the blood-vessel-wall injury contributes to the genesis of different thrombotic disorders, such as microthrombosis, macrothrombosis, fibrin clots, thrombo-hemorrhagic clots, and various endotheliopathic syndromes. This concept based on the blood-vessel-wall model is especially important in the understanding of different phenotypes of stroke and heart attack. Abbreviations: EVT: extravascular tissue; eTF: extravascular tissue factor; SET: subendothelial tissue; sTF: subendothelial tissue factor; RBC: red blood cells; ULVWF: ultra-large von Willebrand factor.

2.2.1. Hemostasis Based on the Blood-Vessel Model

The concept of EA-VMTD cannot be understood without the comprehension of the hemostatic mechanism in vivo. However, hemostasis based on the blood-vessel model is simple and logical if the anatomy and physiology of the blood-vessel wall are understood, as illustrated in **Figure 3**, and the three fundamentals in hemostasis are applied to the thrombogenesis, as summarized in **Table 2**. The first and second principles illustrate how the molecular

event of hemostatic components is initiated in the vascular-wall injury, and the third principle elaborates the physiologic paths of hemostasis that produces the pathologic phenotypes following an intravascular injury [7].

Table 2. Three fundamentals in normal hemostasis.

(1) Hemostatic Principles
(1) Hemostasis can be activated only by vascular injury;
(2) Hemostasis must be activated through the ULVWF path and/or TF path;
(3) Hemostasis is the same process in both hemorrhage and thrombosis;
(4) Hemostasis is the same process in both arterial thrombosis and venous thrombosis;
(5) The level of vascular damage (endothelium/SET/EVT) determines the different clinical phenotypes of hemorrhagic disease and thrombosis.

(2) Major Participating Components		
Components	Origin	Mechanism
(1) ECs/SET/EVT	Blood-vessel wall/EVT	Protective barrier
(2) ULVWF	ECs	Endothelial exocytosis/anchoring and microthrombogenesis
(3) Platelets	Circulation	Adhesion to ULVWF strings/assembling and microthrombogenesis
(4) TF	SET and EVT	Release from tissue due to vascular injury/leading and fibrinogenesis
(5) Coagulation factors	Circulation	Activation of coagulation factors/forming and macrothrombogenesis
(3) Vascular Injury and Hemostatic Phenotypes		
Injury-Induced Damage	Involved Hemostatic Path	Level of Vascular Injury and Examples
(1) Endothelium	ULVWF	Level 1 damage—microthrombosis (e.g., TIA [focal]; Heyde's syndrome [local]; EA-VMTD [disseminated])
(2) Endothelium/SET	ULVWF + sTF	Level 2 damage—macrothrombosis (e.g., AIS; DVT; PTE; AA)

(3) Endothelium/SET/EVT	ULVWF + eTF	Level 3 damage—macrothrombosis with hemorrhage (e.g., THS; THMI)
(4) EVT alone	eTF	Level e damage—fibrin clot disease (e.g., AHS (e.g., SDH; EDH); ICH; organ/tissue hematoma)
Hemostatic Phenotypes	Causes	Genesis
(1) Hemorrhage	External bodily injury	Trauma-induced external bleeding (e.g., accident; assault; self-inflicted injury)
(2) Hematoma	Internal EVT injury	Obtuse trauma-induced bleeding (e.g., tissue and cavitary hematoma; hemarthrosis)
(3) Thrombosis	Intravascular injury	Intravascular injury (e.g., atherosclerosis; diabetes; indwelling venous catheter; surgery; vascular access)

The established dogma of Virchow's triad on thrombosis and thrombogenesis, which includes (1) endothelial injury, (2) the stasis of blood flow, and (3) hypercoagulability, is considered the essential three components initiating the formation of a thrombus [31]. Unfortunately, this conceptual misunderstanding of hemostasis, thrombosis, and coagulation has not only confused the concepts and uses of the terms, "coagulation" and "thrombosis", but it has also hampered the discovery process of the true hemostatic mechanism in vivo to date. This author has attempted to redefine the conceptual connotation of these terms, as shown in **Table 1**.

Abbreviations: AA: aortic aneurysm; AHS: acute hemorrhagic stroke; AIS: acute ischemic stroke; DVT: deep venous thrombosis; ECs: endothelial cells; EDH: epidural hematoma; EVT: extravascular tissue; ICH: intracerebral hemorrhage; PTE: pulmonary thromboembolism; SDH: subdural hematoma; SET: subendothelial tissue; TF: tissue factor; eTF: extravascular TF; sTF: subendothelial TF; ULVWF: ultra-large von Willebrand factor; THS: thrombo-hemorrhagic stroke; THMI: thrombo-hemorrhagic myocardial infarction; TIA: transient ischemic attack.

After the reinterpretation of "DIC" utilizing the differentiating concepts between "coagulation" and "thrombosis", and their implied meanings in relation to hemostasis in vivo and coagulation in vitro, the true identity of "DIC" was found to be "endotheliopathic microthrombosis" rather than the "uncontrolled" activation of coagulation in vitro or in vivo [8] [20]. In other words, "DIC" is an endothelial phenomenon caused by well-defined partial hemostatic process (i.e., ULVWF path) rather than vague in vivo coagulation disorder reflecting in vitro coagulation abnormalities. Thus, "DIC" has been newly defined as EA-VMTD, which explains not only microvascular microthrombosis (i.e., VMTD), but also confirms the molecular changes associated with the disease (i.e., thrombocytopenia, increased activity of FVIII, and overexpression of ULVWF/VWF antigen) [5][6][8][20]. Furthermore, this author was able to link EA-VMTD (microthrombosis of endotheliopathy) to "DIC" (microthrombosis of sepsis) and could separate the "fibrin clots" of fibrin clot disease (i.e., acute promyelocytic leukemia) from the "microthrombi strings" of "DIC", constructing two hemostatic paths in vivo based on the vascular-wall model after analyzing the following inexplicable hemostatic facts. Finally, identified are the true characters of "microthrombosis" and "fibrin clots" according to the "two-path unifying theory" of hemostasis [11][12].

- Microthrombi strings composed of platelet–ULVWF complexes [32][33];
- Documented endothelial exocytosis of ULVWF and FVIII in patient with sepsis and other endotheliopathy [34][35][36];
- The well-known role of ULVWF from ECs in the early phase of hemostasis in external bodily injury;
- Prominent interaction between platelets and ULVWF in a vascular injury, resulting in consumptive thrombocytopenia [37][38];
- The lack of participation of TF in hemostasis producing septic microthrombosis [4];
- TF unavailable in the EC injury but available from the SET/EVT in local bleeding vascular injury;

- Distinctly different disseminated microthrombosis occurring in the microvasculature in sepsis compared with localized macrothrombosis formed after fibrin clots occurring in a local large-vessel injury;
- The irrefutable hemostatic fundamental: "The hemostasis and thrombogenesis can be activated only by a vascular injury".

The three fundamentals in normal hemostasis are perfectly congruous with the "two-path unifying theory" [7]. Further, the above factual components demonstrate that it is impossible to correctly redefine both "microthrombosis" and "macrothrombosis" using the contemporary hemostatic theory which is based on sequential activation via the extrinsic coagulation cascade initiated by the TF–FVIIa complex alone, [11], as well as fibrin clot disease of acute promyelocytic leukemia (APL) [39]. The "two-path unifying theory" of hemostasis can explain every hemostatic phenotype that occurs in microthrombosis, macrothrombosis, and the unique fibrin clot disease. A true in vivo hemostatic theory should be inclusive of the following molecular and physiologic changes of thrombogenesis [6][10][13][14]:

- Damaged ECs from the blood-vessel wall following a vascular injury release ULVWF/FVIII in partnership, which are the essential components, along with platelets, in the formation of microthrombi strings. The process of ULVWF interacting with platelets is called "microthrombogenesis";
- Damaged SET from the blood-vessel wall following a local vascular injury releases TF into circulation, which activates FVIIa and leads to the formation of fibrin clots via the extrinsic cascade with the sequential activation of FIX, FX, FV, FII, and fibrinogen to fibrin. The process is called "fibrinogenesis";
- A local traumatic vascular injury involving ECs and SET produces both microthrombi strings and fibrin clots. The proposed theory is that the forming process of macrothrombosis must be the result of the "unifying mechanism of microthrombi strings and fibrin meshes". This process is called "macrothrombogenesis", in which neutrophil extracellular traps (NETosis) passively participate;
- The understanding of this unifying mechanism is very important in the understanding of arterial and venous combined micro–macrothrombosis, which is a process that should be called micro–macrothrombogenesis.

The above three basic physiological mechanisms of hemostasis in vivo, constructed in **Figure 2** and **Table 3**, can not only explain every hemorrhagic disease and thrombotic disorder, but it can help also interpreting additional complex hemostatic phenotypes in stroke, heart attack, venous thromboembolism (VTE), CVST, gangrene syndromes, and other tissue/organ-specific syndromes [10][40]. Because EC damage is disseminated without SET damage in sepsis, but EC and SET damages are deeper but limited to localized vascular trauma, the microthrombi strings causing microthrombosis must be the result of microthrombogenesis triggered by endotheliopathy, but macrothrombus unified of microthrombi strings and fibrin clots is the result of fibrinogenesis and macrothrombogenesis in locality (**Table 3**). Two hemostatic theories have been identified: (1) the "two-activation theory of the endothelium" to explain inflammation and microthrombosis (e.g., VMTD), and (2) the "two-path unifying theory" of hemostasis to explain the geneses of microthrombosis, fibrin clot disease, and macrothrombosis (e.g., DVT). The former is illustrated in **Figure 1**, and the latter in **Figure 2**. These two hemostatic theories are being applied in identifying the activated hemostatic mechanisms in the different phenotypes of hemostatic diseases. These mechanisms have correctly established the pathogeneses of many poorly defined thrombotic diseases, which include microthrombosis (e.g., septic endotheliopathy, ARDS, HUS, veno-occlusive disease (VOD)

[4][5][6][7][8][9][10], macrothrombosis (e.g., acute ischemic stroke, deep venous thrombosis) [10][12][40], arterial combined micro–macrothrombosis (e.g., symmetrical peripheral gangrene) [6][10], venous combined micro–macrothrombosis (e.g., VTE, CVST) [13][14], MODS [6], and ARDS, with complex forms of thrombosis during the COVID-19 pandemic [7][10], including the following endotheliopathic syndromes in other human diseases.

Table 3. Three thrombogenetic paths and their clinical phenotypes of thrombosis according to the "two-path unifying theory of hemostasis".

Thrombosis Mechanism	Microthrombogenesis	Fibrinogenesis	Macrothrombogenesis
Utilizing hemostatic path	ULVWF path	TF path	Combined ULVWF and TF path
Examples	Sepsis (bacterial, viral, fungal, rickettsial, parasitic) Polytrauma, surgery, transplant Toxin, drug, venom, vaccine Pregnancy Diseases (autoimmune, cancer, diabetes)	APL SDH EDH	DVT (distal) VTE (central DVT/PTE) Arterial thrombosis Peripheral gangrene
Thrombosis character	Microthrombi strings	Fibrin clots	Macrothrombus Combined micro–macrothrombi
Vascular lesion and phenotype example	Focal—retinal microaneurysm Local—hepatic VOD Multifocal—HERNS, Susac syndrome Disseminated—EA-VMTD/MODS	Disseminated—true DIC	Local—distal DVT, arterial thrombosis, AIS
Complex vascular/ hematologic phenotypes	Venous—venous microthrombi (ITP-like syndrome) Arterial—arterial microthrombi (TTP-like syndrome, MODS)	Venous/arterial—DIC (fibrin clot disease)	Combined micro–macrothrombosis Venous—VTE, PTE, CVST Arterial—SPG, limb gangrene

Abbreviations: AIS: acute ischemic stroke; APL: acute promyelocytic leukemia; CVST: cerebral venous sinus thrombosis; DIC: disseminated intravascular coagulation; DVT: deep venous thrombosis; EDH: epidural hematoma; MODS: multiorgan dysfunction syndrome; PTE: pulmonary thromboembolism; ITP: immune thrombocytopenic purpura; PTE: pulmonary thromboembolism; SDH: subdural hematoma; SPG: symmetrical peripheral gangrene; TF: tissue factor; TTP: thrombotic thrombocytopenic purpura; ULVWF: ultra-large von Willebrand factor; VOD, veno-occlusive disease; VTE: venous thromboembolism.

References

1. Hattori, R.; Hamilton, K.K.; McEver, R.P.; Sims, P.J. Complement proteins C5b-9 induce secretion of high molecular weight mul-timers of endothelial von Willebrand factor and translocation of granule membrane protein GMP-140 to the cell surface. J. Biol. Chem. 1989, 264, 9053–9060.

2. Brunn, G.J.; Saadi, S.; Platt, J.L. Differential Regulation of Endothelial Cell Activation by Complement and Interleukin 1α. Circ. Res. 2006, 98, 793–800.

3. Kerr, H.; Richards, A. Complement-mediated injury and protection of endothelium: Lessons from atypical haemolytic uraemic syndrome. Immunobiology 2012, 217, 195–203.

4. Chang, J.C. Thrombocytopenia in critically ill patients due to vascular microthrombotic disease: Pathogenesis based on "two activation theory of the endothelium". Vasc. Dis. Ther. 2017, 2, 2–7. Available online: https://www.oatext.com/pdf/VDT-2-132.pdf (accessed on 13 September 2022).

5. Chang, J.C. TTP-like syndrome: Novel concept and molecular pathogenesis of endotheliopathy-associated vascular micro-thrombotic disease. Thromb. J. 2018, 16, 20.

6. Chang, J.C. Sepsis and septic shock: Endothelial molecular pathogenesis associated with vascular microthrombotic disease. Thromb. J. 2019, 17, 10.

7. Chang, J.C. Acute Respiratory Distress Syndrome as an Organ Phenotype of Vascular Microthrombotic Disease: Based on Hemostatic Theory and Endothelial Molecular Pathogenesis. Clin. Appl. Thromb. Hemost. 2019, 25, 1076029619887437.

8. Chang, J.C. Disseminated intravascular coagulation: New identity as endotheliopathy-associated vascular microthrombotic disease based on in vivo hemostasis and endothelial molecular pathogenesis. Thromb. J. 2020, 18, 25.

9. Chang, J. Pathogenesis of Ebola Viral Haemorrhagic Fever: TTP-like Syndrome Associated with Hepatic Coagulopathy based on "Two Activation Theory of the Endothelium". J. Prev. Infect. Control 2017, 3, 1–4.

10. Chang, J.C. COVID-19 Sepsis: Pathogenesis and Endothelial Molecular Mechanisms Based on "Two-Path Unifying Theory" of Hemostasis and Endotheliopathy-Associated Vascular Microthrombotic Disease, and Proposed Therapeutic Approach with Antimicrothrombotic Therapy. Vasc. Health Risk Manag. 2021, 17, 273–298.

11. Chang, J.C. Hemostasis based on a novel 'two-path unifying theory' and classification of hemostatic disorders. Blood Coagul. Fibrinolysis 2018, 29, 573–584.

12. Chang, J.C. Thrombogenesis and thrombotic disorders based on "two-path unifying theory of hemostasis: Philosophical, physiological and phenotypical interpretation. Blood Coagul. Fibrinolysis 2018, 29, 585–595.

13. Chang, J.C.; Hawley, H.B. Vaccine-Associated Thrombocytopenia and Thrombosis: Venous Endotheliopathy Leading to Venous Combined Micro-Macrothrombosis. Medicina 2021, 57, 1163.

14. Chang, J.C. Pathogenesis of Two Faces of DVT: New Identity of Venous Thromboembolism as Combined Micro-Macrothrombosis via Unifying Mechanism Based on "Two-Path Unifying Theory" of Hemostasis and "Two-Activation Theory of the Endothelium". Life 2022, 12, 220.

15. Esmon, C.T. Crosstalk between inflammation and thrombosis. Maturitas 2004, 47, 305–314.

16. Aksu, K.; Donmez, A.; Keser, G. Inflammation-induced thrombosis: Mechanisms, disease associations and management. Curr. Pharm. Des. 2012, 18, 1478–1493.

17. Engelmann, B.; Massberg, S. Thrombosis as an intravascular effector of innate immunity. Nat. Rev. Immunol. 2013, 13, 34–45.

18. Allam, R.; Kumar, S.V.; Darisipudi, M.N.; Anders, H.-J. Extracellular histones in tissue injury and inflammation. J. Mol. Med. 2014, 92, 465–472.

19. Palankar, R.; Greinacher, A. Challenging the concept of immunothrombosis. Blood 2019, 133, 508–509.

20. Chang, J.C. Disseminated intravascular coagulation (DIC): Is it fact or fancy? Blood Coagul. Fibrinolysis 2018, 29, 330–337.

21. Turner, N.A.; Moake, J. Assembly and activation of alternative complement components on endothelial cell-anchored ultra-large von Willebrand factor links complement and hemostasis-thrombosis. PLoS ONE 2013, 8, e59372.

22. Amari Chinchilla, K.; Vijayan, M.; Taveras Garcia, B.; Jim, B. Complement-Mediated Disorders in Pregnancy. Adv. Chronic Kidney Dis. 2020, 27, 155–164.

23. Ghosh, P.; Sahoo, R.; Vaidya, A.; Chorev, M.; Halperin, J.A. Role of Complement and Complement Regulatory Proteins in the Complications of Diabetes. Endocr. Rev. 2015, 36, 272–288.

24. Xie, C.B.; Jane-Wit, D.; Pober, J.S. Complement Membrane Attack Complex: New Roles, Mechanisms of Action, and Therapeutic Targets. Am. J. Pathol. 2020, 190, 1138–1150.

25. Yu, J.; Abagyan, R.; Dong, S.; Gilbert, A.; Nussenzweig, V.; Tomlinson, S. Mapping the Active Site of CD59. J. Exp. Med. 1997, 185, 745–754.

26. Wu, G.; Hu, W.; Shahsafaei, A.; Song, W.; Dobarro, M.; Sukhova, G.K.; Bronson, R.R.; Shi, G.-P.; Rother, R.P.; Halperin, J.A.; et al. Complement Regulator CD59 Protects Against Atherosclerosis by Restricting the Formation of Complement Membrane Attack Complex. Circ. Res. 2009, 104, 550–558.

27. Blann, A.D. How a damaged blood vessel wall contibutes to thrombosis and hypertension. Pathophysiol. Haemost. Thromb. 2003, 33, 445–448.

28. Teijaro, J.R.; Walsh, K.B.; Cahalan, S.; Fremgen, D.M.; Roberts, E.; Scott, F.; Martinborough, E.; Peach, R.; Oldstone, M.B.; Rosen, H. Endothelial cells are central orchestrators of cytokine amplification during influenza virus infection. Cell 2011, 146, 980–991.

29. Adam, E.; Zacharowski, K.; Miesbach, W. A comprehensive assessment of the coagulation profile in critically ill COVID-19 patients. Thromb. Res. 2020, 194, 42–44.

30. Favaloro, E.J.; Henry, B.M.; Lippi, G. Increased VWF and Decreased ADAMTS-13 in COVID-19: Creating a Milieu for (Micro) Thrombosis. Semin. Thromb. Hemost. 2021, 47, 400–418.

31. Bagot, C.N.; Arya, R. Virchow and his triad: A question of attribution. Br. J. Haematol. 2008, 143, 180–190.

32. Chauhan, A.K.; Goerge, T.; Schneider, S.W.; Wagner, D.D. Formation of platelet strings and microthrombi in the presence of ADAMTS-13 inhibitor does not require P-selectin or beta3 integrin. J. Thromb. Haemost. 2007, 5, 583–589.

33. Tsai, H.-M. Pathophysiology of thrombotic thrombocytopenic purpura. Int. J. Hematol. 2010, 91, 1–19.

34. Nguyen, T.C.; Liu, A.; Liu, L.; Ball, C.; Choi, H.; May, W.S.; Aboulfatova, K.; Bergeron, A.L.; Dong, J.-F. Acquired ADAMTS-13 deficiency in pediatric patients with severe sepsis. Haematologica 2007, 92, 121–124.

35. Karim, F.; Adil, S.N.; Afaq, B.; Haq, A.U. Deficiency of ADAMTS-13 in pediatric patients with severe sepsis and impact on in-hospital mortality. BMC Pediatr. 2013, 13, 44.

36. Fukushima, H.; Nishio, K.; Asai, H.; Watanabe, T.; Seki, T.; Matsui, H.; Sugimoto, M.; Matsumoto, M.; Fujimura, Y.; Okuchi, K. Ratio of von Willebrand Factor Propeptide to ADAMTS13 Is Associated with Severity of Sepsis. Shock 2013, 39, 409–414.

37. Mendolicchio, G.L.; Ruggeri, Z.M. Interaction of von Willebrand factor with platelets and the vessel wall. Hämostaseologie 2015, 35, 211–224.

38. Shim, C.Y.; Ni Liu, Y.; Atkinson, T.; Xie, A.; Foster, T.; Davidson, B.P.; Treible, M.; Qi, Y.; López, J.A.; Munday, A.; et al. Molecular Imaging of Platelet–Endothelial Interactions and Endothelial von Willebrand Factor in Early and Mid-Stage Atherosclerosis. Circ. Cardiovasc. Imaging 2015, 8, e002765.

39. Stein, E.; McMahon, B.; Kwaan, H.; Altman, J.K.; Frankfurt, O.; Tallman, M.S. The coagulopathy of acute promyelocytic leukaemia revisited. Best Pract. Res. Clin. Haematol. 2009, 22, 153–163.

40. Chang, J.C. Stroke Classification: Critical Role of Unusually Large von Willebrand Factor Multimers and Tissue Factor on Clinical Phenotypes Based on Novel "Two-Path Unifying Theory" of Hemostasis. Clin. Appl. Thromb. Hemost. 2020, 26, 1076029620913634.