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REVIEW

# Advances in Research on the Release of von Willebrand Factor from Endothelial Cells through the Membrane Attack Complex C5b-9 in Sepsis

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**Abstract:** Sepsis, a lethal organ dysfunction syndrome driven by aberrant host responses to infection, intertwines excessive inflammatory responses and dysregulated coagulation processes in its pathophysiology. Emerging research reveals the complement terminal membrane attack complex C5b-9 orchestrates ultralarge von Willebrand factor (ULVWF) release from vascular endothelial cells (ECs) through multifaceted mechanisms: C5b-9 compromises EC membrane integrity, activates calcium influx cascades, and provokes NLRP3 inflammasome signaling, triggering massive exocytosis of ULVWF stored within Weibel-Palade bodies (WPBs). When ADAMTS13 activity falters, undegraded ULVWF complexes with platelets to spawn microthrombi, precipitating microvascular occlusion and multiorgan collapse. Strikingly, elevated plasma von Willebrand factor (vWF) antigen levels in sepsis patients correlate robustly with endothelial injury, thrombocytopenia, and mortality—underscoring C5b-9-driven vWF release as a linchpin of septic coagulopathy. Current therapeutic strategies targeting these pathways, including recombinant ADAMTS13 (rhADAMTS13), N-acetylcysteine (NAC), and complement inhibitors like eculizumab, face limitations in clinical translation, necessitating further validation of their efficacy. Additionally, investigating complement regulatory molecules such as CD59 may unlock novel therapeutic avenues. Deciphering the intricate interplay within the C5b-9-vWF axis and advancing precision therapies hold transformative potential for ameliorating sepsis outcomes.

**Keywords:** sepsis, C5b-9 complex, von Willebrand factor, platelet-ULVWF microthrombus, endothelial dysfunction

## Introduction

Sepsis is a life-threatening acute organ dysfunction syndrome resulting from the host's dysfunctional response to infection.[1] In the pathogenesis of sepsis, systemic inflammatory cascades upregulate tissue factor expression, triggering coagulation system activation while suppressing both anticoagulant mechanisms and fibrinolytic pathways. This coordinated dysregulation promotes platelet hyperreactivity and facilitates fibrin-rich thrombogenesis. In parallel, thrombin/Xa/fibrin complexes amplify inflammatory responses through protease-activated receptor (PAR) signaling, inducing endothelial glycocalyx degradation, impaired nitric oxide bioavailability, and activated protein C (APC) dysfunction. These pathophysiological alterations culminate in microcirculatory thrombosis, tissue hypoperfusion, a self-perpetuating pathological loop of inflammation-apoptosis crosstalk, and subsequent multiple organ dysfunction syndrome (MODS) development.[2,3] Sepsis-induced shock and subsequent progression to MODS originate from complex pathophysiological mechanisms including microcirculatory failure (characterized by tissue hypoxia and resultant metabolic dysregulation), profound mitochondrial dysfunction with impaired cellular energy production, progressive immunosuppression accompanied by compromised host defense mechanisms, cross-organ propagation of inflammatory mediators through systemic cytokine storms and chemokine cascades, as well

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as disruption of autonomic nervous system homeostasis leading to dysregulated neuroendocrine-immune axis coordination. These mechanisms, through a complex balance of adaptive compensation and pathological decompensation, collectively drive organ dysfunction.[4–9] Recently, a novel coagulation pathway, the platelet microthrombus pathway theory, has emerged.[10] According to this theory, the C5b-9 complex, activated by the complement system, targets vascular endothelial cells (ECs), stimulating them to release von Willebrand factor (vWF), which interacts with platelets to form ultralarge von Willebrand factor (ULVWF) microthrombi, ultimately contributing to organ dysfunction.[11] However, this theory requires further and more comprehensive investigation. A previous retrospective clinical study revealed that approximately 40% of non-sepsis-induced coagulopathy (SIC) patients with septic shock exhibited elevated vWF antibody expression, which was negatively correlated with platelet count reduction and associated with organ damage and increased mortality. This incidental finding supports the existence of the platelet-ULVWF microthrombus pathway and reinforces the "endothelial dual activation theory" hypothesis.[10,12] This theory refers to the occurrence of endotheliopathy in sepsis, where endothelial dysfunction activates two independent endothelial pathways; the inflammatory pathway and the thrombotic pathway. Therefore, we hypothesize that C5b-9 may mediate the release of vWF from vascular ECs, thereby promoting the formation of platelet-ULVWF microthrombi. Further studies are planned to validate this hypothesis and identify new therapeutic targets for preventing microthrombus formation in sepsis.

## The Role of the C5b-9 Complex in the Complement System During Sepsis

### Formation of C5b-9 in the Complement System

The complement system is a crucial component of the human innate immune system, playing a vital role in maintaining immune balance and defending against infections.[13] In severe conditions such as sepsis and acute respiratory distress syndrome (ARDS), excessive activation or dysregulation of the complement system can lead to pathological conditions, including tissue damage, uncontrolled inflammation, and microvascular leakage syndrome, all of which are closely associated with disease severity and poor prognosis.[14,15] In sepsis, the complement system is primarily activated via the classical pathway, alternative pathway, and mannan-binding lectin (MBL) pathway,[16] which ultimately converge to form C3 convertase and C5 convertase. These convertases initiate the production of anaphylatoxins (C3a and C5a) and opsonins (C3b/iC3b). C3a and C5a bind to specific receptors, triggering the release of inflammatory mediators, stimulating ECs, and promoting cell migration and activation. C5 convertase cleaves C5 into C5a and C5b,[17,18] with C5b subsequently binding to C6 to activate the downstream terminal complement pathway. The C5b-6 complex progressively associates with C7, C8, and C9 in a sequential manner, ultimately forming the C5b-9 complex, also known as the membrane attack complex (MAC)[19] (Figure 1).

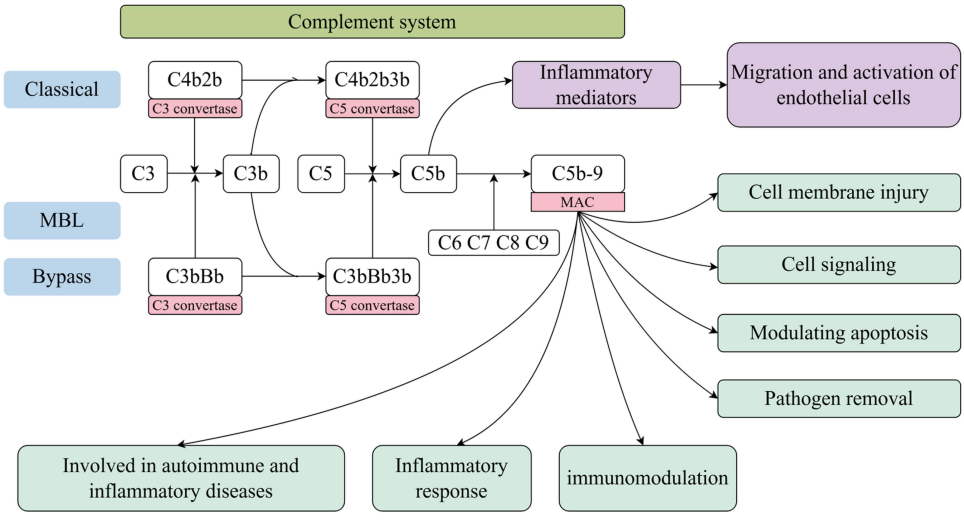


**Figure 1** Formation and main functions of C5b-9. This figure was created by Figdraw.[18–20]
**Note**: It illustrates the formation process of the membrane attack complex C5b-9 through classical pathways, MBL pathways, and bypass pathways, focusing on the roles of C5b and C5b-9 in circulation.

## The Main Function of C5b-9 in the Complement System

C5b-9 plays a pivotal role in the complement system, with its key functions (Figure 1) including: 1. Cell Membrane Damage:[20] The primary function of the C5b-9 complex is to form transmembrane channels on the membranes of target cells, allowing non-selective passage of substances into and out of the cell. This leads to damage of the cell membrane and ultimately results in cell lysis. 2. Cell Signaling:[21] Interaction between the C5b-9 complex and the cell membrane can influence cell signaling, thereby affecting cell behavior and function. 3. Apoptosis Regulation:[22] The C5b-9 complex may play a role in regulating apoptosis, particularly under pathological conditions, where it can either promote or inhibit programmed cell death. 4. Pathogen Elimination:[23] During the immune response, C5b-9 complexes contribute to pathogen elimination by forming pores in the cell membranes of bacteria and viruses, compromising their integrity and leading to pathogen death. 5. Inflammatory Response:[24] The assembly and activation of the C5b-9 complex can initiate an inflammatory response by promoting the release of inflammatory mediators, increasing vascular permeability, and attracting immune cells to the site of infection or injury. 6. Immune Regulation:[25] Complement system activation enhances the immune response, with the formation of C5b-9 being one of the final steps in the complement cascade. This complex regulates immune activity either by directly destroying pathogens or modulating immune cell function. 7. Autoimmune and Inflammatory Diseases:[26,27] Abnormal activation or dysregulation of the C5b-9 complex has been implicated in various autoimmune and inflammatory diseases, including atypical hemolytic uremic syndrome (aHUS), certain nephritides, and vasculitis.

The C5b-9 complex plays a central role in the complement system, directly eliminating pathogens or target cells through the formation of membrane attack complexes. It is also involved in various immunomodulatory and inflammatory processes, playing a critical role in both immune defense and immune damage in diseases such as sepsis.

## The Role of C5b-9 in Various Diseases

In complement-mediated aHUS,[28,29] uncontrolled complement activation is triggered by alterations in complement regulatory factors, leading to excessive deposition of complement components, including C5b-9, in the vascular endothelium. Reviewing 103 patients with acute thrombotic microangiopathy (TMA), 19 patients with aHUS were identified. Multiple markers in the complement activation pathway were tested, including C3a, Bb, C4d, C5a, C5b-9, ADAMTS13 activity, and vWF multimers. These patients had a platelet count of <100 × 10^9/L, serum creatinine >2.25 mg/dL, and ADAMTS13 activity >10%. Compared to patients with thrombotic thrombocytopenic purpura (TTP), aHUS patients generally exhibited elevated levels of complement activation markers before treatment, particularly C5a and C5b-9, which were significantly higher than those with ADAMTS13-deficient TTP. C5b-9 damages ECs by forming membrane pores, impairing endothelial function. It induces ECs to release vWF, stimulates the activity of thrombin and tissue factor (TF), and activates platelet aggregation and fibrin deposition, thereby promoting thrombosis on the endothelial surface. C5b-9 also induces morphological changes in ECs, cell contraction, exposure of procoagulant factors on the basement membrane, and enhances platelet and leukocyte adhesion. Additionally, it stimulates the release of inflammatory and growth factors, leading to microthrombus formation, which results in thrombotic microangiopathies in renal capillaries and small arterial branches, thrombocytopenia, vessel swelling and narrowing, and increased blood flow shear stress. This destruction predominantly affects red blood cells. Moreover, C5b-9 not only damages ECs but also promotes inflammation through chemotaxis, with C3a and C5a exhibiting strong chemotactic effects on phagocytic cells. This triggers the release of histamine from phagocytic cells, increasing small blood vessel permeability, causing kidney damage, and potentially leading to renal failure. The study revealed that aHUS patients showed no abnormal accumulation of ULVWF multimers during acute episodes, strongly indicating these complement biomarkers could prove instrumental in distinguishing aHUS from TTP. Of the 16 aHUS patients undergoing plasma exchange (PEX), 6 (38%) demonstrated positive responses, while among 9 patients treated with eculizumab, 7 (78%) achieved therapeutic efficacy. These findings underscore that C5a and C5b-9—key biomarkers of complement activation—may not only confirm aHUS diagnoses but also sharpen differentiation from clinically similar thrombotic microangiopathies like TTP.[29] The measurement of these markers may help predict the response to complement inhibition therapy.

Studies have also shown that the deposition of C5b-9 and vWF on the vascular endothelium of pre-eclamptic patients is significantly higher compared to normal pregnancy, suggesting that this may be a key factor in the vascular endothelial injury and dysfunction observed in pre-eclampsia.[30] In the context of TMA, C5b-9 deposition may reflect complement-mediated endothelial damage and could be associated with the release of vWF and subsequent platelet aggregation.[31] Thus, C5b-9 plays a critical role in the pathogenesis of aHUS.

## Sepsis and the Role of C5b-9

In sepsis, the complement system plays a protective role by rapidly identifying and eliminating pathogens, with the alternative pathway serving as the primary mechanism for complement activation. The resulting C5b-9 complex targets pathogen cell membranes, forming pores that lead to cell disintegration.[16] While C5b-9 serves a protective function, reduced expression of the complement regulator CD59 (a C5b-9 inhibitor), due to genetic mutations or acquired diseases, can lead to overactivation of C5b-9. This overactivation induces the exposure of phosphatidylserine on platelet surfaces, activating their procoagulant activity and providing a catalytic surface for prothrombin assembly, thereby promoting platelet aggregation. This process plays a role in coagulation regulation and triggers positive feedback in complement activation.[32]

In sepsis, C5b-9 predominantly affects vascular ECs, leading to the following manifestations: 1. Cell Membrane Damage:[33] C5b-9 forms pores in the cell membrane, compromising its integrity. This allows molecules, including water, ions, proteins, and blood cells, to enter or exit the cell, disrupting the internal and external balance of the cell. 2. Increased Cell Permeability:[34] The pores created by C5b-9 increase the permeability of vascular ECs, which can lead to leakage of fluids and proteins, compromising the vascular barrier function and enhancing vessel leakage. 3. Cell Death:[35] The insertion of the C5b-9 complex can directly induce cell death through a programmed process known as complement-mediated cytolysis. 4. Alterations in EC Function:[36,37] C5b-9-induced damage to ECs can lead to significant functional changes, including disruption of vascular tone regulation, impairment of white blood cell adhesion and migration, and hindered vascular repair and regeneration. 5. Changes in Signal Transduction of Vascular ECs:[38,39] The insertion of C5b-9 may activate intracellular signaling pathways in ECs, such as the mitogen-activated protein kinase (MAPK) and extracellular signal-regulated kinase (ERK) pathways, leading to increased production of inflammatory mediators. 6. Imbalance Between Coagulation and Anticoagulation:[40,41] C5b-9 activation of the clotting pathway following endothelial damage promotes thrombosis. Simultaneously, injured ECs may fail to effectively produce anticoagulant and pro-fibrinolytic factors, resulting in a coagulation-anticoagulation imbalance. 7. Overactivation of the Complement System:[39,42] The formation of C5b-9 serves as a signal to further activate the complement system, leading to the generation of additional MAC and exacerbating cell damage. 8. C5b-9 Activation of Calcium ($Ca^{2+}$) Channels:[43] C5b-9 can trigger $Ca^{2+}$ channels in ECs and epithelial cells, activating the NOD-like receptor heat protein domain-associated protein 3 (NLRP3) inflammasome, which subsequently contributes to cell damage. These combined effects promote vascular endothelial dysfunction and damage in conditions such as sepsis and coronavirus disease 2019 (COVID-19), leading to multiple organ dysfunction and failure (Figure 2).

The mechanisms described above have been further supported by numerous cases of COVID-19 sepsis.[44–46] Research indicates that C5b-9 plays the following roles in COVID-19 sepsis: 1. Association with Disease Severity: Elevated C5b-9 levels have been observed in COVID-19 patients, indicating activation of the complement system. This activation correlates with disease severity, with C5b-9 levels decreasing as clinical improvement occurs. Higher C5b-9 levels are associated with a more severe disease phenotype. 2. Correlation with Viral Load: A positive correlation between high viral load and elevated C5b-9 levels suggests that the virus may directly trigger complement system activation. 3. Inflammation and Coagulation Response: This may explain the heightened inflammatory state, altered vascular permeability, and abnormal coagulation observed in COVID-19 patients. 4. Tissue Damage and Microvascular Injury: The observed tissue damage is consistent with microvascular injury, suggesting that C5b-9 may contribute to microvascular damage in COVID-19 sepsis.

A study on preterm infants with sepsis also revealed significantly elevated plasma levels of C-reactive protein (CRP), SC5b-9, and interleukins (IL)-10 and IL-4 in infants with moderate to severe sepsis compared to healthy controls. This

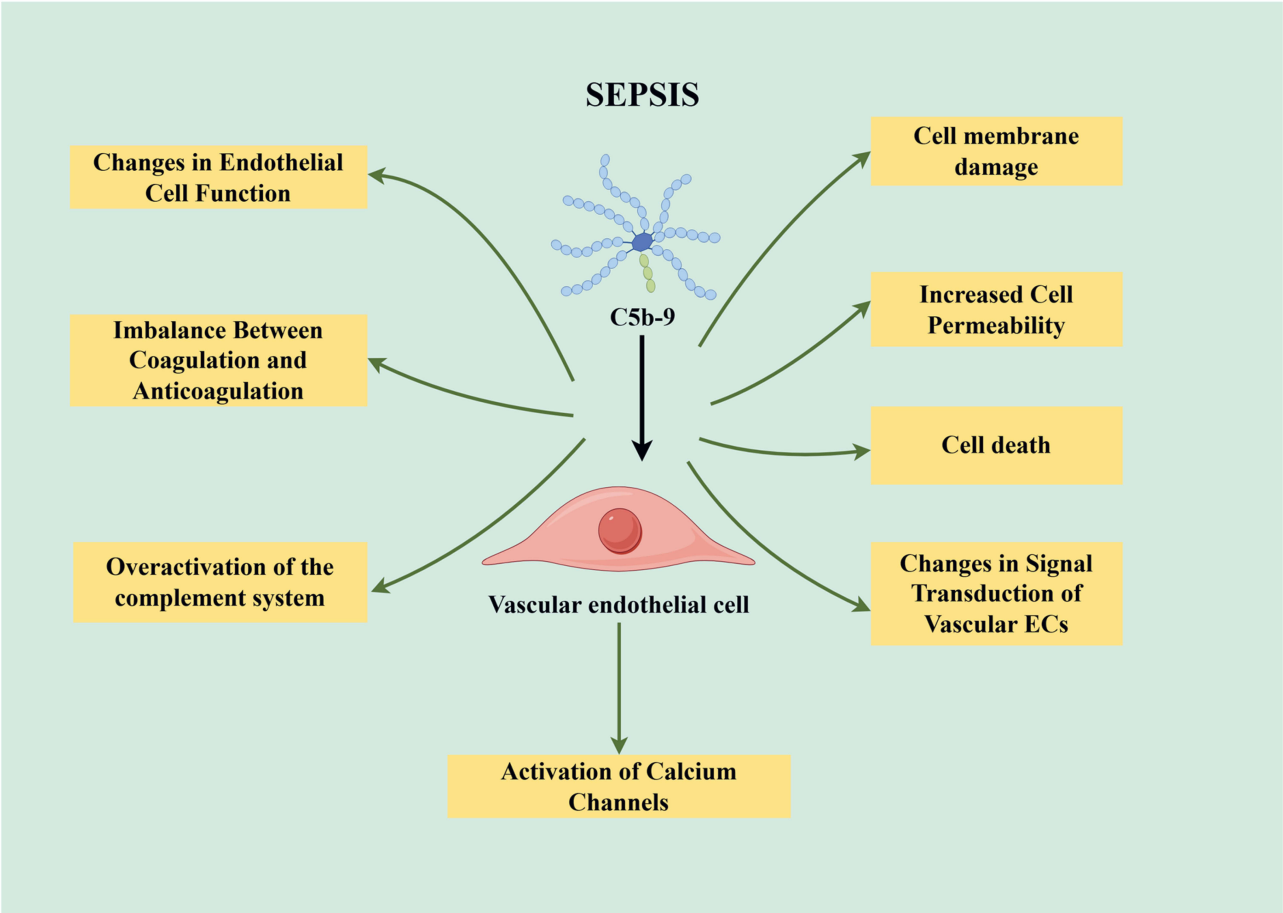


**Figure 2** Effect of C5b-9 on vascular endothelium in sepsis.
**Note**: This figure specifically illustrates the effect of C5b-9 on vascular ECs in sepsis. Created by Figdraw.

suggests that, in the context of neonatal sepsis, SC5b-9, as a marker of complement system activation, plays a crucial role in assessing the severity of sepsis in preterm infants.[47]

## Mechanism of vWF Release by ECs to Form Microthrombi in Sepsis

### Effects of Sepsis on the Vascular Endothelium

Pathogen-associated molecular patterns (PAMPs) in sepsis induce EC inflammation, activate ECs, increase capillary permeability, promote leukocyte adhesion, establish a procoagulant phenotype, and alter vascular tone.[10,48]

In immune-mediated sepsis, EC activation triggers the release of damage-associated molecular patterns (DAMPs) and cytokines, initiating the inflammatory response. Immune cells, such as monocytes and neutrophils, further amplify this response by upregulating receptors and releasing inflammatory mediators, which leads to the shedding or degradation of the glycocalyx on the endothelial surface. This process results in an increased expression of TF and vWF on both endothelial and monocyte surfaces, enhancing platelet adhesion and aggregation, while downregulating anticoagulation and fibrinolysis, thus contributing to abnormal coagulation and thrombosis.[49,50]

Exposure of ECs to serum samples from sepsis patients has been shown to activate the inflammation-related p38MAPK pathway, which is closely associated with EC activation and the inflammatory response.[51]

During acute myocardial infarction, dysregulated complement activation through the C5a:C5a - Receptor (C5aR)1 axis leads to endothelial glycocalyx degradation and endothelial dysfunction.[52] In sepsis, C5a induces inflammatory signaling and apoptosis in PC12 cells through C5aR-dependent signaling, which may be a potential mechanism for adrenal injury in sepsis.[53] C5a and neutrophil C5a receptor play a central role in anti-neutrophil cytoplasmic antibodies


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(ANCA)-mediated neutrophil recruitment and activation. The activation of p38MAPK, ERK, and phosphatidylinositol 3-kinase (PI3K) are important steps in ANCA antigen translocation and C5a-induced ANCA activation of neutrophils. This indicates that C5a can trigger signaling pathway-related responses involving pathways such as p38MAPK.[54] Although these studies do not directly mention it, they reflect the role of C5a in the pathological process of microthrombus formation in sepsis.

In addition to C5b-9, complement activation in sepsis involves other complement components, such as C5a, which activates neutrophils and exacerbates inflammation, ultimately leading to EC activation or injury.[55,56]

Overall, sepsis induces endothelial dysfunction, characterized by EC inflammation, abnormal coagulation, and impaired vascular tone regulation, leading to thrombosis, vasodilation, tissue hypoperfusion, inadequate oxygen delivery, secondary hypotension, and loss of endothelial barrier function.

## Mechanism by Which ECs Release vWF to Form Microthrombi

vWF is a polymeric plasma glycoprotein synthesized by ECs and megakaryocytes.[57] Its biosynthesis is a complex process, involving the removal of signal peptides and propeptides, glycosylation, sulfation, dimerization, and final polymerization. The synthesized vWF polymers are primarily stored in Weibel-Palade bodies (WPBs) of ECs or in the α-granules of megakaryocytes and platelets as ULVWF polymers.[58,59] Upon stimulation by various agonists (such as cytokines or histamine) or fluid shear stress, these ULVWF polymers are rapidly secreted by ECs and anchored to the endothelial surface, forming long, string-like, highly adhesive structures, or are released into the circulation. Under shear stress in blood flow, the anchored and newly released ULVWF polymers undergo further changes.[60,61] vWF is the only known substrate for the metalloproteinase ADAMTS13, which exists in a closed conformation in circulation, with its CUB domain interacting with the septal region. vWF can bind to this closed conformation of ADAMTS13, exposing functional extranuclear sites within the ADAMTS13 spacer and activating the protease.[62] ADAMTS13 cleaves the Tyr1605-Met1606 bond in the vWF A2 domain via its metalloproteinase domain, thereby shortening the vWF polymers. In TTP), when ADAMTS13 is deficient or inactive, ULVWF polymers persist in circulation, and their spontaneous binding to platelets is no longer inhibited. These ULVWF polymers bind to platelets, leading to platelet accumulation, activation, and the formation of platelet-ULVWF microthrombi. These active microthrombi propagate, consuming platelets and resulting in thrombocytopenia, mechanical destruction of red blood cells, and hemolytic anemia. Fragmented red blood cells are visible on peripheral blood smears, and ultimately, platelet-ULVWF microthrombi obstruct blood vessels, causing ischemic organ injury.[57,63]

In a cohort of 152 suspected disseminated intravascular coagulation (DIC) patients, comprehensive monitoring of ADAMTS13-vWF axis markers and DIC biomarkers unveiled striking disparities: vWF:Ag levels surged dramatically while ADAMTS13 activity plunged to critical lows. Prognostic analysis highlighted the platelet count/vWF:Ag ratio as the most potent predictor ($p = 0.037$), surpassing other ADAMTS13-vWF axis metrics including vWF:Ag levels ($p = 0.009$), ADAMTS13 activity/vWF:Ag ratio ($p = 0.037$), and ADAMTS13 activity/vWF:Rco ratio ($p = 0.028$).[64] Intriguingly, human umbilical vein endothelial cell (HUVEC) studies revealed ADAMTS13 secretion persists constitutively regardless of inflammatory triggers, despite HUVEC ADAMTS13 mRNA expression registering at a mere 1:100 ratio relative to vWF monomer subunit expression. Histamine stimulation triggered a surge in vWF chain secretion while paradoxically reducing ADAMTS13-mediated cleavage efficiency at the vWF Y(1605)-M(1606) site. This sustained ADAMTS13 secretion from endothelial cells may preserve low adhesiveness of vWF multimer chains on cellular surfaces, maintaining vascular homeostasis through dynamic molecular regulation.[65] ECs synthesize vWF and complement regulatory factor H (FH). Studies have found that FH and VWF coexist in the leukocytes of HUVECs. The binding of vWF to FH enhances the cofactor activity of FH, downregulates complement activation mediated by factor I, and inhibits vWF proteolysis mediated by ADAMTS13, promoting platelet aggregation.[66]

In typical hemolytic uremic syndrome (STEC-HUS) and aHUS,[67] excessive activation of Shiga toxin (Stx) and the complement system can cause EC damage, which triggers the release of excessive vWF, subsequently interacting with platelets. This disruption of the balance between coagulation and anticoagulation ultimately promotes microvascular thrombosis and leads to organ damage and dysfunction.

In certain infectious diseases,[68] such as bacterial endocarditis, brucellosis, acute glomerulonephritis caused by streptococcal infection, invasive fungal infections (eg, aspergillosis), and viral and rickettsial infections, pathogens can

directly damage ECs, activate the complement system, and induce the release of excessive vWF. The abnormal activation and aggregation of platelets through inflammatory responses and coagulation cascade activation result in microvascular thrombosis, paralleling the pathophysiological processes observed in TTP.

During major cardiovascular surgical trauma,[69,70] ECs are directly damaged, leading to the release of large amounts of ULVWF polymers, decreased ADAMTS13 activity, and platelet adhesion and aggregation, all of which contribute to the formation of microthrombi and result in clinical manifestations similar to TTP.

Therefore, when ECs are damaged by various factors, excessive release of vWF polymers activates platelets. If the quantity and activity of ADAMTS13 are insufficient, platelet-ULVWF microthrombosis occurs, resulting in vascular occlusion and organ dysfunction.

## Study of C5b9-Mediated vWF Release From ECs in Sepsis

### Mechanism by Which C5b-9 Mediates Endothelial vWF Release

The deposition of the C5b-9 complex on the surface of ECs leads to cell damage and activates signaling pathways that mediate vWF secretion through several mechanisms: 1. Direct Attack on the Cell Membrane: The C5b-9 complex can integrate directly into the lipid bilayer of the cell membrane, causing structural changes and forming both single and compound pores.[19] This disrupts the integrity of the cell membrane, resulting in cell lysis and detachment, impairing secretion and anticoagulant functions,[71] and promoting vWF secretion. Additionally, C5b-9 increases EC toxicity.[72] Lactate dehydrogenase (LDH) levels can be used to assess the extent of cell damage. 2. Action on vWF Storage Particles: The C5b-9 complex may directly target vWF storage particles (WPBs) within ECs, leading to the fusion of these particles with the cell membrane and subsequent vWF release.[73] 3. Increased $Ca^{2+}$ Ion Flux and Vesiculation of the Cell Membrane: The deposition of C5b-9 in HUVECs disrupts membrane integrity, alters membrane $Ca^{2+}$ channels, and increases $Ca^{2+}$ flux. This induces vesiculation of membrane particles on the EC surface, triggering the movement and fusion of storage particles with the membrane, leading to vWF secretion or exocytosis.[74,75] 4. Promotion of P-Selectin Expression: C5b-9 deposition may transiently upregulate the expression of P-selectin on the human EC surface. P-selectin and vWF are stored together in WPBs, which also serve as binding sites for monocytes and neutrophils, potentially contributing to vWF release.[73] 5. Involvement of Protein Kinase: The C5b-9-induced secretion response involves cellular protein kinases. Inhibition of cellular protein kinases with ceramide partially reduces C5b-9-induced vWF secretion.[71] Extracorporeal studies have found that the receptor-binding domain (RBD) of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) spike protein is sufficient to induce endothelial cell permeability and vWF secretion through angiotensin-converting enzyme (ACE) 2, in a manner dependent on the activation of Adp-ribosylation factor (ARF) 6. Furthermore, the use of pharmacological inhibitors has revealed a signal cascade downstream of ACE2 involved in SARS-CoV-2 spike protein-induced EC permeability and vWF secretion.[76] 6. Inflammation-Mediated:[55] C5b-9 induces mitochondrial damage and activates the NLRP3 inflammasome, leading to the release of potent pro-inflammatory cytokines, including IL-1β and IL-18. These inflammatory mediators contribute to tissue damage and an inflammatory response, which in turn affects vascular EC function and indirectly increases vWF release from ECs.

A recent study[75] demonstrated that when blood-derived endothelial cells (BOECs) are exposed to C5b-9, membrane integrity is compromised, resulting in membrane leakage, increased permeability, and rapid intracellular $Ca^{2+}$ flux. Despite the continuous rise in intracellular $Ca^{2+}$, membrane leakage ceases within 30 minutes. This response does not result in necrosis or apoptosis, and the cells exhibit a similar ability to repair plasma membrane damage within 20–30 minutes of C5b-9 exposure. The increase in intracellular $Ca^{2+}$ triggers the mobilization of WPBs to the plasma membrane, where they fuse, leading to the secretion of vWF. The fusion of WPBs with the plasma membrane helps repair the damage caused by C5b-9. This repair mechanism enables vascular ECs to resist further damage, repair existing damage, and survive. However, this survival mechanism comes at a cost—the release of large amounts of ULVWF polymers. These polymers must undergo regulated cleavage by ADAMTS13 in the bloodstream. If ADAMTS13 activity is inhibited by environmental factors, or if ULVWF release exceeds the cleavage capacity of ADAMTS13, excessive ULVWF will interact with activated platelets, leading to the formation of platelet-ULVWF microthrombi. Thus, C5b-9-mediated endothelial release of vWF acts as a protective mechanism, albeit at the expense of intravascular microthrombus formation, ultimately leading to microvascular occlusion and organ dysfunction.

In 2017, Chang JC et al proposed the "endothelial dual activation theory",[10–12,77,78] suggesting that in sepsis, the C5b-9 complex formed by complement activation induces endothelial damage, leading to both structural and biological changes in ECs, resulting in molecular dysfunction. This damage activates two concurrent pathways: the inflammatory and micro-thrombotic pathways. The activated inflammatory pathway triggers the release of various pro-inflammatory cytokines, including IL-1, IL-6, tumour necrosis factor (TNF) -α, and interferon (IFN) -γ, contributing to the inflammatory response. The molecular response of the activated microthrombotic pathway involves the exocytosis of large amounts of ULVWF from WPBs in ECs, which subsequently activates platelets. If metalloproteinase ADAMTS13 is insufficient to cleave the excess exocytosed ULVWF, the ULVWF anchors to the damaged endothelial membrane, forming slender lines and recruiting a large number of activated platelets. The interaction of these components leads to the formation of platelet-ULVWF complexes, which eventually evolve into microclots that adhere to the damaged ECs and occlude microvessels, resulting in organ dysfunction.

However, the specific processes, mechanisms, and consequences of the platelet-ULVWF microthrombus pathway mediated by complement-induced EC injury remain insufficiently explored at the molecular and cellular levels.

## In Sepsis, C5b-9 Mediates Endothelial vWF Release to Promote Microthrombosis

In summary, C5b-9 has been shown to directly or indirectly mediate the release of vWF by vascular ECs, thereby promoting platelet microthrombus formation in various diseases associated with complement activation (Figure 3). A significant body of research has confirmed this mechanism in patients with COVID-19 sepsis,[79] yet studies examining this pathway in sepsis caused by a broad range of bacterial infections remain limited. In a previous retrospective study, 147 patients diagnosed with

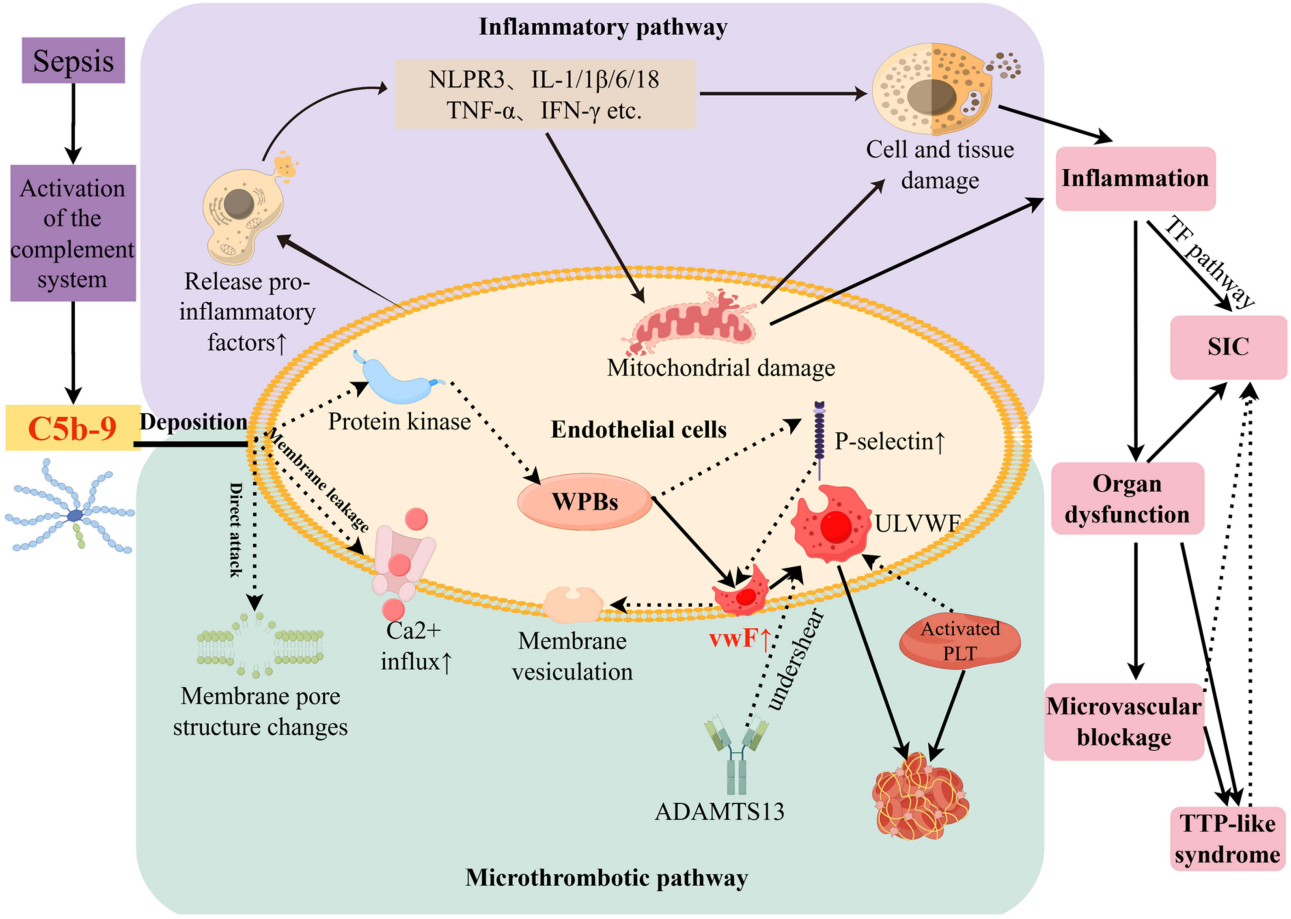


**Figure 3** Molecular mechanism diagram of microthrombus formation by EC secretion of vWF mediated by C5b-9 in sepsis.
**Note**: NLPR3: NOD-like receptor family, pyrin domain containing 3; IL: Interleukin; WPBs: Weibel-Palade bodies; SIC: sepsis-induced coagulopathy. Created by Figdraw.

septic shock were admitted to the Intensive Care Unit at the Second Affiliated Hospital of Kunming Medical University. Elevated vWF antigen expression was observed in all patients, indicating endothelial injury caused by septic shock, which resulted in the release of substantial amounts of vWF. Additionally, 64 SIC patients with TF pathway-activated fibrin thrombosis were identified using the SIC score. There were another 65 patients exhibited thrombocytopenia, increased vWF antigen expression, and concurrent MODS. These findings suggest that endothelial injury led to the formation of platelet-ULVWF microthrombi, resembling a TTP-like syndrome,[77] which was associated with organ dysfunction and increased mortality. It is evident that vWF antigen expression is closely linked to endothelial and organ damage in sepsis, with complement activation playing a crucial role in the pathogenesis of sepsis. In the early stages of sepsis, the expression of vWF antigen increases, characterized by microthrombi composed of platelet-ULVWF complexes, which ultimately lead to a massive consumption of platelets and MODS.[11] Its hematological phenotype is very similar to that of TTP, but the pathophysiological mechanisms are completely different, hence it is called TTP-like syndrome.[10,77,80] When vascular injury reaches subendothelial tissue (SET) and extravascular tissue (EVT), a large amount of TF is released, activating the TF pathway, forming "large thrombi", and ultimately leading to SIC.[11,81] Therefore, it can be concluded that C5b-9 may directly or indirectly mediate the release of vWF by vascular ECs, promoting platelet-ULVWF microthrombus formation, blocking microvessels, and ultimately causing organ dysfunction in sepsis caused by bacterial infection. Therefore, reducing the expression of vWF in patients with sepsis, improving thrombocytopenia, and alleviating organ dysfunction will play a significant role in improving patient outcomes.

## Therapeutic Advances in the C5b-9 EC vWF Release Pathway

Currently, no clear treatment protocol exists for the formation of platelet-ULVWF microthrombi and the associated organ dysfunction resulting from this pathway. Although the complement system plays a crucial role in early immune defense, caution is needed when considering anti-complement therapy in sepsis. Given that platelet-ULVWF microthrombosis arises from excessive vWF secretion by damaged ECs and a relative deficiency of ADAMTS13, existing therapeutic strategies focus on the following directions: 1. Recombinant ADAMTS13 (rhADAMTS13): ADAMTS13/rhADAMTS13 is used to inhibit the ULVWF pathway and prevent the accumulation of excess ULVWF polymers, thereby preventing microthrombus formation. In animal models, prophylactic administration of rhADAMTS13 protects ADAMTS13 knockout mice from TTP-like syndrome and reduces the incidence and severity of TTP, although clinical use has not yet been established. However, rhADAMTS13 could theoretically represent the optimal approach for preventing and treating endotheliopathy-associated vascular microthrombotic disease (EA-VMTD).[82,83] 2. Disulfide Bond Reduction Mucolytic Therapy: N-acetylcysteine (NAC) is the most commonly used reducing agent. By reducing disulfide bonds in ULVWF polymers, NAC inhibits vWF-dependent platelet aggregation and collagen binding, thereby mitigating microthrombus formation.[84–87] NAC is an inexpensive, widely available drug, clinically used for treating chronic obstructive pulmonary disease (COPD), cystic fibrosis, bronchiectasis, and other conditions, with a high therapeutic safety profile. 3. Caplacizumab Treatment: Caplacizumab is a humanized, bivalent, variable-domain immunoglobulin fragment (Nanobody, Ablynx) that prevents microvascular thrombosis by targeting the A1 domain of vWF, thereby blocking the interaction of vWF polymers with platelet glycoprotein Ib-IX-V receptors. Significant clinical benefits have been observed in acquired TTP, further supporting the role of the ULVWF pathway in TTP-like syndrome and EA-VMTD.[88] 4. Therapeutic Plasma Exchange (TPE): As an alternative to ADAMTS13 therapy, TPE has demonstrated effectiveness in treating TTP and TTP-like syndrome. TPE can supplement ADAMTS13, reduce microthrombus formation, and improve outcomes in conditions such as ARDS and other organ syndromes.[89–91]

In addition to complementing ADAMTS13 therapy, inhibition of C5b-9 generation and activation can be targeted from the upstream pathway, as shown in Table 1. Eculizumab, a recombinant humanized IgG2/4 monoclonal antibody, binds to the human complement C5 protein, inhibiting the activation of the C5b-9 terminal complement complex. This prevents complement overactivation, thereby reducing complement-mediated inflammation and cell damage. In the treatment of TMA (cTMA) mediated by complement gene variations, eculizumab has shown significant hematological and renal responses, leading to favorable therapeutic outcomes. However, its response rate in secondary TMA (sTMA) remains low.[92] sTMAs arise from underlying medical conditions such as infections, autoimmune disorders, or therapeutic interventions. Resolution typically follows the cessation of these triggers. However, eculizumab—a targeted complement

**Table 1** Studies Examining Treatment of C5b-9 Mediating ECs Releasing vWF in Sepsis

| Author | Year | Model | Drugs/ PROCEDURES | Target | Curative Effect |
|---|---|---|---|---|---|
| Alexandra Schiviz[82] | 2012 | ADAMTS13 KO mice | rhADAMTS13 | vWF, ADAMTS13 | The administration of therapeutic doses significantly decreased both the incidence and severity of TTP, with efficacy closely correlated to the treatment interval. Although this strategy has not yet been implemented in clinical practice, it presents substantial potential as a novel therapeutic target. |
| Marijke Peetermans[83] | 2020 | S. aureus sepsis both in patients and in mice | ADAMTS13 | vWF, ADAMTS13 | The ratio of vWF to ADAMTS13 in patients with bacteraemia was strongly associated with severe disease outcomes. In vWF-/- mice, enhanced bacterial clearance, reduced mortality, and diminished organ microthrombosis were observed, highlighting the essential role of vWF in the development of infection-related complications. |
| Sultan Mehmood Kamran[89] | 2020 | 280 Covid-19 hospitalized patients in a single centre in Pakistan | TPE | ADAMTS13 | Early initiation of TPE was significantly associated with improved overall survival, faster resolution of cytokine release syndrome (CRS), reduced time to hospital discharge, and a notable decrease in microthrombus formation. |
| Faryal Khamis[90] | 2020 | 31 Covid-19 patients in the Royal Hospital of Oman | TPE | ADAMTS13 | TPE significantly decreased 28-day mortality and overall mortality rates, improved respiratory function, and significantly enhanced extubation rates. |
| Junmei Chen[84] | 2011 | Human plasma and mice | NAC | ULVWF, ADAMTS13 | NAC effectively reduces soluble plasma-type vWF polymers in a concentration-dependent manner and rapidly degrades ULVWF polymer chains extruded from activated ECs. Additionally, NAC inhibits vWF-dependent platelet aggregation and collagen binding, demonstrating its potential as a potent modulator of platelet function. |
| Gerardo Cabanillas[85] | 2015 | A patient with recurrent TTP following the failure of plasma exchange (PE) treatment, despite therapy with steroids, rituximab, cyclophosphamide, vincristine, and azathioprine. | NAC | ADAMTS13 | NAC treatment at a dose of 150 mg/kg was initiated on day 135 for a duration of 10 days, in conjunction with PE and low-dose steroids. The platelet count fully recovered, and the patient was successfully discharged. |
| Claudia Tersteeg[86] | 2016 | Preclinical mouse and baboon models of TTP | NAC | ULVWF, ADAMTS13 | Prophylactic administration of NAC, in the absence of concurrent plasmapheresis, effectively prevented the severe manifestations of TTP in mice. However, NAC did not alleviate the acute symptoms of TTP in either mice or baboons. |
| Amihai Rottenstreich[87] | 2015 | 3 patients with TTP were initially treated with PE, corticosteroids, and other immunosuppressants, in combination with NAC. | NAC | ADAMTS13 | A significant clinical improvement was observed in both symptoms, with platelet counts and ADAMTS13 activity levels returning to normal concurrently. |
| M. Scully[88] | 2019 | In a double-blind, controlled trial, randomly assigned 145 patients with TTP to receive caplacizumab or placebo during PE and for 30 days thereafter. | Caplacizumab | ADAMTS13 | In patients with TTP, treatment with caplacizumab was associated with a more rapid normalization of platelet counts. The combined incidence of TTP-related death, recurrence, or thromboembolic events during treatment was significantly low. Moreover, the recurrence rate of TTP during the study was notably lower compared to the placebo group. |
| Christof Aigner[92] | 2022 | Between 2012 and 2019, among patients treated with eculizumab, 15 were diagnosed with TMA, 6 with sTMA, and 2 with C3 glomerulopathy (C3G). | Eculizumab | C5b-9 | For cTMA patients who do not respond to plasma therapy, eculizumab is considered the treatment of choice. However, the response rates in patients with sTMA and C3G have been notably low. |
| Toshiyuki Ohta[93] | 2015 | A 4-month-old boy who developed aHUS presenting with undetectable C3 protein. | Eculizumab | C3 | Control of severe hypertension (HTN) and cessation of peritoneal dialysis. |

(*Continued*)

**Table 1** (Continued).

| Author | Year | Model | Drugs/ PROCEDURES | Target | Curative Effect |
|---|---|---|---|---|---|
| Naoko Ito[94] | 2016 | 10 children with aHUS were treated with eculizumab. 7 patients developed resistance to plasma therapy, while 3 remained dependent on it. Genetic mutations associated with the disease were identified in 5 patients, and 2 patients tested positive for autoantibodies against FH. Additionally, three patients had a family history of TMA. | Eculizumab | C5b-9 | Following the initiation of eculizumab, all patients achieved immediate hematological remission and were able to successfully discontinued plasma therapy. 9 patients regained renal function, while 2 ultimately progressed to end-stage renal disease (ESRD), necessitating long-term renal replacement therapy (RRT). No patient experienced a relapse of TMA during conventional eculizumab therapy, and no serious adverse events were reported during the follow-up period. |
| Masayoshi Okumi[95] | 2016 | A 22-year-old male patient with ESRD and aHUS, who experienced recurrent aHUS after renal transplantation and cTMA, was treated with PE in combination with eculizumab (900 mg) and followed up for 5 years. | Eculizumab | C5/C5b-9 | At 40 months, GBx showed no signs of mild interstitial fibrosis, TMA, nephritis, or glomerulitis. No significant adverse events or abnormal laboratory findings were observed, and renal function remained stable and intact. |
| Hironori Nakamura[96] | 2018 | A rare case involved a 76-year-old male patient with IgA nephropathy and TMA, who presented with aHUS linked to a mutation in the FH gene. | Eculizumab | C3 | Light microscopy confirmed the diagnosis of HUS, while immunofluorescence analysis detected the presence of IgA and C3. Genetic analysis revealed a p.Arg1215Gln mutation in the FH gene, and treatment with eculizumab has been ongoing for five months. |

inhibitor for treating complement-mediated TMA—paradoxically heightens infection risks due to its suppression of complement proteins, which are vital for immune defense. Eculizumab was approved in Japan in 2013 for the treatment of complement-mediated aHUS, with confirmed efficacy and safety in both children[93,94] and adults.[95,96] CD59, a C5b-9 inhibitor, is a membrane glycoprotein that prevents the formation of the MAC C5b-9 by integrating into the complex and blocking the uptake and insertion of C9 molecules during the C5b-8 phase. This inhibits C5b-9 formation and protects ECs from damage. In rat models, CD59 has been shown to protect glomerular ECs from immune-mediated TMA-induced damage. However, no large-scale clinical trials have directly targeted CD59 as a therapeutic option, which may represent a promising avenue for future research.[28]

## Summary

In sepsis, the hyperactivation of the complement system triggers rampant formation of MAC C5b-9, which assails vascular ECss and ignites catastrophic pathological cascades, emerging as a pivotal orchestrator of multiorgan failure. Mounting clinical evidence reveals that elevated C5b-9 concentrations demonstrate striking correlations with disease severity, cytokine storms, coagulopathic derangements, and microbial load, cementing its dual role as a prognostic beacon and therapeutic lodestar. Molecularly, C5b-9 subverts endothelial homeostasis through four-pronged warfare: unleashing torrents of proinflammatory cytokines to fuel systemic inflammaging; fracturing endothelial junctions to exacerbate vascular permeability; and crippling thrombomodulin-driven anticoagulant machinery, thereby turbocharging the coagulation-inflammation nexus. These synergistic endothelial insults coalesce into microvascular thrombosis and organ necrosis, with ruthless efficiency in renal, pulmonary, and hepatic territories. Rooted within this intricate mechanistic tapestry, we posit that C5b-9 orchestrates the pathological unleashing of vWF from ECs—relentlessly propelling platelet-ULVWF microclot formation and wholesale capillary obliteration. To decrypt this axis, imminent studies will map: the temporal dance between CD59 regulator and C5b-9 storm kinetics; ADAMTS13 protease failure as a microthrombosis perpetuator; dose-time-response relationships governing C5b-9-induced vWF multimer metamorphosis; and topographic alignment between microthrombus hotspots and organ injury signatures. Therapeutic innovation will

dual-wield: C5b-9 neutralization via monoclonal antibodies/complement inhibitors (anti-C5 biologics) to dismantle microthrombotic networks, coupled with ADAMTS13 rescue or vWF blockade strategies. The paradigm-shifting potential of this work resides in decoding the complement-endothelium-hemostasis triad: multidimensional profiling of C5b-9/vWF/ADAMTS13 dynamics may unlock precision prognostics; combinatorial C5b-9 inhibition (eculizumab analogs) and vWF pathway correction (recombinant ADAMTS13) could outmaneuver traditional anticoagulant pitfalls; while C5b-9 trajectory-guided chronotherapeutics and CRISPR-engineered CD59 enhancements might birth a new epoch of targeted critical care.

As the molecular keystone bridging endothelial cataclysm and microthrombotic avalanches in sepsis, C5b-9 unveils therapeutic frontiers for resuscitating failing organs. Silencing C5b-9 generation, resuscitating ADAMTS13 proteostasis, or intercepting malignant vWF surges could forge revolutionary interventions for microcirculatory catastrophe. This conceptual metamorphosis not only carries seismic potential to redefine sepsis trajectories but also illuminates fundamental mechanisms underlying thrombotic-inflammatory pandemonium—from aHUS to TMA—heralding an age of molecularly sculpted therapeutics.

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## Disclosure

The authors report no conflicts of interest in this work.

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