



Role of microthrombosis in coronary microvascular dysfunction

Junjie Bei¹, Houyuan Hu^{2,3,4}

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Microthrombosis, coronary microvascular dysfunction, inflammation, platelets, neutrophil extracellular traps, high mobility group box 1, biomarkers

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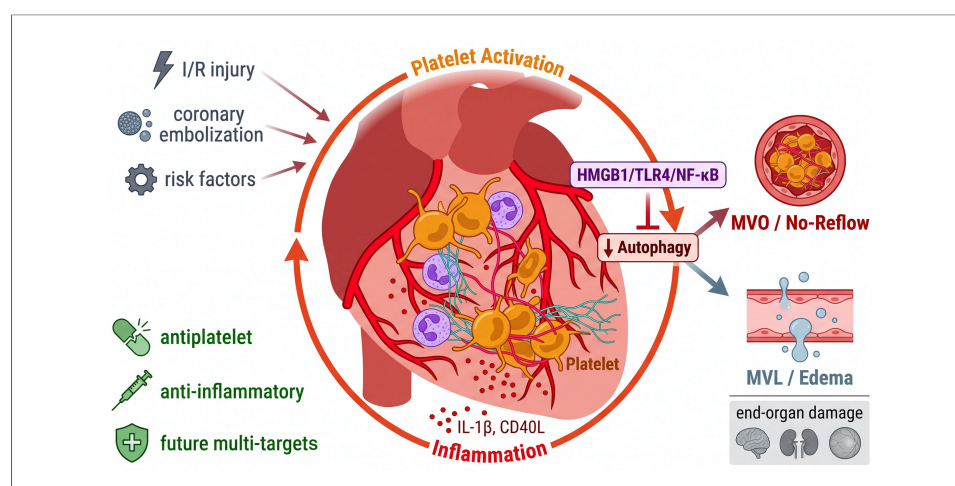
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Abstract

Coronary microvascular dysfunction is a common and prognostically important event of ischemic heart disease, yet effective therapeutic strategies remain limited. Microthrombosis is a pivotal driver of microvascular obstruction and no-reflow. This review focuses on the self-amplifying loop between inflammation and platelet activation that sustains microvascular occlusion. Within this loop, myocardial inflammatory signaling suppresses protective autophagy, predominantly in cardiomyocytes, and promotes endothelial dysfunction. The resulting prothrombotic endothelial surface supports activated platelet adhesion, inflammatory mediator release, and platelet-leukocyte aggregation. Neutrophil-derived web-like structures, known as neutrophil extracellular traps (NETs), further enhance platelet aggregation and fibrin deposition. These thromboinflammatory processes lead to microinfarcts, contractile dysfunction, and elevated cardiac troponin, and extend to multiple organs, particularly in patients with common cardiometabolic risk factors such as hypertension, diabetes, dyslipidemia, and hyperhomocysteinemia. Platelet-derived microparticles, platelet-monocyte aggregates,



¹Department of Cardiovascular Medicine, Guangxi Zhuang Autonomous Region Corps Hospital of Chinese People's Armed Police Forces, Nanning 530003, Guangxi, China.

²Department of Cardiovascular Medicine, Southwest Hospital, Third Military Medical University (Army Medical University), Chongqing 400038, China.

³Key Laboratory of Geriatric Cardiovascular and Cerebrovascular Disease, Ministry of Education of China, Chongqing 400038, China.

⁴Key Laboratory of Chronobiology and Cardiometabolic Disease, Chongqing Education Commission of China, Chongqing 400038, China.

Correspondence to: Dr. Houyuan Hu, Department of Cardiovascular Medicine, Southwest Hospital, Third Military Medical University (Army Medical University), 29 Gaotanyan Street, Shapingba District, Chongqing 400038, China. E-mail: hhy66@tmmu.edu.cn

and soluble activation markers show promise as diagnostic biomarkers, but further clinical validation is required. Current antiplatelet, anti-inflammatory strategies, or ischemic conditioning are limited by bleeding risk, narrow therapeutic windows, or inconsistent efficacy. We outline future directions, including developing validated biomarker panels, multi-target therapies that block key inflammatory pathways or NET formation, and human-relevant microphysiological models. Understanding the positive feedback cycle between inflammation and platelets is essential for developing novel diagnostic and therapeutic approaches to mitigate microvascular damage in coronary artery disease as well as other thromboinflammatory disorders.

INTRODUCTION

Coronary artery disease (CAD) remains a leading cause of morbidity and mortality worldwide. Timely restoration of epicardial blood flow by primary percutaneous coronary intervention (PCI) or thrombolysis is the standard of care for acute myocardial infarction (AMI). However, successful reopening of the culprit artery does not always translate into adequate myocardial perfusion. Up to 50% of patients with AMI may develop microvascular damage despite an open culprit artery, i.e., no-reflow phenomenon, which is associated with larger infarct size, adverse left ventricular (LV) remodeling, and increased mortality^[1,2]. A major contributor to this damage is coronary microembolization (CME), which occurs in up to 25% of patients undergoing primary PCI^[3]. Microvascular damage clinically manifests as microvascular obstruction (MVO, also termed “no-reflow”) and microvascular leakage (MVL)^[4,5]. Both MVO and MVL are independent predictors of poor prognosis, yet effective therapies remain limited. Being regarded as the central driver of MVO, microthrombosis has its pathogenesis profoundly amplified by several underlying common clinical conditions, including hypertension, which promotes endothelial dysfunction and vascular remodeling^[6]; diabetes mellitus, commonly associated with platelet hyperreactivity and a prothrombotic milieu^[7,8]; and hyperhomocysteinemia, with impaired fibrinolysis and augmented oxidative stress^[9].

This review examines the role of microthrombosis in driving MVO, with particular emphasis on the self-amplifying loop between inflammation and platelet activation. We synthesize recent evidence to show how inflammatory signaling sustains platelet reactivity while platelet-derived mediators in turn amplify endothelial dysfunction, perpetuating MVO. Breaking this cycle offers a unifying framework for understanding microvascular injury across diverse clinical settings and points toward new diagnostic and therapeutic strategies for CAD.

MICROTHROMBOSIS AS THE CORE OF MVO AND NO-REFLOW

Microthrombosis refers to the formation of platelet-rich fibrin thrombi within vessels smaller than 200 µm in diameter, impairing nutrient delivery and causing tissue ischemia and microinfarction. In the coronary microcirculation, microthrombi physically occlude capillaries and arterioles, directly contributing to MVO with tissue “no-reflow” phenomenon. As discussed in this review, this process is not limited to acute coronary syndrome (ACS); microthrombosis occurs in a wide range of clinical conditions including hypertension, diabetes mellitus, antiphospholipid syndrome, coronavirus disease 2019 (COVID-19), sepsis, heart failure, and systemic inflammatory diseases. Among the multiple contributors to MVO, such as endothelial swelling, vasoconstriction, and external compression, microthrombosis is recognized as the central pathological driver. In addition to the formation of platelet aggregates or platelet-neutrophil aggregates, plaque rupture releases aggregate debris and microparticles into the coronary microcirculation^[10,11]. Concurrently, ischemia-reperfusion (I/R) injury activates the coagulation cascade, exposes tissue factor (TF), and promotes platelet adhesion to damaged endothelium, resulting in formation of stable fibrin-rich microthrombi^[12]. In animal models of CME, injection of microspheres leads to patchy microinfarcts, impaired LV function, and elevated circulating levels of cardiac troponin, directly mimicking microthrombi-mediated myocardial injury^[13]. Autopsy studies of severe COVID-19 revealed von Willebrand

Factor (vWF)-rich and neutrophil extracellular trap (NET)-rich thrombi occluding microvessels^[14], underscoring the broad relevance of microthrombosis in cardiovascular pathology. A further example is Kawasaki disease, a systemic vasculitis in children, which also features persistent coronary microvascular dysfunction and microthrombosis independent of conventional atherosclerotic risk factors^[15,16].

PATHOPHYSIOLOGY OF MICROTHROMBOSIS: FROM ENDOTHELIAL INJURY TO STABLE MICROTHROMBI

Microthrombus formation in the coronary microcirculation follows a stepwise cascade, starting from endothelial injury and culminating in stable platelet-fibrin aggregates. The process involves the release of embolic materials from ruptured plaques, activation of the coagulation cascade, platelet adhesion and aggregation, and amplification by platelet-derived microparticles (PMPs). Importantly, microthrombosis exacerbates MVL, forming a vicious cycle.

Sources of microemboli

The embolic material responsible for MVO is heterogeneous, originating from rupture of upstream unstable atherosclerotic plaque, which not only releases plaque debris but also triggers the formation of platelet-neutrophil aggregates and sheds endothelial-derived microparticles (EMPs) and PMPs into the coronary microcirculation^[10,11]. A clinical study in ST-segment elevation myocardial infarction (STEMI) patients found that intracoronary levels of EMPs and PMPs are elevated and correlate with thrombus burden^[11]. These cellular and subcellular components physically occlude capillaries and arterioles, contributing directly to the “no-reflow” phenomenon^[5].

Endothelial injury and activation of coagulation cascade

Endothelial dysfunction is a critical initiating event in the pathogenesis of microthrombosis [Figure 1]^[17]. Upon endothelial injury, the potent procoagulant protein TF is exposed, triggering the extrinsic coagulation cascade. In a study involving clinical cohorts and animal models^[12], elevated levels of trimethylamine N-oxide (TMAO), a gut microbiota-derived and oxidized metabolite, are associated with increased risk of major adverse cardiovascular events (MACE); the animal experiments further demonstrate that TMAO dose-dependently induces expression of TF and vascular cell adhesion molecule-1 (VCAM-1) in human microvascular endothelial cells, thereby enhancing arterial thrombosis potential. This endothelial disruption is often exacerbated by underlying conditions, such as hypertension, which promotes oxidative stress and reduces nitric oxide (NO) bioavailability, further predisposing the microvasculature to a prothrombotic status^[6]. In addition, damaged endothelium releases high mobility group box 1 (HMGB1) as a damage-associated molecular pattern (DAMPs), which further promotes inflammatory signaling (see Section “The HMGB1/TLR4/NF- κ B pathway: a key signaling axis” and Figure 1).

Platelet adhesion, activation, and aggregation

Following endothelial injury, platelet adhesion is primarily mediated by the glycoprotein Ib-IX-V complex (GPIb-IX-V) through binding to vWF that anchors on the exposed subendothelial matrix [Figure 2]. This interaction is amplified by high shear stress. In a mouse model of venothrombosis, inflammatory mediators can induce CD40L-mediated release of ultra-large vWF that strings from venous endothelium and avidly binds to platelets^[18]. Subsequent platelet activation is reinforced by signaling pathways involving Toll-like receptor 4 (TLR4) and leads to the conformational activation of integrin α IIB β 3 (the GPIIb/IIIa receptor). Upon activation, this integrin binds to fibrinogen, forming a molecular bridge crosslinking adjacent platelets, thereby consolidating stable platelet aggregation^[19] [Figure 2]. In a mouse model of endotoxemia, Rumbaut *et al.* demonstrated that lipopolysaccharide (LPS) enhances formation of venular platelet thrombus via a TLR4-dependent mechanism^[20]. Platelet surface P-selectin (CD62P) is rapidly expressed upon activation but is transient and quickly shed. Importantly, hyperactive platelets are a hallmark of

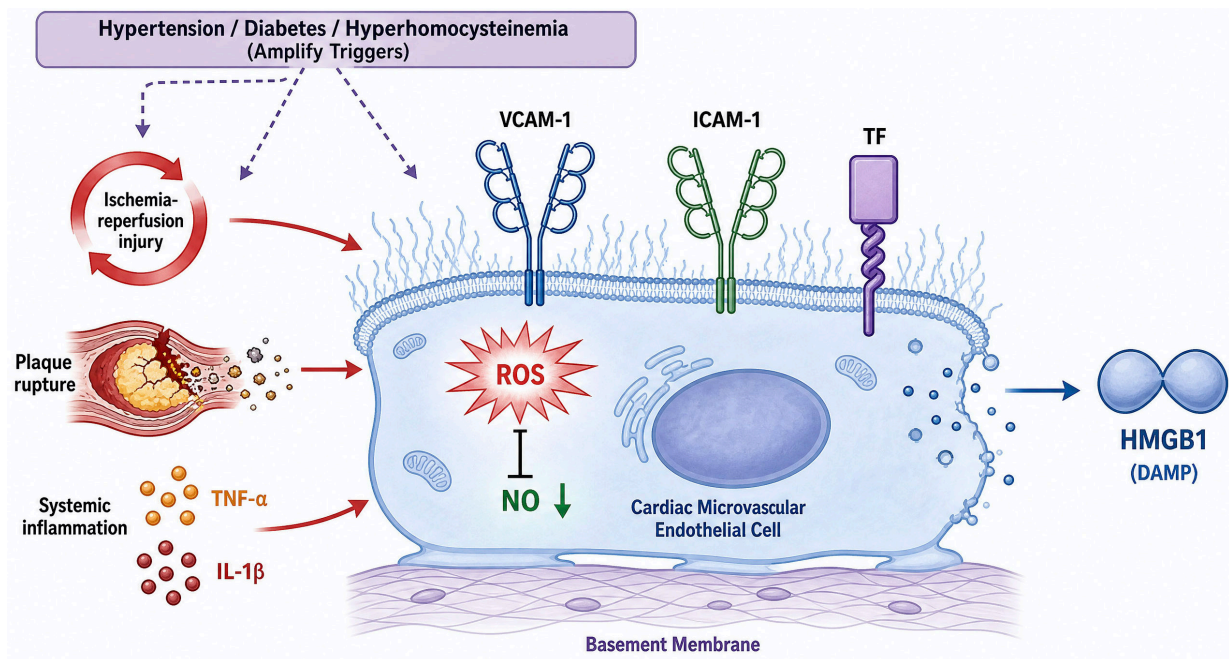


Figure 1. Triggers and endothelial dysfunction. Diverse initiating stimuli, including ischemia-reperfusion injury, plaque rupture, and systemic inflammation ($\text{TNF-}\alpha$, $\text{IL-1}\beta$), all amplified by hypertension, diabetes, and hyperhomocysteinemia, converge on the coronary microvascular endothelium. This results in oxidative stress ($\text{ROS}\uparrow/\text{NO}\downarrow$), upregulation of adhesion molecules (VCAM-1, ICAM-1), TF exposure, and release of HMGB1 as a damage-associated molecular pattern, establishing a prothrombotic and proinflammatory microenvironment. VCAM-1: Vascular cell adhesion molecule-1; ICAM-1: intercellular adhesion molecule-1; TF: tissue factor; HMGB1: high mobility group box 1; $\text{TNF-}\alpha$: tumor necrosis factor- α ; $\text{IL-1}\beta$: interleukin-1 β ; ROS: reactive oxygen species; NO: nitric oxide.

prothrombotic conditions like diabetes, significantly amplifying this response^[21].

Role of platelet microparticles and fibrin deposition

Upon activation, platelets shed submicron vesicles known as PMPs. Experimental studies show that these vesicles carry platelet surface antigens such as GPIIb/IIIa, P-selectin, and CD40L (see Section “Platelets as bidirectional mediators of inflammation” for detailed signaling mechanisms), and expose phosphatidylserine on their outer leaflet [Figure 2]^[22]. The exposure of phosphatidylserine provides a high-affinity surface for the assembly of tenase and prothrombinase complexes, thereby accelerating thrombin generation and fibrin deposition. Beyond promoting thrombin generation, PMPs themselves can bind directly to fibrin fibers, as shown in an experimental thrombosis model where PMPs generated during thrombus formation were found to associate with local fibrin^[23]. Additionally, PMPs have been shown to increase platelet and fibrin deposition on arterial walls, thereby promoting mural thrombus formation^[24].

As depicted in Figure 3, there exists a multi-step cascade covering endothelial activation, platelet aggregation, neutrophil extracellular trap formation (NETosis), and thrombus formation, in which PMPs greatly amplify the coagulation cascade and consolidate the nascent platelet aggregate into a stable microthrombus. The prothrombotic role of PMPs is supported by human pathological evidence: autopsy studies of severe COVID-19 have identified vWF-rich thrombi together with PMPs in the pulmonary microvasculature, suggesting that PMPs cooperate with endothelial-derived vWF and NETs to propagate microvascular occlusion^[14,25]. Thus, PMPs function not merely as passive by-products of platelet activation but as active participants that promote stable microthrombus formation.

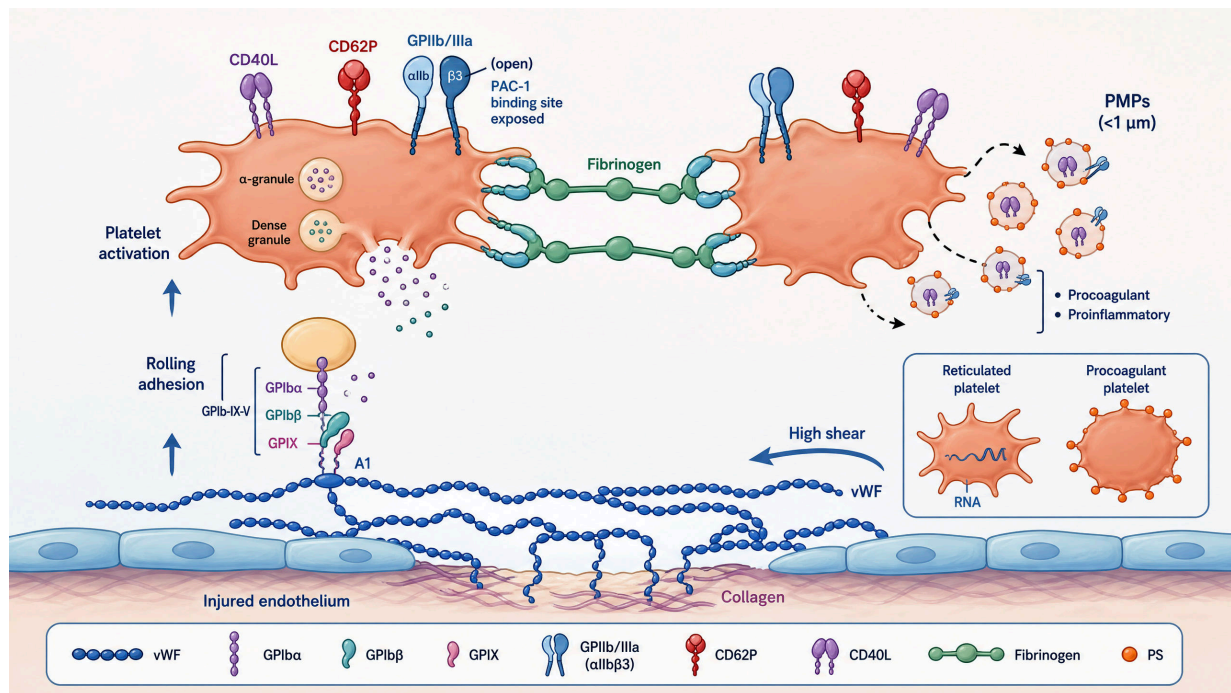


Figure 2. Platelet adhesion, activation and platelet-derived microparticles. Platelets initially tether to exposed vWF via the GPIb-IX-V receptor under high shear stress, then undergo activation marked by GPIIb/IIIa (integrin $\alpha\text{IIb}\beta 3$) conformational change detected by PAC-1 (a monoclonal antibody that specifically recognizes the active conformation of integrin $\alpha\text{IIb}\beta 3$), CD62P and CD40L expression, with fibrinogen crosslinking adjacent platelets to consolidate aggregates. Activated platelets additionally shed procoagulant (phosphatidylserine-exposing) and proinflammatory (CD40L-bearing) microparticles (PMPs), which further amplify both thrombosis and inflammation. vWF: von Willebrand factor; GPIb-IX-V: glycoprotein Ib-IX-V complex; GPIIb/IIIa: glycoprotein IIb/IIIa (integrin $\alpha\text{IIb}\beta 3$); CD62P: P-selectin; CD40L: CD40 ligand; PMPs: platelet-derived microparticles; PS: phosphatidylserine.

Interactions of microthrombi with MVL

The process of microthrombosis is closely intertwined with MVL, creating a vicious cycle that worsens tissue perfusion. I/R injury damages endothelial cell-cell junctions and degrades the glycocalyx, a surface layer of cross-linked glycoproteins and glycolipids, leading to increased vascular permeability and interstitial edema^[5]. Tissue edema compresses microvessels externally, worsening luminal obstruction from microthrombi and contributing to the “no-reflow” phenomenon. In a mouse cardiac I/R model, the MVL-affected area exceeds the infarction and MVO regions, and MVL severity correlates with LV dilatation and functional decline^[4].

Activated platelets and PMPs directly modulate endothelial barrier function through multiple mechanisms. Shedding from activated platelets, PMPs carry surface molecules such as P-selectin, CD40L, and GPIIb/IIIa that can interact with endothelial cells^[26] (Figure 2, right portion depicting PMP surface molecules). Studies in human umbilical vein endothelial cells found that PMPs dose-dependently promoted tube formation, migration, and proliferation, effects that were mediated by upregulation of matrix metalloproteinase (MMP)-2 and MMP-9 via extracellular signal-regulated kinase signaling, and inhibition of these MMPs abolished the pro-angiogenic and pro-migratory effects of PMPs^[26].

The interaction between platelets and endothelial cells downregulates protein expression of tight junctions (claudin-5, occludin, zonula occludens protein-1) and adherens junctions (VE-cadherin), resulting in paracellular gap formation and hyperpermeability^[5,27]. In cardiac microvascular endothelial cells, I/R injury induces cytoskeletal reorganization and gap junction disassembly, leading to increased permeability and extravasation^[4]. Peptide hormone relaxin has been shown to counteract these detrimental effects: *in vitro*, it

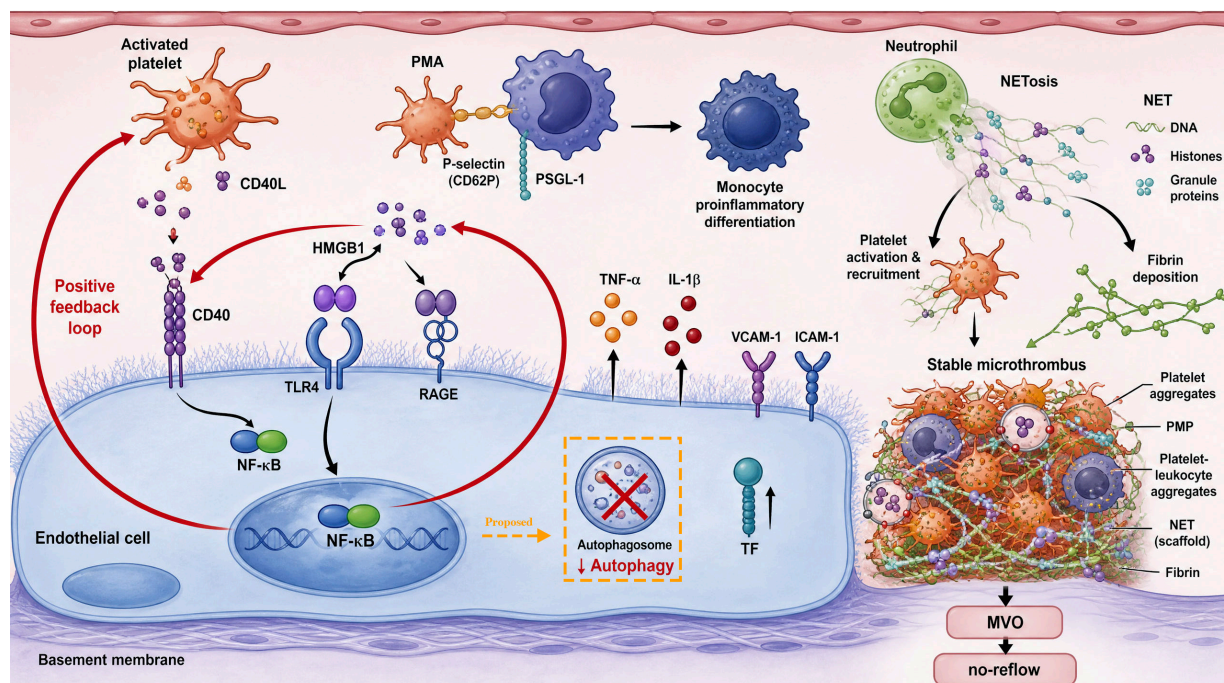


Figure 3. Platelet-mediated inflammation, HMGB1/TLR4/NF- κ B axis, and microthrombosis. The central positive feedback loop between inflammation and platelet activation is driven by the HMGB1/TLR4/NF- κ B pathway in the endothelial cell, which upregulates pro-inflammatory cytokines (TNF- α , IL-1 β) and adhesion molecules (VCAM-1, ICAM-1) while suppressing protective autophagy. Within the vascular lumen, activated platelets release CD40L and IL-1 β that further amplify this axis via endothelial CD40, form proinflammatory PMAs through P-selectin/PSGL-1 interaction, and shed PMPs into blood circulation. Neutrophils release NETs (DNA with bead-like histones) that capture platelets and promote fibrin deposition. All components converge within the lumen to form a stable microthrombus containing platelet aggregates, PMPs, platelet-leukocyte aggregates, NET scaffolds, and fibrin strands, ultimately causing MVO and no-reflow. The orange dashed box/arrow and the “Proposed” label indicate that the illustrated HMGB1/TLR4/NF- κ B-mediated autophagy suppression is based on the evidence from whole-myocardium studies (predominantly cardiomyocytes), and definitive endothelial-specific verification is yet to be collected. Solid arrows denote established endothelial signaling events. HMGB1: High mobility group box 1; TLR4: toll-like receptor 4; RAGE: receptor for advanced glycation end products; NF- κ B: nuclear factor kappa-B; TNF- α : tumor necrosis factor- α ; IL-1 β : interleukin-1 β ; VCAM-1: vascular cell adhesion molecule-1; ICAM-1: intercellular adhesion molecule-1; TF: tissue factor; CD40L: CD40 ligand; CD40: CD40 receptor; PSGL-1: P-selectin glycoprotein ligand-1; PMPs: platelet-derived microparticles; PMAs: platelet-monocyte aggregates; NET: neutrophil extracellular trap; MVO: microvascular obstruction.

attenuates hypoxia-reoxygenation, H_2O_2 , or cytokine-induced endothelial hyperpermeability independently of NO but with preservation of VE-cadherin; in a mouse cardiac I/R model, it reduces Evans blue extravasation by 56% in jeopardized myocardium, decreases leukocyte density and cytokine expression, and partially preserves LV contractile function while limiting chamber dilatation^[28].

Activated platelets also release vasoconstrictors such as thromboxane A2 and endothelin-1, exacerbating microvascular constriction and the “no-reflow” phenomenon^[2]. In the context of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, pericyte dysfunction plays a critical role in MVVL. Using a vascular organoid model, researchers demonstrated that pericytes preferentially take up SARS-CoV-2 viral particles due to high expression of angiotensin-converting enzyme 2 (ACE2), leading to pericyte detachment, damage and cell death, disruption of pericyte-endothelial cell crosstalk, microvascular endothelial hyperpermeability, and enhancement of platelet recruitment and vWF deposition in capillaries^[29]. Endothelial dysfunction in severe COVID-19 is further characterized by decreased expression of CD31 (an endothelial marker) and high expression of vWF, as observed in post-mortem cardiac tissues from 88 patients, directly linking viral infection to a prothrombotic endothelial phenotype^[30].

Table 1. Roles and points of convergence of molecular pathways in microthrombosis

Role in microthrombosis	Pathway	Key molecules/factors and points of crosstalk	Principal contribution
Primary driver and execution core	Endothelial activation and platelet adhesion	TF, VCAM-1, ICAM-1, vWF, ROS, NO; GPIb-IX-V, αIIbβ3, fibrinogen, P-selectin, CD40L	Initiates and executes microthrombus formation; links a prothrombotic endothelial surface to stable platelet-fibrin aggregates ^[6,12,18,19,31]
Dominant inflammatory amplifier	HMGB1-TLR4-NF-κB signaling	HMGB1, TLR4, NF-κB, RAGE, PMPs, TF, VCAM-1, ICAM-1	Sustains inflammation, suppresses protective autophagy, and promotes a procoagulant endothelial phenotype; PMP-derived HMGB1 exacerbates MVO ^[13,20,61,63]
Secondary amplifier and co-stimulator	Amplification mediated by CD40L/CD40, P-selectin/PSGL-1, and microparticles	CD40L, CD40, P-selectin, PSGL-1, PMPs, PMAs, TF, NET formation	Reinforces the core axis, recruits leukocytes, and bridges thrombosis with inflammation ^[11,22,32,33,100]
Scaffolding and thrombus stabilization	NET-vWF-fibrin scaffold	NETs, vWF, fibrin, factor XII	Forms NET- and vWF-rich microthrombi; promotes fibrin deposition and MVO ^[14,50,55,111]

TF: Tissue factor; VCAM-1: vascular cell adhesion molecule-1; ICAM-1: intercellular adhesion molecule-1; vWF: von Willebrand factor; ROS: reactive oxygen species; NO: nitric oxide; GPIb-IX-V: glycoprotein Ib-IX-V complex; αIIbβ3: integrin αIIbβ3; CD40L: CD40 ligand; CD40: CD40 receptor; P-selectin: CD62P; PSGL-1: P-selectin glycoprotein ligand-1; PMPs: platelet-derived microparticles; PMAs: platelet-monocyte aggregates; HMGB1: high mobility group box 1; TLR4: Toll-like receptor 4; NF-κB: nuclear factor kappa-B; RAGE: receptor for advanced glycation end products; NETs: neutrophil extracellular traps; MVO: microvascular obstruction.

INFLAMMATION AND PLATELETS: THE POSITIVE FEEDBACK LOOP DRIVING MICROTHROMBOSIS

Inflammation and platelet activation are not independent processes but rather fuel each other in a vicious cycle. The following subsections examine the key cellular and molecular interactions that sustain this positive feedback loop, including inflammation-induced endothelial dysfunction, the inflammatory phenotypes of platelets, neutrophil-mediated inflammatory responses, and specific inflammatory signaling cascades. Rather than acting as parallel or independent modules, these pathways function primarily as inflammatory amplifiers, secondary co-stimulators, or context-restricted modulators that converge on the core endothelial-platelet axis described in Section “PATHOPHYSIOLOGY OF MICROTHROMBOSIS: FROM ENDOTHELIAL INJURY TO STABLE MICROTHROMBI”. Their relative roles and points of crosstalk are summarized in [Table 1](#).

Platelets as bidirectional mediators of inflammation

Platelets are not merely passive targets of inflammatory signals but also actively sense and amplify inflammation through reciprocal interactions with immune cells and the endothelium. Activated platelets function as genuine immune cells, expressing pattern recognition receptors such as TLR4 that can be directly engaged by pathogen-associated molecular pattern (PAMPs) or DAMPs^[20]. In murine models, endotoxin (LPS) enhanced venular platelet thrombus formation via a TLR4-dependent mechanism, and this effect was independent of neutrophils, demonstrating that platelets can directly sense inflammatory signals without requiring intermediary immune cells^[20]. Upon activation, platelets release a wide array of inflammatory mediators stored in their α- and dense granules, including soluble CD40 ligand (sCD40L), interleukin-1β (IL-1β), platelet factor 4, and thrombospondin-1, which act on endothelial cells and leukocytes to augment inflammation^[31].

Critical to the proinflammatory properties of platelets is platelet-derived CD40L that binds to CD40 receptors on endothelial cells and monocytes, triggering nuclear factor kappa-B (NF-κB)-dependent expression of cytokines, chemokines, and TF, thereby amplifying the inflammatory cascade together with a procoagulant state ([Figure 3](#), left portion depicting CD40/CD40L axis)^[22]. In an experimental study using staphylococcal superantigen-like protein 5-induced platelet microparticles, CD40L on PMPs was shown to

directly interact with CD40 on monocytes, leading to activation of the TNF receptor-associated factor 6/NF- κ B signaling pathway and a robust production of IL-1 β , tumor necrosis factor- α (TNF- α), monocyte chemoattractant protein-1, and MMP-9^[22]. Blockade of CD40L or CD40 significantly reduced these proinflammatory responses, highlighting the CD40/CD40L axis as a key driver of platelet-mediated inflammation (Figure 3, left portion depicting the CD40/CD40L axis).

P-selectin on activated platelets can bind to P-selectin glycoprotein ligand-1 (PSGL-1) on monocytes or neutrophils, forming platelet-leukocyte aggregates^[32]. Genetic deficiency of Mac-1 (CD11b/CD18) or mutation of its binding site on platelet GPIb α markedly delayed thrombosis in murine models without affecting hemostasis, indicating that platelet-leukocyte adhesion is a specific and potentially targetable component of the thromboinflammation cascade^[32]. These platelet-monocyte aggregates (PMAs) promote monocyte differentiation toward a proinflammatory phenotype and enhance the release of MMPs, such as MMP-9, which degrade the extracellular matrix and facilitate vascular remodeling and further inflammation^[33,34]. In patients with type 2 diabetes mellitus, platelet hyperreactivity is particularly pronounced, which contributes to an exaggerated I/R injury response and greater risk of both microvascular and macrovascular complications, underscoring the clinical importance of platelet-mediated inflammation in common metabolic diseases^[7,21].

Beyond these classical pathways, several non-canonical mechanisms have recently been identified to link platelet activation to microthrombosis. In platelets from patients with STEMI, stress-activated protein kinase-interacting protein 1 (Sin1), an essential component of mammalian target of rapamycin complex 2, was found to be phosphorylated at T86, and such phosphorylation correlated with no-reflow phenomena after revascularization^[35]. Importantly, platelet-specific Sin1 deficiency protects mice from ischemia-induced microvascular embolization and subsequent heart dysfunction, and Sin1-mediated outside-in signaling through integrin α IIb β 3 is a critical regulator of platelet-driven microthrombosis^[35]. In a rat model of type 2 diabetes, fibrinogen-like protein 2 is highly expressed in cardiac microvascular endothelial cells. Its upregulation is switched on by local or circulating TNF- α and is associated with cardiac hyaline microthrombosis and impaired diastolic and systolic function^[36]. In Kawasaki disease, thymic stromal lymphopoietin has been identified as a platelet-activating cytokine that induces mitophagy via a novel pathway and promotes thrombosis^[37], representing another non-canonical pathway by which inflammatory mediators directly drive platelet-mediated microvascular injury.

Platelet subclasses in thromboinflammation

Circulating platelets are not a homogeneous population but exhibit considerable functional and maturational heterogeneity^[38]. Based on functional characteristics, platelets can be categorized into procoagulant, aggregatory, and secretory subtypes, each contributing distinctly to thrombus formation and inflammatory modulation^[38]. Among these, procoagulant platelets are particularly relevant to microthrombosis. Marked by sustained Ca²⁺ elevation and surface exposure of phosphatidylserine, procoagulant platelets provide a catalytic platform for tenase and prothrombinase complexes, thereby accelerating thrombin generation and fibrin deposition at sites of microvascular injury^[39]. This procoagulant phenotype positions these cells at the dynamic intersection between coagulation and inflammation, as their activation is potently modulated by inflammatory mediators, and they in turn release signals that perpetuate endothelial dysfunction^[39].

Platelets can also be classified by maturational stage into reticulated (immature) and non-reticulated (mature) platelets. Reticulated platelets (RPs)—newly released from megakaryocytes and rich in residual messenger RNA—exhibit a prothrombotic transcriptome with enrichment of receptors critical for platelet activation and aggregation^[40], and demonstrate heightened propensity for arterial thrombus incorporation under high-shear conditions comparable to those of stenotic coronary arteries^[41] (Figure 2, left portion

depicting GPIb-IX-V/vWF interaction under high shear stress). Clinical studies have revealed that elevated RP content correlates with increased platelet reactivity and reduced efficacy of standard dual antiplatelet therapy in patients with CAD^[42], and that the immature platelet fraction functions as an independent long-term predictor of MACE in ACS patients^[43]. Multi-omic profiling of RPs has further identified selectively enriched glycoprotein VI (GPVI) and phosphatidylinositol 3-kinase (PI3K) signaling pathways, whose pharmacological inhibition effectively reduces RP-mediated aggregation and thrombus recruitment^[44].

Inflammation-induced endothelial dysfunction

The inflammatory mediators released by activated platelets, together with other inflammatory cytokines such as IL-1 β and TNF- α , activate NF- κ B in endothelial cells, leading to the upregulation of adhesion molecules including intercellular adhesion molecule-1 (ICAM-1), VCAM-1, and P-selectin^[6] (Figure 1, right portion showing NF- κ B-driven upregulation of VCAM-1 and ICAM-1). This inflammatory activation creates a pro-adhesive surface that captures circulating platelets and leukocytes, initiating the thromboinflammatory cascade. TMAO directly promotes this prothrombotic transformation by dose-dependently inducing expression of TF and VCAM-1 in human microvascular endothelial cells. Notably, this effect persists despite antiplatelet therapy, indicating that TMAO-enhanced thrombosis is mediated at least in part through endothelial pathways beyond platelet inhibition^[12].

Hypertension and other metabolic risk factors exacerbate endothelial dysfunction through multiple molecular mechanisms. Increased reactive oxygen species (ROS) production via NADPH oxidase activation reduces NO bioavailability, uncouples endothelial nitric oxide synthase (eNOS), and promotes angiotensin II-mediated vasoconstriction, all of which shift the endothelium toward a proinflammatory and prothrombotic phenotype^[6]. The loss of normal antithrombotic and anti-inflammatory functions of endothelial cells leads to dysregulation of coagulation, complement activation, and platelet aggregation in the microvasculature, a state now recognized as central to thromboinflammation across a broad range of disorders including sepsis, I/R injury, and the antiphospholipid syndrome^[45]. In patients with antiphospholipid syndrome as an autoimmune disorder, persistent endothelial dysfunction, accelerated intimal hyperplasia, and secretion of inflammatory products are hallmark features that predispose to microvascular thrombosis and cardiovascular events^[46].

Beyond classical oxidative stress pathways, iron metabolism has emerged as a key regulator of inflammation and thrombosis. Iron overload promotes ROS generation via the Fenton reaction, activating NF- κ B and exacerbating endothelial dysfunction^[47]. In this context, ferroptosis—an iron-dependent, lipid peroxidation-driven cell death—disrupts endothelial integrity, promotes phosphatidylserine externalization and expression of TF, thereby amplifying prothrombotic signals. Use of ferroptosis inhibitors restores endothelial homeostasis in thrombotic models^[48,49].

NETs promote platelet aggregation and fibrin deposition

Neutrophils recruited to sites of microvascular injury undergo a specialized form of cell death called NETosis, releasing NETs composed of decondensed DNA, histones, and granule proteins^[17]. Acting as a physical scaffold, NETs capture and directly activate platelets, promote platelet adhesion and aggregation, and initiate the intrinsic coagulation pathway via factor XII, ultimately leading to enhanced fibrin deposition (Figure 3, lower right area depicting NETs and fibrin scaffold). In mouse models of myocardial I/R injury, NETs were identified within the microvasculature of the ischemic zone, and treatment with DNase to degrade NETs reduced MVO and improved myocardial perfusion^[50]. In sepsis and I/R injury models, similar benefits have been observed with DNase treatment, highlighting the therapeutic potential of targeting NETs in thromboinflammatory diseases^[45].

There is strong clinical evidence for the association between NETosis and microthrombosis across diverse inflammatory conditions. In severe COVID-19, post-mortem analyses have demonstrated NET-rich thrombi occluding microvessels, with vWF and NETs synergistically contributing to thrombotic microangiopathy^[14]. This thromboinflammatory state extends to post-acute sequelae of COVID-19 (long COVID), where ongoing NET-driven pathology is strongly indicated by persistent neutrophil activation, elevated platelet-neutrophil aggregates, and circulating microclots^[51]. In addition to NETosis, SARS-CoV-2 infection directly amplifies endothelial injury through viral invasion of ACE2-expressing cells, triggering inflammation, microthrombosis, and angiogenesis^[52]. Consistent with this, post-mortem studies of COVID-19 patients consistently demonstrate small coronary vessel microthrombosis alongside a systemic inflammatory infiltrate dominated by CD3+/CD8+ cytotoxic lymphocytes and CD68+ macrophages^[53], underscoring the broad cardiovascular complications of this thromboinflammatory state^[54]. Beyond COVID-19, NETs have been implicated in atrial fibrillation—where levels of NETs in left atrial blood samples correlate with those of vWF and cardiac injury markers^[55]—as well as in vaccine-associated thrombosis, trauma-induced hypercoagulability, and complement-mediated thrombotic microangiopathy, where NETs cooperate with vWF and activated complement to promote MVO^[56–58]. In the failing heart due particularly to ischemic cardiomyopathy, endothelial vWF expression and Weibel-Palade bodies are markedly increased and correlate with coronary microthrombi^[59].

The HMGB1/TLR4/NF- κ B pathway: a key signaling axis

HMGB1 is a nuclear protein that functions as a DAMP when released from injured, necrotic, or activated cells. Once in the extracellular space, HMGB1 triggers robust inflammatory responses by binding to TLR4 and the receptor for advanced glycation end products (RAGE)^[60,61] (Figure 3, left-hand pathway depicting HMGB1/TLR4/NF- κ B signaling). In a rat model of CME induced by LV injection of 4000 polyethylene microspheres (45 μ m diameter) into the LV cavity, HMGB1 expression was significantly upregulated and translocated from the nucleus to the cytoplasm, accompanied by increased expression of TLR4 and NF- κ B and a marked decrease in autophagic activity in myocardial tissue (predominantly in cardiomyocytes) as reflected by reduced LC3BII/LC3BI and Beclin-1 protein levels^[13].

Yang *et al.* reported in a CME model that pharmacological inhibition of HMGB1 using glycyrrhizin administered 30 min before CME markedly reduced HMGB1 nuclear export, suppressed TLR4 and NF- κ B expression, and significantly restored autophagic activity^[13]. This restoration of autophagy was associated with substantial cardioprotection, including improved left ventricular ejection fraction (LVEF) and reduced microinfarct size (see Section “Myocardial level: microinfarction, contractile dysfunction, and biomarker elevation” for details). Critically, these protective effects were completely abrogated by pretreatment with the autophagy inhibitor 3-methyladenine, demonstrating that the beneficial actions of HMGB1 inhibition are mediated through restoration of autophagic flux rather than direct anti-inflammatory effects alone^[13] (Figure 3, left-hand pathway depicting HMGB1/TLR4/NF- κ B signaling). It should be noted that the evidence for HMGB1/TLR4/NF- κ B-mediated autophagy suppression in this model is derived from whole myocardial tissue; hence, it is likely that cardiomyocytes are the primary cell type for the observed autophagic changes^[13,62]. However, recent studies have established that HMGB1-TLR4 signaling is functionally active in microvascular endothelial cells, inducing proinflammatory and procoagulant phenotypes^[63], and that HMGB1 can regulate autophagy in cardiac microvascular endothelial cells under stress conditions^[64]. It remains to be determined whether HMGB1/TLR4/NF- κ B directly suppresses autophagy in cardiac microvascular endothelial cells, and whether the observed effects are mediated indirectly via paracrine signals from neighboring cells. Endothelial-specific genetic deletion of HMGB1 or TLR4 in future studies will be essential to confirm the cell-autonomous function of this signaling axis and to dissect direct versus indirect regulatory mechanisms. In the failing human heart—independent of COVID-19—microthrombi are increased 7- to 9-fold compared to control hearts, and this is associated with a 3.5- to 5-fold increase in

Weibel-Palade bodies and vWF expression, especially in ischemic cardiomyopathy. PECAM-1 is also upregulated but apparently insufficient to compensate for the prothrombotic shift^[59]. These findings underscore that the HMGB1-mediated inflammatory pathway and vWF-related prothrombotic pathway are conserved mechanisms of microthrombosis across different etiologies of heart failure^[59,60].

In macrophages, HMGB1 is known to promote the secretion of matrix vesicles via the RAGE/p38 MAPK/neutral sphingomyelinase 2 pathway, leading to ectopic mineralization and further vascular dysfunction^[65]. In RAW264.7 macrophages, HMGB1 stimulation significantly increased secretion of matrix vesicles that exhibited higher activity of tissue non-specific alkaline phosphatase and greater potential to initiate mineralization when tested in collagen-coated dishes or subcutaneous injection models *in vivo*^[65]. These findings indicate that HMGB1's prothrombotic and proinflammatory roles extend beyond the immediate injury phase to include long-term vascular remodeling and calcification, the processes known to exacerbate atherosclerosis progression and heart failure.

The interdependent cycle of inflammation, platelet activation, endothelial barrier disruption, and thrombosis thus represents a central therapeutic target (see also the reciprocal interactions between microthrombosis and MVL in Section "Interactions of microthrombi with microvascular leakage"). Interruption of any node in this cycle - whether by antiplatelet agents (aspirin, P2Y₁₂ inhibitors, GPIIb/IIIa antagonists), anti-inflammatory biologics (IL-1/IL-6 inhibitors, HMGB1 inhibitors such as glycyrrhizin), or targeted inhibition of neutrophil NETosis - holds promise for mitigating microvascular damage in AMI, COVID-19, and other thromboinflammatory disorders.

CONSEQUENCES OF MICROTHROMBOSIS: MYOCARDIAL INJURY AND ORGAN DYSFUNCTION

Microthrombosis not only compromises coronary flow but also triggers direct myocardial damage and remote organ dysfunction. The following subsections detail the consequences at the myocardial level - including microinfarction, contractile failure, and biomarker elevation - as well as the multi-organ involvement and the amplifying effects of underlying diseases such as hypertension, diabetes, and hyperhomocysteinemia.

Myocardial level: microinfarction, contractile dysfunction, and biomarker elevation

Microthrombosis directly contributes to myocardial injury by forming microinfarcts characterized by small, discrete areas of cardiomyocyte necrosis resulting from occlusion of the coronary microvasculature. In animal models of CME, this histologic damage translates into profound contractile dysfunction—marked by a substantial reduction in LVEF and progressive LV dilatation—along with elevated serum cardiac troponin I levels, confirming ongoing cardiomyocyte necrosis^[13]. Notably, HMGB1 inhibition with glycyrrhizin significantly attenuates both microinfarct burden and LV functional decline, whereas co-administration of the autophagy inhibitor 3-methyladenine abrogates these protective effects, indicating that autophagy restoration is a critical downstream mediator of HMGB1-driven myocardial injury^[13]. Using the same rat CME model, Su *et al.* further demonstrated that oxidative stress induced by USP16 S-nitrosylation disrupted glutathione homeostasis, and that blocking this modification alleviated cardiac dysfunction^[66]. In line with these preclinical findings, clinical observations show that the degree of contractile dysfunction correlates with the extent of MVO in STEMI patients undergoing primary PCI^[67].

Biochemical markers of myocardial injury, particularly cardiac troponin I, are significantly elevated in the setting of microthrombosis. In the CME rat model, serum cardiac troponin I levels increased markedly compared to sham controls, reflecting ongoing cardiomyocyte necrosis^[13]. In patients with STEMI, the

Table 2. Mechanisms of microthrombosis across different organs

Organ	Microvascular pathology	Key drivers	Shared mechanisms with heart	Unique features	References
Heart	MVO; MVL; no-reflow; microinfarcts	Platelet activation; NETs; HMGB1/TLR4 signaling; endothelial dysfunction	-	Ischemia-reperfusion injury; cardiomyocyte necrosis; troponin elevation	[4,5,13,14,15]
Kidney	Glomerular capillary thrombosis; tubular injury; proteinuria	Endothelial injury; complement activation (C5b-9); RAAS activation	Endothelial dysfunction; platelet activation; oxidative stress	Prominent complement involvement; hyperfiltration; proteinuria	[6,58,72]
Brain	White matter hyperintensities; lacunar infarcts; microhemorrhages	Lipohyalinosis; fibrinoid necrosis; endothelial dysfunction	Oxidative stress; reduced NO bioavailability	Hypertension-induced small vessel remodeling; blood-brain barrier disruption	[6]
Lung	Microvascular thrombosis; alveolar-capillary injury; pulmonary hypertension	NETs; vWF; complement activation; endothelial activation	vWF/NETs-mediated thromboinflammation	Pressure overload (HF)-induced endothelial activation; CD40 deficiency phenotype; SARS-CoV-2 tropism	[14,58,75]
Retina	Microhemorrhages; cotton-wool spots; increased permeability	Endothelial dysfunction; oxidative stress	NO reduction; ROS increase	Visible surrogate of systemic microvascular damage; correlates with cardiovascular and renal outcomes	[6]

MVO: Microvascular obstruction; MVL: microvascular leakage; NETs: neutrophil extracellular traps; HMGB1: high mobility group box 1; TLR4: toll-like receptor 4; RAAS: renin-angiotensin-aldosterone system; NO: nitric oxide; ROS: reactive oxygen species; vWF: von Willebrand factor; HF: heart failure; CD40: CD40 receptor; SARS-CoV-2: severe acute respiratory syndrome coronavirus 2.

presence of MVO detected by cardiac magnetic resonance (CMR) is associated with higher peak troponin levels, larger infarct size, and worse long-term clinical outcomes, including a higher incidence of MACE^[1,68]. Moreover, intracoronary levels of PMPs and EMPs are positively correlated with thrombus score and corrected TIMI frame count (a measure of coronary blood flow velocity) and independently associated with angiographic and electrocardiographic MVO, suggesting that microparticles not only serve as markers but may also directly participate in the pathogenesis of MVO^[11].

Microthrombosis in multi-organs

Thromboinflammatory processes that drive microthrombosis in the heart are not organ-specific, and similar mechanisms operate in other vascular beds, leading to multi-organ dysfunction in systemic conditions. Table 2 summarizes detailed mechanisms involved across organs. Exemplifying this systemic nature is Kawasaki disease, in which ultrastructural studies of endomyocardial biopsies have directly demonstrated platelet aggregation with thrombosis in coronary microvessels, along with endothelial injury, microvascular dilatation, and luminal stenosis—changes that persist for years after disease onset and correlate with myocardial fibrosis and dysfunction^[15]. These microvascular lesions are accompanied by systemic vascular dysfunction, including reduced coronary flow reserve and persistent endothelial impairment even in patients without coronary aneurysms^[16,69]. The persistent platelet activation state in Kawasaki disease—now recognized as a paradigm shift from merely increased platelet counts to functional activation^[70]—drives thromboinflammatory cascades through platelet-leukocyte aggregates, NLRP3 inflammasome activation, and IL-1 β production, directly linking platelet biology to vascular injury and microthrombosis^[71].

In the kidney, hypertension induces microvascular remodeling and rarefaction, characterized by a decreased vessel density and increased wall-to-lumen ratio in resistance arterioles^[6]. These structural changes, combined with endothelial dysfunction and activation of the renin-angiotensin system, lead to glomerular hyperfiltration, proteinuria, and progressive chronic kidney disease. Microthrombi within the renal microcirculation have been documented in severe COVID-19 autopsies, contributing to the onset of acute kidney injury^[72]. In the brain, hypertension is a major risk factor for cerebral small vessel disease, which is

characterized on MRI as white matter hyperintensities, lacunas, microhemorrhages, and microinfarcts^[6]. These microvascular lesions, driven by lipohyalinosis and fibrinoid necrosis of small arterioles, are associated with cognitive decline, dementia, and an increased risk of ischemic and hemorrhagic stroke. In the retina, hypertensive retinopathy - marked by increased vascular permeability, microhemorrhages, and cotton-wool spots - serves as a visible indicator of systemic microvascular damage and predicts cardiovascular and renal complications^[6].

Activation of the complement pathway differentially contributes to microvascular versus macrovascular thrombosis. In patients with complement-mediated thrombotic microangiopathy, higher urinary sC5b-9 levels and renal C5b-9 deposition correlate with renal microthrombosis, while plasma levels of C5a and renal expression of C5aR1 are associated with macrovascular thrombosis, suggesting that targeting individual complement components could yield more precise antithrombotic effects^[58]. In primary antiphospholipid syndrome, pulmonary microthrombosis, stroke and deep vein thrombosis are common clinical manifestations, and microvascular involvement frequently underlies multiorgan failure in the catastrophic variant^[73,74].

Underlying diseases exacerbate microthrombosis

Pre-existing conditions such as hypertension, diabetes mellitus, hyperhomocysteinemia, and dyslipidemia profoundly amplify the risk and severity of microthrombosis.

Hypertension, as detailed in Section “Inflammation-induced endothelial dysfunction”, promotes endothelial dysfunction, vascular remodeling, and rarefaction, all of which predispose to microthrombosis. CD40 knockout mice developed profound pulmonary microthrombosis when subjected to pressure overload induced by transverse aortic constriction (TAC)^[75]. This prothrombotic phenotype was not attributable to differential LV dysfunction, as CD40 deficiency did not affect LV remodeling and function after TAC. Rather, it was accompanied by enhanced pulmonary oxidative stress, endothelial activation (upregulation of VCAM-1 and ICAM-1), and platelet hyperresponsiveness (augmented aggregation to thrombin, collagen, and ADP), indicating a direct prothrombotic effect of CD40 loss on the pulmonary microvasculature under pressure overload^[75].

Diabetes mellitus induces a state of platelet hyperreactivity, endothelial dysfunction, and impaired fibrinolysis, collectively known as a prothrombotic milieu^[7,8]. Diabetic patients have elevated levels of circulating PMPs and EMPs, which correlate with the presence and severity of microvascular complications such as retinopathy and nephropathy^[76,77]. In myocardial I/R injury, diabetes worsens microvascular damage through mechanisms involving enhanced oxidative stress, impaired mitophagy, and reduced endothelial barrier function^[21,78]. Clinically, diabetes has also been identified as an independent predictor of MVO after primary PCI^[79].

Hyperhomocysteinemia is a further underappreciated risk factor for coronary microvascular dysfunction due to several molecular mechanisms. First, hyperhomocysteinemia impairs annexin A2-dependent fibrinolysis: in hyperhomocysteinemic mice, tPA-dependent plasmin activation, an annexin A2-dependent process, is abolished, leading to perivascular fibrin accumulation and impaired angiogenesis^[80]. Second, during cardiac I/R, hyperhomocysteinemia synergizes with mobilized copper ions (Cu^{2+}) to induce necroptosis of cardiac microvascular endothelial cells via peroxynitrite generation from NADPH oxidase and eNOS^[81]. Third, hyperhomocysteinemia activates the lncRNA MEG3/microRNA-223/NLRP3 inflammasome axis, leading to endothelial cell pyroptosis with reduced microvascular density^[82]. Fourth, hyperhomocysteinemia increases oxidative stress and thromboxane A_2 activity while reducing NO bioavailability, promoting vasomotor dysfunction and a prothrombotic state^[9]. Finally, hyperhomocysteinemia-induced microvascular dysfunction

Table 3. Molecular pathways of major risk factors exacerbating microthrombosis

Risk factor	Shared pathways	Unique pathways	Key
Hypertension	ROS↑, NO↓, endothelial dysfunction, platelet activation	RAAS activation, vascular remodeling, rarefaction, CD40 deficiency-related pulmonary thrombosis	[6,75]
Diabetes mellitus	ROS↑, NO↓, endothelial dysfunction, platelet hyperreactivity	AGEs, impaired mitophagy, platelet PMP/EMP elevation, microvascular complications (retinopathy, nephropathy)	[7,8,21,76,77]
Hyperhomocysteinemia	ROS↑, oxidative stress, endothelial dysfunction	Impaired annexin A2-dependent fibrinolysis, Cu ²⁺ -induced necroptosis, lncRNA MEG3/miR-223/NLRP3 pyroptosis, SP1/STAT3/KCa3.1/eNOS axis	[9,80,81,82,83]
Dyslipidemia	ROS↑, oxidative stress, endothelial dysfunction	Enhanced free radical production, leukocyte recruitment, platelet adhesion; oxLDL-induced endothelial damage and platelet hyperreactivity via CD36; elevated lipoprotein(a)-mediated antifibrinolytic effects and oxidized phospholipid carriage	[84,85,86]
Kawasaki disease	Platelet activation, inflammation, NLRP3 inflammasome	TSLP-induced mitophagy, persistent endothelial dysfunction even without aneurysms	[15,16,37,70,71]

ROS: Reactive oxygen species; NO: nitric oxide; RAAS: renin-angiotensin-aldosterone system; AGEs: advanced glycation end-products; PMP: platelet-derived microparticle; EMP: endothelial-derived microparticle; lncRNA: long non-coding RNA; MEG3: maternally expressed gene 3; miR-223: microRNA-223; NLRP3: NOD-like receptor family pyrin domain containing 3; SP1: specificity protein 1; STAT3: signal transducer and activator of transcription 3; KCa3.1: calcium-activated potassium channel KCa3.1; eNOS: endothelial nitric oxide synthase; oxLDL: oxidized low-density lipoprotein; TSLP: thymic stromal lymphopoietin.

after AMI can be reversed by a protective transcriptional axis driven by the transcription factor SP1 (specificity protein 1), which activates STAT3/KCa3.1/eNOS signaling^[83].

Dyslipidemia is an important modifiable risk factor that predisposes to microthrombosis. Dyslipidemia induces a proinflammatory and prothrombotic phenotype through enhanced production of free radicals, leukocyte recruitment, and platelet adhesion^[84]. Oxidized low-density lipoprotein (oxLDL) directly damages the endothelial monolayer, facilitates thrombocyte adhesion, and promotes the formation of microthrombi. Moreover, hypercholesterolemia contributes to platelet hyperreactivity via direct oxLDL-platelet membrane interaction through scavenger receptors such as CD36, and oxLDL has been shown to induce platelet activation and arterial thrombosis^[85]. Among lipid abnormalities, lipoprotein(a) has recently emerged as an independent risk factor for coronary microvascular dysfunction. Acting as a carrier of oxidized phospholipids, elevated lipoprotein(a) promotes endothelial dysfunction, oxidative stress, and microthrombotic susceptibility through its antifibrinolytic effects^[86]. These findings suggest that lipoprotein(a) screening may be valuable in patients with microvascular angina. (Table 3 provides a summary of the shared and unique molecular pathways of these risk factors involved).

In summary, underlying diseases, as specified above, amplify microthrombosis through shared pathways of oxidative stress, endothelial dysfunction, platelet activation, and impaired endogenous protective mechanisms, making them critical determinants of clinical outcomes in patients with AMI, heart failure, and systemic inflammatory conditions.

PLATELET-RELATED BIOMARKERS FOR THE DETECTION OF MICROTHROMBOSIS

Detecting microthrombosis in patients remains challenging, but platelet-related biomarkers offer promising non-invasive tools. The following subsections review the most studied candidates, including PMPs and related platelet parameters, soluble activation markers, cellular aggregates, and surface receptor expression, along with their clinical correlations with MVO, no-reflow, and long-term prognosis. (A comparative overview of these biomarkers is provided in Table 4 at the end of this section).

Table 4. Comparison of platelet-related biomarkers for microthrombosis detection

Biomarker	Specimen type	Assay method	Association with MVO/no-reflow	Specificity	Clinical utility	Key limitations
PMPs (CD41+/Annexin V+)	Plasma (platelet-poor)	Flow cytometry	Correlates with TS, cTFC, MBG, and STR ^[91]	Moderate (also elevated in inflammation, diabetes, cancer)	Candidate prognostic marker for MACE	High pre-analytical variability; lack of standardized reference ranges ^[92]
EMPs (CD31+/CD42-)	Plasma (platelet-poor)	Flow cytometry	Correlates with TS and MVO indices ^[91]	Moderate (also elevated in endothelial injury)	Marker of endothelial dysfunction	Similar pre-analytical issues to PMPs
PMAAs (CD14+CD61+ aggregates)	Whole blood	Flow cytometry	Correlates with MVO, inflammation, and disease severity ^[32,94,98,99]	High (aggregate-specific)	Sensitive indicator of ongoing thromboinflammation	Requires immediate sample processing; labor-intensive
sP-selectin (sCD62P)	Serum/plasma	ELISA	Independent predictor of long-term MACE after PCI ^[96]	Low (not platelet-specific)	Easy to measure; commercially available	Also from endothelial cells; affected by renal function
sCD40L	Serum/plasma	ELISA	Associated with MVO in AMI ^[79]	Low (multiple cell sources)	Reflects both platelet and inflammatory activation	Less specific; affected by sample handling
Activated GPIIb/IIIa (PAC-1 binding)	Whole blood	Flow cytometry	Residual platelet activation may contribute to MVO ^[101]	High (activation-specific)	Direct measure of platelet aggregation	Transient expression; requires immediate processing
IPF	Whole blood	Flow cytometry	Independent long-term predictor of MACE in ACS (IPF > 3.45% is cut-off) ^[43]	Moderate (also elevated in other conditions)	May aid long-term MACE risk stratification	Relatively new; cut-off values need validation
MPV	Whole blood	Automated hematology analyzer	Predicts Killip class ≥ 2 ^[93] ; may contribute to MVO ^[94]	Low (influenced by multiple factors)	Widely available, inexpensive	Non-specific; affected by splenic release, demographics

MVO: Microvascular obstruction; PMPs: platelet-derived microparticles; TS: thrombus score; cTFC: corrected TIMI frame count; MBG: myocardial blush grade; MACE: major adverse cardiovascular events; EMPs: endothelial-derived microparticles; PMAAs: platelet-monocyte aggregates; sP-selectin: soluble P-selectin; sCD62P: soluble CD62P; PCI: percutaneous coronary intervention; sCD40L: soluble CD40 ligand; AMI: acute myocardial infarction; GPIIb/IIIa: glycoprotein IIb/IIIa; PAC-1: monoclonal antibody against active glycoprotein IIb/IIIa; IPF: immature platelet fraction; ACS: acute coronary syndrome; MPV: mean platelet volume; STR: ST-segment resolution.

PMPs and related platelet parameters

Because PMPs are released by activated platelets and can be quantified in peripheral blood, they have been extensively investigated as circulating biomarkers of microthrombosis^[87]. Flow cytometric enumeration of PMPs (defined as CD41-positive, annexin V-positive particles < 1 μm) allows assessment of *in vivo* platelet activation and thrombogenicity. Studies on STEMI patients have consistently shown that intracoronary levels of PMPs exceed those in the systemic circulation and correlate with local thrombotic activity, as reflected by MVO indices (myocardial blush grade ≤ 2 and incomplete ST-segment resolution (STR)) and by SYNTAX score in those with ACS^[11,88]. A meta-analysis further confirmed that plasma PMP concentrations are significantly elevated in ACS patients compared to those with stable angina or healthy controls^[89], and elevated baseline PMP levels correlate with increased MACE risk during long-term follow-up, encompassing death, recurrent myocardial infarction (MI), and heart failure hospitalization. Elevated PMP levels have also been reported in hypertension^[90], diabetes^[76,77], and COVID-19^[91], where they correlate with disease severity and adverse outcomes. Despite their promise, routine clinical adoption of PMP quantification is hampered by pre-analytical variability, lack of standardized reference ranges, and limited sensitivity of conventional flow cytometers^[92].

Beyond PMPs, other platelet-related parameters have demonstrated prognostic utility. Admission mean platelet volume (MPV), a surrogate of platelet size and reactivity, independently predicts Killip class ≥ 2 ^[93]. Mechanistically, AMI triggers the splenic release of larger, more reactive platelets; splenectomy attenuates the rise in MPV, reduces cardiac platelet and leukocyte accumulation, and limits the infarct size^[94]. In patients with coronary artery calcification, the PDW/HDL-C ratio (where PDW reflects platelet size heterogeneity) has been proposed as a simple, inexpensive prognostic index^[95]. Additionally, immature platelet fraction, which quantifies circulating RPs, has emerged as an independent long-term predictor of MACE in a 5-year ACS cohort^[43], supporting its utility for risk stratification in microthrombosis.

Soluble platelet activation markers: soluble P-selectin (sP-selectin), CD40L, and thrombospondin-1

Upon activation, platelets secrete or shed soluble forms of membrane proteins that can be measured in plasma or serum. sP-selectin (also known as sCD62P) is derived mainly from activated platelets and is elevated in ACS, atrial fibrillation, and peripheral artery disease. In a prospective cohort of 733 patients undergoing PCI, elevated sP-selectin levels were associated with a significantly increased risk of long-term MACE after a mean follow-up period of 9.7 years^[96]. Furthermore, in patients with coronary chronic total occlusion (CTO) undergoing technically challenging PCI, higher Japanese CTO scores and longer procedure duration were associated with significantly elevated platelet P-selectin positivity 2 days after the intervention and increased sP-selectin levels 3–6 months later, indicating sustained platelet activation^[97]. Similarly, sCD40L is predominantly released from activated platelets and has potent proinflammatory properties by inducing endothelial cells to express adhesion molecules and TF^[46]. In the setting of CME and I/R, increased plasma levels of sCD40L correlate with platelet activation and MVO. Consistent with this, a proof-of-concept study in STEMI patients showed that higher levels of sCD40-L, hs-CRP, and VCAM-1 within aspirated coronary thrombi were associated with MVO^[79]. Thrombospondin-1, another α -granule protein, is involved in platelet aggregation, angiogenesis, and TGF- β activation, and has been explored as a biomarker of adverse cardiac remodeling. While these soluble markers are easier to measure by ELISA and are commercially available, they are less specific for microthrombosis than cell-associated or microparticle-based markers because they can be released by other cells and are affected by renal function and sample handling.

PMAs and Platelet-leukocyte aggregates

PMAs are a sensitive indicator of *in vivo* platelet activation and cell-cell crosstalk. Both cell types form primarily through binding via platelet P-selectin to monocyte PSGL-1, with additional stabilization by Mac-1 (CD11b/CD18) on leukocytes interacting with platelet GPIb α ^[32]. Elevated PMAs are present in patients with ACS, diabetes, and post-coronary stenting, and they have been linked to a prothrombotic and proinflammatory phenotype. In patients with COVID-19, massive image-based single-cell profiling revealed the presence of excessive circulating platelet-neutrophil aggregates and PMAs in nearly 90% of patients, and the concentration of these aggregates was strongly associated with disease severity, mortality, respiratory failure, and markers of endothelial dysfunction^[98]. Platelet-neutrophil aggregates are similarly elevated in STEMI, sepsis, and severe COVID-19 and are being explored as flow cytometric biomarkers for NETs-mediated microthrombosis, although pre-analytical challenges remain to be addressed, particularly the need for immediate sample processing to avoid spontaneous *in vitro* aggregate formation (see Sections “Platelets as bidirectional mediators of inflammation” and “Neutrophil extracellular traps promote platelet aggregation and fibrin deposition” for mechanistic details). The persistence of platelet-leukocyte aggregates has also been documented in long COVID, suggesting ongoing thromboinflammation^[51]. The time course of PMA formation is rapid. In AMI patients, circulating PMAs increase as early as 1–3 h post-MI and return to baseline within 24 h; antiplatelet therapy with clopidogrel or P2Y₁₂ inhibitors effectively reduces PMA formation, leukocyte infiltration into the infarcted myocardium, and expression of inflammatory mediators^[94,99]. From a clinical perspective, detection of PMAs by flow cytometry is more robust than single platelet activation markers, as the larger aggregates facilitate gating and reduce the confounding effects of

platelet fragmentation. Importantly, this advantage does not obviate the need for careful sample handling to prevent *in vitro* aggregation artifacts. The requirement for immediate sample processing and specialized equipment still limits its routine use.

Beyond flow cytometric enumeration of PMAs, recent work has leveraged platelet transcriptomics to capture the thromboinflammatory axis at a molecular level. In a longitudinal cohort study^[100], Beitzén-Heineke *et al.* defined high vs. low monocyte platelet aggregates (MPA) status based on consistently elevated MPA levels over two time points^[100]. High MPA individuals showed increased expression of platelet activation markers (P-selectin, PAC-1, CD40L) and enriched immune signaling pathways. Using the platelet transcriptome, the authors derived a 42-gene thromboinflammation platelet signature (TIPS) that correlated strongly with MPA levels, was reproducible over time, and was significantly elevated in patients with COVID-19 and in those with MI. Importantly, in a prospective cohort of patients undergoing lower extremity revascularization (median follow-up 18 months), higher TIPS independently predicted future cardiovascular events after adjusting for age, sex, race, and ethnicity. Notably, TIPS was modifiable by ticagrelor but not by aspirin. These findings extend the concept of PMAs from a cellular aggregate to a transcriptomic signature, offering a novel tool for cardiovascular risk stratification and highlighting the potential of platelet RNA-based diagnostics.

Platelet surface receptor expression: activated GPIIb/IIIa, P-selectin, and TLR4

Flow cytometric assessment of platelet surface receptor expression provides a direct snapshot of platelet activation status (for the mechanistic roles of these receptors, see Section “Platelet adhesion, activation, and aggregation”). Activated GPIIb/IIIa, detected by PAC-1 antibody that recognizes the active conformation of integrin α IIb β 3, is a highly specific marker of ongoing platelet aggregation. In patients with ACS, early administration of eptifibatide (a GPIIb/IIIa antagonist) rapidly inhibited PAC-1 binding and ADP-induced aggregation, but did not affect CD62P or platelet-leukocyte aggregate formation, indicating that different activation pathways are not equally blocked by a single antiplatelet agent^[101]. The clinical relevance of GPIIb/IIIa inhibition as a strategy to reduce MVO was subsequently evaluated in the REVERSE-FLOW trial (see Section “Antiplatelet therapy” for detailed results and discussion). Platelet surface P-selectin (CD62P) is another standard activation marker, but it is transiently expressed and rapidly shed, making it less reliable than PAC-1 for detecting ongoing aggregation. TLR4 expression on platelets is upregulated in inflammatory states. In a study of 40 MI patients and 40 matched controls, Barillà *et al.* demonstrated that platelet TLR4 is activated in MI patients, as evidenced by increased TIRAP phosphorylation and TIRAP-MyD88 interaction, and that circulating LPS may serve as a trigger for this activation; *ex vivo* inhibition of TLR4 reduced collagen-induced platelet aggregation, suggesting that TLR4 contributes to platelet hyperreactivity after MI^[102]. Although flow cytometry-based assays for these surface markers are highly informative in research settings, their clinical application is hindered by the need for rapid sample processing, antibody panels, and technical expertise.

Challenges and strategies for clinical implementation

Despite their promise, the clinical translation of platelet-related biomarkers is hindered by profound pre-analytical variability. Sampling timing is critical: PMAs peak at 1–3 h post-MI and return to baseline within 24 h^[94], while MPV decreases by approximately 16% within the first 24 h^[93]. Sampling site also matters, as intracoronary levels of PMPs and EMPs are significantly higher than peripheral venous levels^[11,88]. Processing variables, including anticoagulant type, centrifugation speed, and time to analysis, further affect reproducibility^[103]. These factors, combined with the lack of standardized protocols across studies, have prevented the establishment of uniform clinical detection criteria and validated reference ranges.

To address these barriers and facilitate clinical application, the following strategies are recommended. (1) Standardized pre-analytical protocols should be adopted, specifying anticoagulant (citrate preferred),

centrifugation protocol (e.g., 2,500 g for 15 min twice for platelet-free plasma), and time to analysis (fresh samples preferred)^[103]; (2) Stratified reference ranges should be established for each biomarker, separately defined by sampling site (intracoronary vs. peripheral) and sampling time relative to symptom onset and reperfusion, using large, well-characterized cohorts; (3) MVO risk stratification could categorize patients into low-, intermediate-, and high-risk groups. This can be achieved by developing risk prediction models that incorporate multiple biomarkers and potentially integrate other readily available parameters such as D-dimer to provide individualized risk scores (e.g., via logistic regression with nomogram presentation). Prospective validation in multicenter cohorts will be essential to confirm the clinical utility of these biomarkers and define their role in clinical decision-making.

THERAPEUTIC STRATEGIES TARGETING MICROTHROMBOSIS

Several therapeutic strategies have been explored to prevent or reverse microthrombosis, ranging from conventional antiplatelet drugs to statins, anti-inflammatory agents, mechanical conditioning, and more recently targeted local thrombolysis. The following subsections summarize the findings and limitations of each approach, highlighting recent clinical trials and emerging targets. A structured summary of key clinical trials is provided in [Table 5](#) at the end of this chapter.

Antiplatelet therapy

Antiplatelet agents are the cornerstone of pharmacological intervention against microthrombosis in ACS and following PCI. Aspirin irreversibly inhibits cyclooxygenase-1, reducing thromboxane A₂ production and attenuating platelet aggregation. P₂Y₁₂ inhibitors (clopidogrel, prasugrel, ticagrelor) block the ADP receptor, thereby suppressing platelet activation. In a randomized, blinded trial of ACS patients undergoing stenting, prasugrel and ticagrelor achieved stronger platelet inhibition than clopidogrel and were associated with transient improvements in microvascular reactive hyperemia measured by laser Doppler, suggesting a direct beneficial effect on microvascular function^[104]. However, this microvascular effect was lost one day after stenting, indicating that sustained protection may require more prolonged or additional strategies. GPIIb/IIIa antagonists (e.g., eptifibatide, abciximab) block the final common pathway of platelet aggregation. Early administration of eptifibatide rapidly and profoundly inhibited platelet aggregation and reduced the expression of activated GPIIb/IIIa (PAC-1 binding) as well as CD51/61 and CD42b^[101]. Nevertheless, platelet-leukocyte aggregate formation continued to rise within 24 h despite eptifibatide therapy, suggesting that GPIIb/IIIa blockade alone does not fully disrupt the inflammation-platelet cycle. The clinical impact of GPIIb/IIIa inhibition on MVO was evaluated in the REVERSE-FLOW trial^[105], which randomized 120 AMI patients with angiographic MVO after PCI to bailout GPIIb/IIIa inhibitor (eptifibatide or tirofiban) versus optimal medical therapy. Although the primary endpoint of the infarct size was not reduced, the treatment group showed significantly fewer patients with CMR-derived MVO (74.5% vs. 92.2%; $P = 0.017$) and a smaller MVO extent (2.1% vs. 3.4% of the LV; $P = 0.025$), but with an increased risk of non-life-threatening bleeding (22.6% vs. 6.9%; $P = 0.016$). This dissociation, with improvement in a mechanistic surrogate without translating into infarct size limitation, might be attributable to a few reasons. GPIIb/IIIa inhibitors were administered as bailout therapy after reperfusion, a time point at which the cellular events driving cardiomyocyte necrosis had already occurred. MVO reflects microvascular perfusion defects rather than myocardial necrosis per se, and improvement in MVO may not reverse established infarct size. Indeed, in a mouse model of cardiac I/R of 1 h/24 h, the size of MVO was much smaller than that of the infarct size (36% vs. 47%) of the risk zone^[4]. Further, the increased bleeding risk may partially offset the microvascular benefits. Thus, antiplatelet strategies aimed at myocardial salvage may require earlier administration or combination with agents targeting the inflammation-platelet axis, rather than bailout inhibition after MVO has already developed. Current guidelines recommend dual antiplatelet therapy for secondary prevention of arterial thrombosis in ACS; however, direct clinical evidence remains lacking on reduction in MVO or MVL by specifically antiplatelet regimens. Beyond their antithrombotic action, antiplatelet drugs may exert

Table 5. Summary of completed key clinical trials targeting microthrombosis in STEMI/ACS

Trial	Population	Intervention	Primary endpoint	Key findings	References
REVERSE-FLOW	STEMI (<i>n</i> = 120) with angiographic MVO	Bailout GPIIb/IIIa inhibitor (eptifibatide/tirofiban) vs. optimal medical therapy	Infarct size (%LV by CMR)	Neutral for infarct size; reduced CMR-MVO extent (2.1% vs. 3.4% LV; <i>P</i> = 0.025); increased non-life-threatening bleeding (22.6% vs. 6.9%; <i>P</i> = 0.016)	[105]
STRIVE	STEMI (<i>n</i> = 210) with large thrombus burden	Intracoronary low-dose alteplase (10/20 mg) vs. placebo	Composite: 30-day MACE, MBG 0/1, distal embolization, or incomplete STR	Neutral (53.3% vs. 52.9%; RR 1.00; <i>P</i> > 0.99); trend toward more VF with alteplase (10.2% vs. 1.4%; <i>P</i> = 0.06)	[134]
ASSAIL-MI	STEMI (<i>n</i> = 199)	IV tocilizumab (280 mg) vs. placebo before primary PCI	Myocardial salvage index (CMR)	Improved myocardial salvage index (difference 5.6 percentage points; <i>P</i> = 0.04); neutral for infarct size; mechanistic sub-studies showed NETs suppression and monocyte phenotype modulation	[109,110,111,112]
T-TIME	STEMI (<i>n</i> = 440)	Low-dose intracoronary alteplase vs. placebo	MVO (%LV mass) by CMR at 2-7 days after randomization	Neutral; no reduction in MVO (20 mg vs. placebo: 3.5% vs. 2.3%, <i>P</i> = 0.32; 10 mg vs. placebo: 2.6% vs. 2.3%, <i>P</i> = 0.74)	[135]
STREAM	STEMI (<i>n</i> = 1892)	Pharmacoinvasive strategy (tenecteplase + early PCI) vs. primary PCI	Composite of death, shock, CHF, or reinfarction at 30 days	Neutral; provides context for fibrinolytic strategies in the reperfusion era	[136]
LIPSIA CONDITIONING	STEMI (<i>n</i> = 696)	Combined RIC + postconditioning vs. postconditioning vs. control	Myocardial salvage index (CMR)	Improved myocardial salvage index with combined therapy; no significant effect on infarct size or MVO	[142]
POST	STEMI (<i>n</i> = 700)	Ischemic postconditioning vs. control	Myocardial reperfusion (STR, MBG)	Neutral for primary endpoint; no significant difference in infarct size or MVO by CMR substudy	[138]
CONDI-2/ERIC-PPCI	STEMI (<i>n</i> = 5401)	RIC vs. sham	Cardiac death or hospitalization for heart failure at 12 months	Neutral (informs clinical utility of RIC)	[143]

STEMI: ST-segment elevation myocardial infarction; ACS: acute coronary syndrome; MVO: microvascular obstruction; GPIIb/IIIa: glycoprotein IIb/IIIa; LV: left ventricular; CMR: cardiac magnetic resonance; MACE: major adverse cardiovascular events; MBG: myocardial blush grade; STR: ST-segment resolution; RR: risk ratio; VF: ventricular fibrillation; IV: intravenous; PCI: percutaneous coronary intervention; NETs: neutrophil extracellular traps; CHF: congestive heart failure; RIC: remote ischemic conditioning; PPCI: primary percutaneous coronary intervention.

additional anti-inflammatory benefits: in a mouse MI model, clopidogrel, but not aspirin, significantly reduced cardiac rupture incidence (from 45% to 10%) and decreased intramural platelet thrombus formation, accompanied by suppressed systemic and cardiac inflammatory responses^[99,106].

Another potential therapeutic avenue targets the splenic release of platelets. In a study by Gao *et al.*, AMI triggered the release of larger, more reactive platelets from the spleen; splenectomy attenuated the rise in MPV, reduced circulating platelet-monocyte conjugation as well as cardiac accumulation of platelets and leukocytes, limited infarct size, and alleviated cardiac dilatation and dysfunction^[94]. Importantly, interventions that are not directly antiplatelet - such as β -blockers or ACE inhibitors - also provided cardioprotection, suggesting that modulating splenic platelet release could be a novel therapeutic strategy^[94].

Looking forward, novel antiplatelet agents targeting platelet receptors distinct from conventional pathways are being explored. A recent review highlights potential benefits of inhibitors against GPVI, protease-activated receptor 4, GPIb-IX-V, and serotonin receptor 2A, as well as P-selectin inhibitors, which have

shown efficacy in reducing microvascular thrombosis in sickle cell disease and could translate into benefits in ACS^[107]. Notably, patients with elevated RPs may derive particular benefit from these emerging strategies, as RPs show reduced responsiveness to P2Y₁₂ inhibitors and aspirin, correlating with persistent thromboinflammatory activity despite dual therapy^[42]. Multi-omic profiling of RPs has further identified selectively enriched GPVI and PI3K signaling, while pharmacological inhibition (e.g., glenzocimab or PI3K blockade) effectively reduces RP-mediated aggregation and thrombus recruitment^[44]. However, translating these insights into clinical practice must contend with the persistent bleeding risk that undermines the utility of intensified antiplatelet regimens, particularly in patients at high bleeding risk^[45]. In this context, the natural compound formononetin has recently been shown to inhibit platelet activation and NETosis via the CD36/extracellular signal-regulated kinase 5 pathway, and attenuate myocardial I/R injury without prolonging bleeding time, suggesting a potentially safer antiplatelet strategy^[108].

Anti-inflammatory therapy

Given the central role of inflammation in driving microthrombosis, targeting inflammatory cytokines and their signaling pathways has emerged as a promising therapeutic approach. In the ASSAIL-MI trial, a double-blind, randomized study of 199 STEMI patients, intravenous administration of tocilizumab (an interleukin-6 receptor inhibitor) prior to primary PCI improved the myocardial salvage index measured by CMR compared to placebo^[109]. However, a subsequent sub-study found that tocilizumab did not significantly affect circulating levels of endothelial- or platelet-derived CXC-chemokines (CXCL4, CXCL5, CXCL6, CXCL7, CXCL12), and none of these chemokines were associated with the infarct size, MVO, or troponin T levels^[110]. Notably, heparin administration in this trial significantly influenced chemokine levels, highlighting a potential confounding factor in biomarker studies. These findings suggest that the beneficial effects of tocilizumab in STEMI are not mediated through changes in these specific CXC-chemokines, and the exact mechanisms remain to be elucidated. Mechanistic sub-studies have revealed that tocilizumab attenuates neutrophil counts and suppresses the formation of NETs^[111], and reshapes monocyte transcriptome profiles toward a cardioprotective phenotype, potentially through downregulation of SOCS3, with *in vitro* evidence showing direct limitation of cardiomyocyte apoptosis^[112]. These immunomodulatory effects appear to protect the jeopardized myocardium within the area at risk, thereby improving myocardial salvage index. The reduction in final infarct size, however, may require larger sample sizes to achieve statistical significance.

Glucagon-like peptide-1 receptor agonists (GLP-1 RAs) have emerged as a class of agents with anti-inflammatory and vascular protective effects beyond glycemic control. The SELECT trial demonstrated that semaglutide 2.4 mg once weekly reduced the risk of MACE by 20% in patients with established cardiovascular disease and overweight or obesity but without diabetes^[113], while the SURPASS-CVOT trial showed that tirzepatide was non-inferior to dulaglutide with respect to cardiovascular outcomes in patients with type 2 diabetes and atherosclerotic cardiovascular disease^[114]. Mechanistically, GLP-1 receptor activation attenuates platelet aggregation and thrombosis through cAMP/PKA-dependent signaling^[115], reduces microvascular thrombosis and nitro-oxidative stress in endotoxemic models^[116], and modulates prothrombotic and atherosclerotic mechanisms associated with epicardial fat, neutrophils, and endothelial cells^[117]. Although their specific impact on coronary microvascular dysfunction requires further investigation, GLP-1 RAs represent a promising class of agents that target both metabolic dysregulation and thromboinflammation.

HMGB1/TLR4/NF- κ B pathway has shown compelling preclinical results. In a rat model of CME, treatment with the HMGB1 inhibitor glycyrrhizin (10 mg/kg, intravenous) significantly reduced microinfarct size by ~20% and improved LVEF by ~18.5% compared to untreated controls, effects that were mediated through restoration of protective autophagy^[13]. Blockade of HMGB1 also suppressed TLR4 and NF- κ B expression, reducing the proinflammatory and prothrombotic milieu. Glycyrrhizin has a well-established safety profile,

making it an attractive candidate for clinical translation. Despite these encouraging preclinical data, several translational barriers must be considered. Glycyrrhizin can cause dose-dependent mineralocorticoid-like effects, including hypertension, hypokalemia, and fluid retention, which may limit its safety window in patients with cardiovascular disease, particularly those with heart failure or renal impairment^[118]. Additionally, glycyrrhizin doses above 90 mg/kg have been shown to produce significant hemorrhagic effects in animal models^[119], raising concerns about bleeding risk. The optimal dosing regimen in humans also remains undefined, as most preclinical studies employed pre-treatment protocols not directly translatable to the clinical setting. These considerations underscore the need for careful dose-finding studies and bleeding risk monitoring before clinical translation can be pursued.

Another promising anti-inflammatory and microvascular protective agent is relaxin. Relaxin directly inhibits platelet aggregation and suppresses platelet release from megakaryocytes^[120,121], and mitigates I/R-induced endothelial barrier disruption and inflammation. In a mouse cardiac I/R model, relaxin reduced the no-reflow zone of the LV by 38% and Evans blue extravasation by 56%, increased open capillaries, reduced leukocyte density and cytokine gene expression, mitigated I/R-induced loss of VE-cadherin, and at 2 weeks partially preserved LV contractile function while limiting chamber dilatation^[28]. A recent systematic review further supports that pretreatment with relaxin mitigates coronary microvascular injury induced by I/R^[122]. Other anti-inflammatory strategies include IL-1 β inhibition (e.g., canakinumab), which has been shown to reduce cardiovascular event rates in the CANTOS trial, although its effects on MVO specifically have not been fully characterized. Complement inhibitors and neutrophil elastase inhibitors are also being explored to prevent NETosis-mediated microthrombosis^[17]. However, for NETs-targeted strategies, additional translational challenges exist. DNase I has a short plasma half-life, which may limit its therapeutic efficacy^[123]. Systemic NETs degradation could release histones and other pro-inflammatory contents, potentially exacerbating inflammation^[124]; and the balance between NETosis inhibition and preservation of neutrophil host defense raises concerns about infection risk^[125]. Safety concerns also extend to bleeding, as DNase I has been associated with lung hemorrhage in murine models and with hemorrhagic transformation when administered with delay in ischemic stroke models^[126,127]. These safety and pharmacokinetic considerations highlight the need for rigorous risk-benefit assessment before clinical translation.

Antithrombotic effects of statins

Beyond their primary lipid-lowering action, statins exert clinically important antithrombotic effects that are particularly relevant to microvascular protection. The clinical significance of these effects is underscored by the observation that long-term nonadherence to statin therapy is associated with significantly increased mortality. In patients with ACS, nonadherence is associated with a nearly threefold increase in mortality (14.9% vs. 4.9%) compared with adherent patients^[128]. Among survivors of MI, adherence of less than 50% is associated with significantly increased risks of both all-cause mortality (HR 1.58) and cardiovascular mortality (HR 1.60)^[129]. Furthermore, in a large Korean multicenter registry of AMI survivors, postdischarge statin withdrawal was associated with higher mortality than continued statin treatment (HR 3.45), primarily as the result of increased cardiac mortality (HR 4.65)^[130]. These findings highlight the critical need to communicate the full spectrum of statin benefits beyond cholesterol lowering to improve patient persistence, particularly in patients at high risk for MVO.

In addition to lowering lipid levels, statins exert multiple pleiotropic effects that directly modulate the thrombotic and inflammatory milieu. Statins improve endothelial function by enhancing NO bioavailability and reducing oxidative stress^[131]. They also directly inhibit platelet activation, attenuate vascular inflammation, stabilize atherosclerotic plaques, and downregulate TF expression, leading to reduced thrombin generation and enhanced fibrinolysis^[132]. Collectively, these interrelated actions contribute to improved microvascular perfusion and have been associated with reduced no-reflow incidence after AMI.

Emphasizing these antithrombotic and pleiotropic benefits in clinical practice may help improve both patient adherence and microvascular outcomes.

Intracoronary low-dose thrombolysis: from *in vitro* promise to clinical trial

An alternative pharmacological strategy to directly dissolve microthrombi is the local delivery of low-dose thrombolytics into the infarct-related artery. Using an *in vitro* microfluidic model of MVO, a short 90-s exposure to microdoses of alteplase reduced microthrombus volume by up to 75%, at a dose 1,300-fold lower than conventional intravenous thrombolysis^[133]. The STRIVE trial, the first randomized controlled trial testing this approach in patients, was a multicenter, double-blind, placebo-controlled study that enrolled 210 STEMI patients with a large thrombus burden. Patients received either alteplase 10, 20 mg, or saline placebo directly into the culprit artery after reperfusion. The primary composite outcome (MACE at 30 days, myocardial blush grade 0/1, distal embolization, or incomplete STR) occurred in 53.3% of both alteplase groups versus 52.9% of the placebo group (RR 1.00; 95%CI: 0.76-1.31; $P > 0.99$). Results were neutral for all individual components and both doses. A trend toward more ventricular fibrillation was observed with alteplase (10.2% vs. 1.4%; $P = 0.06$), while bleeding events were rare^[134]. The neutral outcome of STRIVE does not support routine use of intracoronary low-dose alteplase in STEMI.

The discrepancy between promising *in vitro* results and the clinical failure highlights the gap between preclinical models and human pathophysiology. Unlike the relatively uniform fibrin-rich thrombi generated in microfluidic systems, MVO in patients is driven by a heterogeneous mixture of fibrin-rich thrombi, platelet aggregates, NETs, and endothelial swelling—components largely resistant to alteplase. Moreover, drug delivery to already obstructed microvessels is technically challenging, and the injection itself may have direct irritant effects on ischemic myocardium, as suggested by the trend toward increased episodes of ventricular fibrillation. Administration after reperfusion occurs after microthrombi have become structurally stabilized, further limiting the window for effective thrombolysis. Patient selection based on angiographic thrombus burden also does not reliably identify those in whom MVO is primarily fibrin-driven. The outcomes of STRIVE are consistent with prior neutral trials, including T-TIME^[135] and STREAM^[136], and collectively argue that targeting fibrin alone is insufficient to overcome the complex pathophysiology of MVO, reinforcing the need for strategies simultaneously addressing multiple components of the thromboinflammatory network.

Non-drug interventions under ischemic conditioning

Non-drug strategies that leverage the heart's endogenous protective mechanisms offer a non-pharmacological approach to reducing microthrombosis. Ischemic preconditioning (IPC) - brief, repetitive episodes of I/R before a sustained ischemic insult - has been shown to attenuate MVO and MVL in animal models. A key mediator of IPC is macrophage migration inhibitory factor (MIF): in a mouse cardiac I/R model, IPC's protective effects (reduced infarct size, myocyte apoptosis, ROS production, and inflammatory infiltration) were completely lost in MIF knockout mice, and IPC required MIF to activate the reperfusion injury salvage kinase (RISK) pathway and AMP-activated protein kinase^[137]. IPC, applied at the onset of reperfusion, has been shown to be cardioprotective in some animal studies, but has yielded inconsistent results in clinical trials^[138], possibly due to differences in protocols or patient populations studied.

Remote ischemic conditioning (RIC) - transient I/R of a limb (e.g., via blood pressure cuff inflation/deflation) - is more clinically feasible and has been shown to reduce MVO and myocardial edema. Its mechanisms involve circulating humoral factors, including microparticles and exosomes, that modulate endothelial function and platelet reactivity^[139], though the precise mediators and their clinical relevance remain poorly defined. In STEMI patients, RIC before primary PCI reduced the infarct size and edema measured by CMR in some studies^[140,141]. However, the LIPSIA CONDITIONING trial reported an

improvement in myocardial salvage index without significant reductions in infarct size or MVO^[142], and two large multicenter trials (CONDI-2/ERIC-PPCI) did not show improved clinical outcomes^[143].

The inconsistency between positive preclinical findings and neutral large-scale trials highlights several translational challenges specific to RIC. First, preclinical studies are typically conducted in young, healthy, genetically homogeneous animals, whereas STEMI patients usually present with multiple comorbidities (diabetes, hypertension, advanced age) and concomitant medications (antiplatelet agents, statins, β -blockers) that can blunt the cardioprotective signaling pathways activated by RIC^[144]. Second, the timing and delivery of RIC in clinical practice are logistically difficult to standardize; heterogeneity in ischemia duration, anesthetic exposure, and the precise timing of conditioning stimuli relative to reperfusion may all contribute to variable efficacy^[144]. Third, the robust mechanistic rationale for RIC, rooted in well-characterized signaling pathways such as the RISK pathway and the survivor activating factor enhancement pathway, may not be fully translational when these pathways are already impaired by comorbidities or pharmacological interference^[145]. These considerations suggest that RIC, as currently applied, may require optimized patient selection, refined conditioning protocols, or combination with pharmacological agents that sensitize cardioprotective pathways, rather than uniform application across all STEMI patients.

As reviewed above, extensive clinical findings underscore a recurring theme: single-agent strategies, although targeting distinct components of the thromboinflammatory cascade, have consistently failed to translate mechanistic benefits into improved clinical outcomes. This sobering reality is reflected in the current guideline landscape. The ESC 2023 guidelines explicitly identify MVO associated with primary PCI as an unmet clinical need and call for preclinical and clinical research to evaluate cardioprotective therapies^[146]. With respect to specific interventions, GP IIb/IIIa inhibitors should be considered (Class IIa, Level C) in the setting of no-reflow or thrombotic complications during PCI, while routine thrombus aspiration is not recommended (Class III, Level A)^[146]. The 2025 ACC/AHA guidelines similarly discourage routine manual thrombus aspiration (Class III, Level A)^[147]. Notably, no specific recommendations exist for anti-inflammatory or NETosis-targeted therapies targeting MVO, reflecting the gap between promising trial results and robust clinical evidence.

SUMMARY AND FUTURE PERSPECTIVES

This final section summarizes the pathophysiological core of microthrombosis, reflects on the current clinical landscape of biomarkers and therapies, and outlines future directions for diagnostic strategies, multi-target therapeutics, microphysiological systems and special population cohorts.

MVO and microthrombosis as pathophysiological core

Microthrombosis originates from endothelial injury and is sustained by a positive feedback loop between platelet activation and inflammation, leading to MVO, MVL, and multi-organ dysfunction. Endothelial dysfunction, characterized by enhanced oxidative stress and upregulation of adhesion molecules, reduces NO bioavailability and creates a prothrombotic cellular surface. Platelets then adhere via GPIb-IX-V/vWF, become activated, and release inflammatory mediators that further amplify endothelial activation and recruit leukocytes, forming platelet-leukocyte aggregates and NETs. The HMGB1/TLR4/NF- κ B pathway suppresses protective autophagy in myocardial tissue, predominantly in cardiomyocytes, exacerbating myocardial injury. Together, these mechanisms result in microinfarcts, contractile dysfunction, elevated cardiac troponin, and - through shared vascular beds - damage to the kidney, brain, and retina, especially in patients with underlying hypertension, diabetes, or hyperhomocysteinemia.

Current clinical status quo

Platelet-related biomarkers, including PMPs, PMAs, sP-selectin, and activated GPIIb/IIIa, enable detection of microthrombosis and correlate with cardiac no-reflow and adverse outcomes. However, this class of biomarkers lacks routine clinical validation due to pre-analytical variability, instrument sensitivity limits, and lack of standardized reference ranges. Existing antiplatelet therapies reduce thrombotic events but do not fully disrupt the inflammation-platelet cycle, and carry a risk of bleeding. This incomplete efficacy may be partly explained by the existence of functionally and maturationally distinct platelet subpopulations that exhibit differential responses to standard antiplatelet agents and contribute to persistent thromboinflammatory activity despite therapeutic interventions. Anti-inflammatory agents show promise in preclinical and early-phase clinical studies but have not yet demonstrated consistent benefit in large clinical trials taking MVO as the endpoint. The failure of intracoronary thrombolysis in the STRIVE trial further illustrates the gap between preclinical promise and clinical reality. Mechanical conditioning is safe and inexpensive, but its inconsistent efficacy based on large trials highlights the need for optimized protocols and better patient selection. Collectively, current therapies are limited by their inability to adequately counteract the multifaceted nature of microvascular injury, and effective strategies have yet to achieve durable suppression of this self-perpetuating cycle.

Future directions

Early identification of patients at risk for microthrombosis requires validated, clinically deployable biomarker panels. Future work should focus on constructing composite risk scores integrating: (1) platelet activation markers (PMPs, sP-selectin), (2) endothelial injury markers (EMPs, vWF), and (3) NETosis markers (cell-free DNA, MPO-DNA complexes). These panels should be validated in prospective cohorts and combined with culprit artery blood sampling during PCI to capture local thrombotic activity. Integration with non-invasive imaging, including CMR for MVO or intramyocardial hemorrhage, and real-time myocardial contrast echocardiography, would further enhance risk stratification and enable early identification of patients who might benefit from targeted adjunctive therapies.

Multi-target therapeutic strategies are needed to address the multifactorial nature of MVO in the setting of AMI with clinically relevant therapeutic regimens. Testable approaches include: (1) combination regimens pairing P2Y₁₂ inhibitors with anti-inflammatory agents to simultaneously block platelet activation and inflammation, (2) HMGB1/TLR4 pathway inhibitors such as glycyrrhizin, which require dose-finding studies to define safety windows given their mineralocorticoid-like and hemorrhagic effects at higher doses, (3) NETosis-targeted agents including DNase I and neutrophil elastase inhibitors, and (4) natural compounds with multi-target effects that warrant investigation in preclinical models of MVO and, if successful, early-phase clinical trials^[148].

To bridge the gap between preclinical promise and clinical translation, human-relevant microphysiological systems should be developed. Coronary microvasculature-on-a-chip models incorporating patient-derived endothelial cells, pericytes, and circulating blood components under controlled flow conditions can model key features of MVO, including platelet adhesion, NETosis, and fibrin deposition. These platforms enable: (1) high-throughput screening of candidate drugs and drug combinations, (2) patient-specific assessment of thrombotic risk and therapeutic response, and (3) mechanistic dissection of the thromboinflammatory cascade in a controlled, reproducible environment. Standardization and validation against clinical outcomes will be essential for their integration into drug development pipelines.

Finally, large-scale, well-characterized clinical cohorts are needed to address critical knowledge gaps in special populations. Priority research directions include: (1) prospective studies in patients with post-infection sequelae to clarify the role of persistent microvascular thrombosis and NETosis in driving

cardiovascular symptoms, (2) cohort studies in patients with comorbidities that amplify microthrombosis risk, including hypertension, diabetes and chronic kidney disease, to validate biomarker panels and risk scores in these high-risk groups, and (3) randomized controlled trials in high-risk MVO populations testing whether adjunctive therapies targeting specific nodes of the thromboinflammatory network improve clinical outcomes beyond current standard of care, with mechanistic sub-studies to confirm target engagement and validate biomarkers as surrogate endpoints.

CONCLUSION

Microthrombosis is a central and self-amplifying driver of coronary microvascular dysfunction and no-reflow, sustained by the interplay among endothelial injury, platelet activation, leukocyte recruitment, NETosis, and the HMGB1/TLR4/NF- κ B pathway. This thromboinflammatory cascade is further amplified by common cardiometabolic and inflammatory comorbidities and extends beyond the heart to multiple vascular beds. Although platelet-related biomarkers and single-target therapies show promise, current strategies remain limited by pre-analytical variability, bleeding risk, and inconsistent clinical translation. Future progress will require validated biomarker panels, endothelial-specific mechanistic clarification, and human-relevant microphysiological models to guide effective, multi-target therapeutic strategies.

DECLARATIONS

Authors' contributions

Conceptualization, supervision, writing - review & editing: Hu H

Writing - original draft, visualization: Bei J

Availability of data and materials

Not applicable.

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During the preparation of this manuscript, the AI tool DeepSeek (version V3.2, released 2025-12-01) was used solely for language editing and polishing. The graphical abstract and [Figures 1-3](#) were created with PicDoc (<https://www.picdoc.cn>, beta version), an AI-powered text-to-visualization tool. These AI tools did not influence the study design, data collection, analysis, interpretation, or the scientific content of the work. All authors take full responsibility for the accuracy, integrity, and final content of the manuscript.

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Conflicts of interest

All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

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