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REVIEW ARTICLE

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Pathophysiology and targeted treatment of cholesterol crystal embolism and the related thrombotic angiopathy

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Abstract

Cholesterol crystal (CC) embolism is a complication of advanced atherosclerotic plaques located in the major arteries. This pathological condition is primarily induced by interventional and surgical procedures or occurs spontaneously. CC can induce a wide range of tissue injuries including CC embolism syndrome, a spontaneous or intervention-induced complication of advanced atherosclerosis, while treatment of CC embolism has remained empiric. Vascular occlusions caused by CC embolism may exceed the ischemia tolerance of many tissues, particularly when small arteries are affected. The main approach to CC embolism is primary prophylaxis in patients at risk by stabilizing atherosclerotic plaques and avoiding unnecessary catheter interventions. During CC embolism, the use of platelet inhibitors to avoid abnormal activation and aggregation and anticoagulants may reduce the risk of vascular occlusions and tissue ischemia. This probably explains the relatively low prevalence of clinical manifestations of CC embolism, which are frequently found in autopsy studies. In this review, we summarized the current knowledge on the pathophysiology of CC embolism syndrome deriving from clinical observations and experimental mouse models. Furthermore, we described the risk factors of CC embolism in humans as well as the experimental studies based on empiric treatments. We also discuss potential therapeutic interventions based on recent experimental data and emerging drug options evolving from other research domains. Given the substantial unmet medical need to improve the outcomes of CC embolism, the identification of effective treatment strategies is urgently needed.

KEYWORDS
atherosclerosis, cholesterol crystal embolism, injury, ischemia, vascular occlusion

Abbreviations: ADP, Adenosine diphosphate; AKI, Acute kidney injury; ATP, Adenosine triphosphate; CC, Cholesterol crystal; CRP, C reactive protein; eNOS, Endothelial NO synthase; FGN, Fibrinogen; GFR, Glomerular filtration rate; GP, Glycoprotein; HUS, Hemolytic uremic syndrome; KLF2, Krüppel-like Factor 2; LDL, Low-density lipoproteins; NO, Nitric oxide; PAR-Gq, Protease-activated receptor; PS, Phosphatidylserine; SMC, Smooth muscle cell; TF, Tissue factor; TMA, Thrombotic microangiopathies; TNFR, Tumor necrosis factor receptor; tPA, Tissue-type plasminogen activator; TTP, Thrombotic thrombocytopenic purpura; vWF, von-Willebrand-Factor.

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1 | INTRODUCTION

Cholesterol crystal (CC) embolism is a complication of advanced atherosclerosis and can affect almost every organ through microvascular obstructions and ischemic tissue necrosis.[1,2] CC embolism originating from the atheromatous plaques of the abdominal aorta affects lower limbs ("blue toe syndrome") as well as solid organs such as the kidney (AKI), the pancreas (ischemic pancreatitis), and the small intestines (intestinal ischemia).[3,4] When the source of CC is plaques in the ascending aorta, ocular or cerebral embolism can occur as well as ischemic lesions in the upper limbs.[5] The pathological consequences of CC embolism are frequently fatal when ischemic necrosis of the small intestines leads to bacterial peritonitis or when bilateral kidney embolism leads to uremia.[6] Milder episodes of CC embolism to non-vital organs are frequently undetected. Indeed, autopsy studies report a high prevalence of CC embolisms which are not recognized during a lifetime.[7]

So far, treatment of CC embolism has remained empiric due to a limited number of randomized controlled trials.[8] Hence, only three trials are available (www.clinicaltrials.gov) in this pathological context: a study testing prednisolone therapy (NCT01452100), a study testing the efficacy of blood purification (NCT01726868), and a diagnostic study of magnetic resonance imaging following angiographies and angioplasty-stenting of the renal artery (NCT00027469).

In this review, we describe the risk factors and pathophysiological nature of CC embolism syndrome. We present new experimental and conceptual progress about the different steps of disease pathogenesis, including the molecular mechanisms of CC embolism initiation, CC embolism-induced thrombosis and thromboinflammation, ischemic injury, and cell death. In addition, we analyze the effects of anti-inflammatory and anti-platelet agents at each stage of CC embolism progression, potentially providing novel preventive and therapeutic approaches for patients diagnosed with CC embolism syndrome.

2 | RISK FACTORS FOR CC EMBOLISM

The incidence of clinically evident CC embolism syndrome has been reported to vary between 0.09% and 2.9%.[9–11] Notably, the true incidence is probably much higher since many cases of CC embolism syndrome are often overlooked as reported by autopsy studies.[12,13] Several risk factors contribute to the development of CC embolism syndrome, including interventional vascular procedures, cardiovascular surgery, hypertension, diabetes mellitus, gender, age, and the use of anticoagulants and thrombolytic treatments (Figure 1).[14–17]

Gender-specific risk factors not only contribute to different development of atherosclerosis but also indirectly promote CC embolism, associated with other risk factors, such as smoking, dyslipidemia, hypertension, and diabetes mellitus.[18] Males tend to develop atherosclerotic plaques earlier and have a higher plaque burden compared to females.[19–21] Males also develop more non-culprit lesions, coronary artery lesions, frequent plaque ruptures, larger total necrotic core volume, and more comorbidities.[19,22]

Age is another important risk factor in CC embolism syndrome. Spontaneous plaque ruptures are predominantly observed in patients over 60 years old, with an incidence of 0.79%–3.4%.[23]

Smoking is another risk factor for CC embolism syndrome. Various chemical components, such as nicotine, present in tobacco smoke directly cause endothelial damage and enhance inflammatory responses in the vascular wall due to the oxidant and proinflammatory properties of nicotine molecules.[24–27] The size of calcified atherosclerotic plaques is directly proportional to the number of cigarettes consumed per day. Moreover, smoking cessation is one of the most important health interventions for reducing the risks of cardiovascular diseases, cancer, and mortality at any age.[28]

Surgical interventions involving the aorta or its major branches are associated with the highest risk of plaque rupture and CC embolism. Abnormalities consistent with CC embolism were observed in 21.7% of the autopsy series

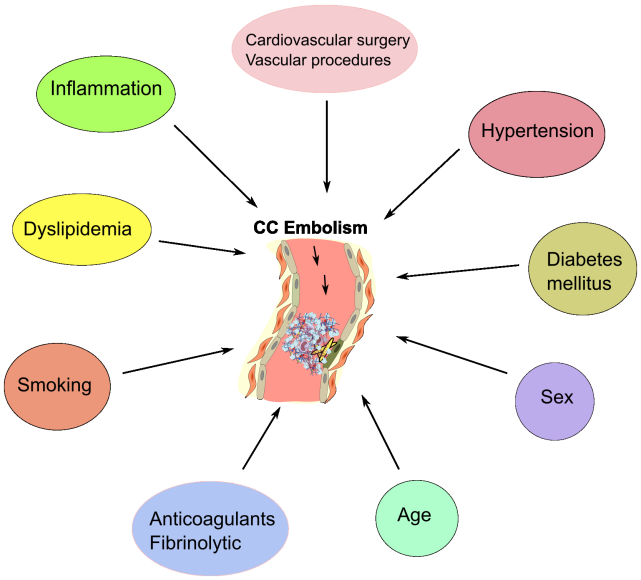

FIGURE 1 Risk factors for CC embolism. Several modifiable and non-modifiable risk factors are involved in CC embolism syndrome.

from patients who underwent myocardial revascularization or heart valve surgery.[29] Coronary revascularization surgery-related CC embolism (26.1%) was three times more common than valve surgery (8.9%).[29] In a prospective study of 1786 patients undergoing cardiac catheterization in Japan, the incidence of CC embolism syndrome was reported as 1.4%.[8] Abdominal aortic aneurysm (AAA) is also a causative factor for the development of CC embolism, 2.9% of AAA patients with CC embolism was detected.[11] However, coronary angioplasty is a lower risk factor, only 0.09% of patients developed CC embolism.[30] Interventional procedures or cardiovascular surgery are the most common risk factors, it may also trigger spontaneous plaque rupture, and the outcome is in 70% of cases generally.[30]

Thrombolytic therapy is also associated with CC embolism syndrome.[31] Anticoagulants and fibrinolytics may promote CC embolism syndrome by causing plaque hemorrhage and plaque rupture.[6,32] However, the exact pathomechanisms, linking anticoagulants/fibrinolytics and CC embolism syndrome remain unclear.[8,33]

Increased inflammation is also a critical risk factor for CC embolism syndrome. Patients with CC embolism syndrome have often significantly higher levels of C reactive protein (CRP) in their plasma compared to those without CC embolism syndrome (0.7 vs 2.4 mg/dL).[8] Therefore, increased CRP levels are considered an independent predictor of CC embolism syndrome (OR 4.6).[8]

3 | CLINICAL MANIFESTATIONS OF CC EMBOLISM

The kidney is a commonly affected organ by CC emboli, but only around 50% of patients experience noticeable clinical symptoms.[34] The onset of symptoms can vary, i.e. some patients exhibit symptoms shortly after CC embolism, while others may experience a delay of several weeks to months.[6,34,35] Kidney CC embolism primarily manifests in the following conditions: (i) AKI accompanied by symptoms originating from multiple sites or organs, typically resulting from either larger arteries or the emboli, (ii) subacute kidney injury attributed to either the inflammatory response caused by CC emboli or the consecutive lodging of new emboli, (iii) chronic kidney disease, characterized by kidney ischemia or vascular sclerosis, is often asymptomatic because CC emboli are mainly detected in kidney biopsies or autopsies.[36] CC embolism can affect the cerebral and retinal arteries, deriving from the ascending aorta and proximal aortic arch. Symptoms include psychological disturbances, headaches, neurological deficits, temporary vision loss and mild paralysis of the lower extremities.[37] Hollenhorst plaques are known as diagnostic features of CC embolism occurring in retinal arteries.[38] Embolism affecting the arteries of the kidneys and arteries below the abdomen, including the legs and feet, typically originates from the descending thoracic and abdominal aorta.[37,39] Patients with CC embolism can also experience gastrointestinal complications, characterized by mucosal ulceration, bleeding, diarrhea, and stomach pain.[40] Skin lesions, such as livedo reticularis, cyanosis, and ulcers, are observed in 35–96% of patients.[41] Other symptoms associated with CC embolism are hemoptysis, femoral head necrosis, dyspnea and rhabdomyolysis.[41]

4 | MOLECULAR MECHANISMS AND PATHOGENESIS OF CC EMBOLISM

4.1 | Rupture of vulnerable plaques

The relationship between CC embolism and aortic atherosclerosis was first suggested in 1945 by Flory.[42] This concept was later studied by Kealy who demonstrated the presence of advanced plaques in several cases of peripheral embolism, often accompanied by the formation of aneurysms.[43] CC embolism occurs when small atheromas found in major arteries, including the aorta, break and release CC into smaller arteries.[44] The central pathology underlying atherosclerosis, a chronic condition, involves the formation of atheromatous or fibrofatty plaques. These plaques are the primary pathological feature in atherosclerosis, and their instability can lead to the release of CC emboli, contributing to CC embolism. Lipids, inflammatory infiltrates, smooth muscle cells (SMCs) and connective tissues are present within the plaques. Two simultaneous mechanisms contribute to plaque rupture: the gradual loss of SMCs from the fibrous cap and the degradation of the collagen-rich cap matrix, leading to thinning of the fibrous cap.[45] At the rupture site, SMCs are usually absent and ruptured caps contain fewer SMCs than intact caps.[46] Activated macrophages and foam cells can degrade the collagen matrix components of the fibrous cap in the lesion.[47] Once the atherosclerotic plaque ruptures, it becomes exposed to the vascular endothelium bloodstream, leading to partial or complete occlusion of the blood vessel lumen. In addition, macrophages and SMCs at the rupture site can produce tissue factors (TF), contributing to the procoagulant phenotype and the development of acute coronary syndrome, which is the main clinical manifestation of atherosclerotic progression (Figure 2). The onset of acute coronary diseases can be triggered by simple daily activities or the circadian rhythm, which has a higher incidence in the morning.[48] Increased heart rate and blood pressure can lead to plaque rupture, with enhanced

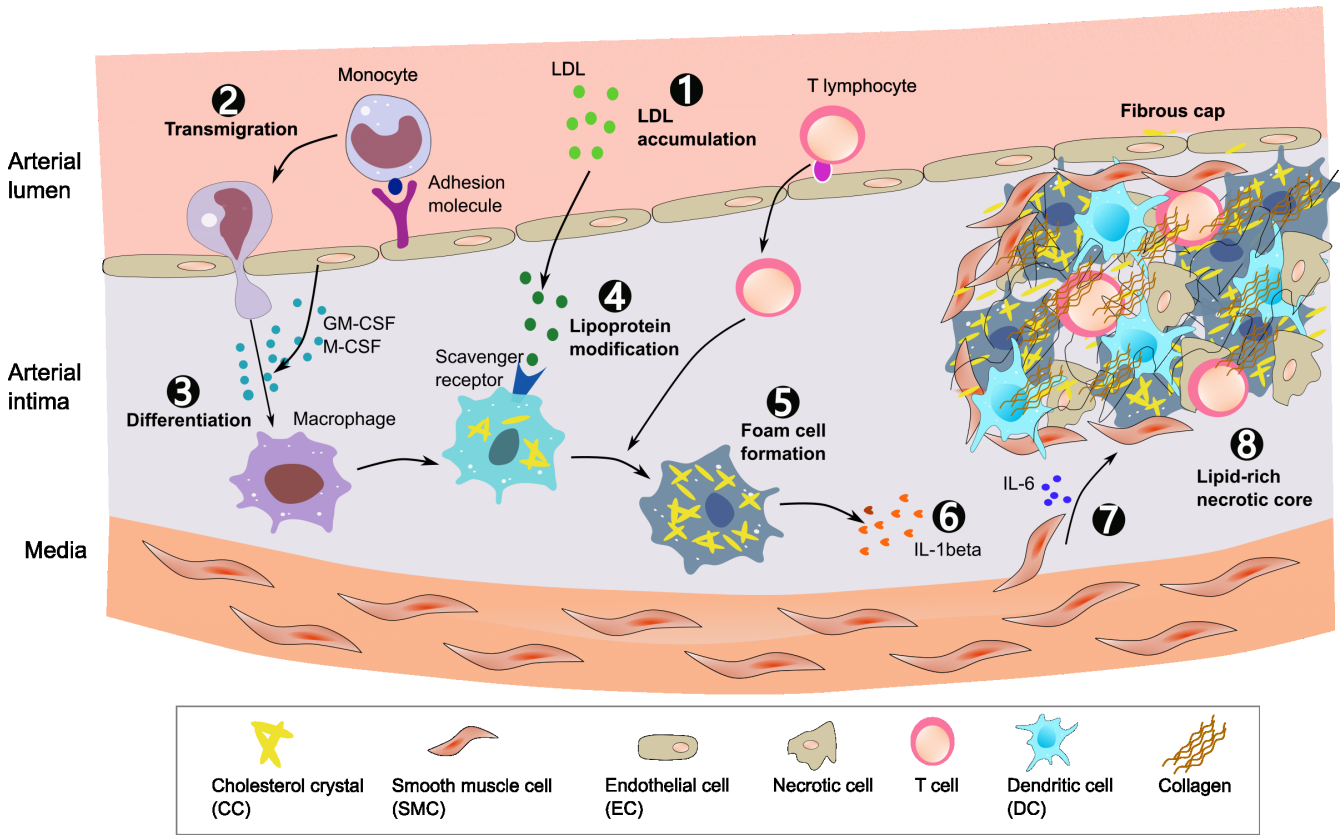

FIGURE 2 The progression of atherosclerotic plaque in the artery. LDL retention in the intima initiates atherosclerosis development, where they can undergo oxidative and other modifications that can render them pro-inflammatory and immunogenic. Accumulation of LDL leads to the upregulation of adhesion molecules on the endothelial surface and the recruitment of monocytes to the forming lesion. In the subendothelial space, monocytes differentiate into macrophages in response to M-CSF and GM-CSF produced by endothelial cells. These macrophages express scavenger receptors that can uptake lipoproteins leading to the formation of foam cells. Cholesterol crystals form in foam cells resulting in the release of IL-1β and IL-6. Both IL-1β and IL-6 exert proinflammatory effects. As the lesion advances, SMCs and macrophages can undergo cell death including apoptosis. The debris from dead and dying cells accumulates, forming the necrotic, lipid-rich core of the atheroma. LDL: low-density lipoprotein, M-CSF: Macrophage colony-stimulating factor, GM-CSF: Granulocyte-macrophage colony-stimulating factor, IL-1β: Interleukin 1 beta, IL-6: Interleukin 6. SMC: Smooth muscle cell.

coagulability and platelet reactivity further amplifying the thrombotic response to the ruptured plaques.[49,50] Various factors influence the vulnerability of plaques to rupture, including the expression of adhesion molecules, local cytokine release, endothelial cell dysfunction, activation of monocytes and macrophages, complement activation and the presence of proteolytic enzymes. Collectively, these factors contribute to the destabilization of plaques, thereby increasing the risk of rupture.[51]

4.2 | CC-induced vascular occlusion

During CC embolism, the hemostatic balance is disturbed by the rupture of the endothelial layer, leading to the exposure of the vascular extracellular matrix. Following vessel injury, subendothelial matrix proteins, including collagen, become exposed to the flowing blood.[52] Exposed collagen anchors von-Willebrand-Factor (vWF) and initiates interactions between platelet glycoprotein (GP) Ibα and vWF, as well as GPVI and collagen, which are crucial steps in platelet activation.[52] Platelets also express various integrins on their surface, which, upon outside-in activation by their extracellular ligands, promote platelet adhesion to the injured vessel wall. Additionally, activated platelets release numerous bioactive molecules and secondary mediators, such as fibrinogen (FGN), vWF, adenosine diphosphate/adenosine triphosphate (ADP/ATP), and serotonin, from their alpha (α) and dense delta (δ) granules.[53] These molecules further enhance the prothrombotic process, promoting the recruitment of circulating platelets to the developing thrombi. The extrinsic and intrinsic blood coagulation pathways trigger the second wave of hemostasis, leading to the generation of thrombin. Thrombin, in turn, converts soluble fibrinogen into fibrin, thereby reinforcing platelet activation and clot formation. Activated platelets also expose phosphatidylserine (PS) on their surface, facilitating the binding of coagulation factors that

stimulate thrombin generation and activate the coagulation cascade. Recently, we provided compelling evidence regarding the significant impact of CC on platelet adhesion and activation, thereby promoting prothrombotic functions. While CC alone does not induce platelet activation or adhesion, upon stimulation with thrombin, CC exerts a potent effect on platelets, leading to their activation and degranulation. This dynamic process results in the release of mediators such as FGN and ATP, thereby fostering a prothrombotic microenvironment. CC stimulates platelet activation through the GPVI-ITAM and PAR-Gq signaling pathways, ultimately leading to the inside-out activation of integrin αIIbβ3 and the exposure of P-selectin.[54,55] Collectively, these intricate mechanisms contribute to enhanced platelet reactivity and the formation of fibrin clots.

Thromboinflammation, a term used to describe the simultaneous activation of thrombotic and inflammatory responses, is observed in various diseases, including CC embolism.[56,57] The presence of CC emboli within arterioles triggers a series of inflammatory reactions, including endothelial dysfunction, cytokine release, immune response and the formation of intravascular thrombi.[54,56] In the initial 24 h, neutrophils are the first inflammatory cells to infiltrate the affected arterioles, followed by the infiltration of monocytes that differentiate into macrophages and form foreign body giant cells responsible for engulfing large CCs. Platelet–neutrophil interactions contribute to the activation and release of proinflammatory cytokines and chemokines, which further promote thrombus formation and exacerbate the inflammatory response.[58] Using the kidney CC embolism model, we recently showed that extracellular traps play a critical role in the formation of emboli. Indeed, the observed immunothrombotic complication was associated with the presence of platelets, red blood cells, leukocytes, fibrin mesh and extracellular DNAs that are released by damaged endothelial cells, neutrophils and activated platelets.[54,56] Thus, the reciprocal interaction between platelets, innate immune cells and activated vascular endothelium influences both the thrombotic potential and immune response, thereby linking thrombosis to the inflammatory site (Figure 3).

Although thrombosis is a characteristic feature of thrombotic microangiopathies (TMAs) observed in CC embolism syndrome, not all cases of thrombosis are classified as TMAs. TMAs were introduced to specifically refer to a group of disorders that share the common feature of microvascular thrombosis and associated organ dysfunction. Other forms of TMAs can be regulated by different mechanisms depending on underlying conditions (platelet consumption, erythrocyte fragmentation, ischemic injury, thrombotic thrombocytopenic purpura (TTP), antiphospholipid syndrome, hemolytic uremic syndrome (HUS).[59]

4.3 | Programmed cell death pathways in CC embolism

The subsequent inflammatory response can be influenced by the programmed cell death pathways, detected in the parenchymal and tubular cells during kidney ischemia. The molecular and cellular mechanisms of ischemic necrosis are well-studied in many thromboinflammatory diseases, including myocardial infarction, ischemic stroke, and acute kidney injury. The measurement of infarct size is the primary endpoint of most experiments predicting long-term organ functionality in the heart, brain, or kidney. Tissue hypoxia in the kidney first impairs the functional capacity of parenchymal cells by depleting ATP but prolonged ischemia leads to ischemic cell necrosis. Different tissues have a different sensitivity to ischemia time but without therapeutic revascularization, CC embolism-related vascular occlusions may exceed ischemia tolerance of most tissues, especially when small ends are affected, e.g., in the brain, the skin, the small intestine, and the kidney. Ischemic necrosis is not a passive process but involves numerous signaling pathways of regulated necrosis discovered and characterized during the last decade (Figure 4). This chapter describes the various types of reprogrammed cell deaths that arise in response to proinflammatory and prothrombotic stimuli during CC embolism.

4.3.1 | Necroptosis

Necroptosis is a programmed form of necrosis, or inflammatory cell death, triggered by outside-in signaling pathways downstream effectors of pro-inflammatory death receptors such as tumor necrosis factor receptor 1 (TNFR1).[60,61] When caspase-8-mediated apoptosis is deactivated, receptor-interacting protein (RIP)-kinase 1 and RIP-kinase 3 dissociate from the receptor complex.[61] Consistently, *Mlkl*-deficient mice are protected from CC embolism-related ischemic necrosis of the kidney, indicating the central role of necroptosis in this process. *Mlkl*-deficient mice are also protected from ischemic necrosis of the kidney induced by transient kidney pedicle clamping as well as from ischemic necrosis in the brain and the heart induced by other means, e.g., arterial ligation.[54] This suggests a general contribution of the necroptosis signaling pathway to ischemic tissue necrosis.

4.3.2 | Apoptosis

The first known form of programmed or regulated cell death was apoptosis, which for a long was defined by

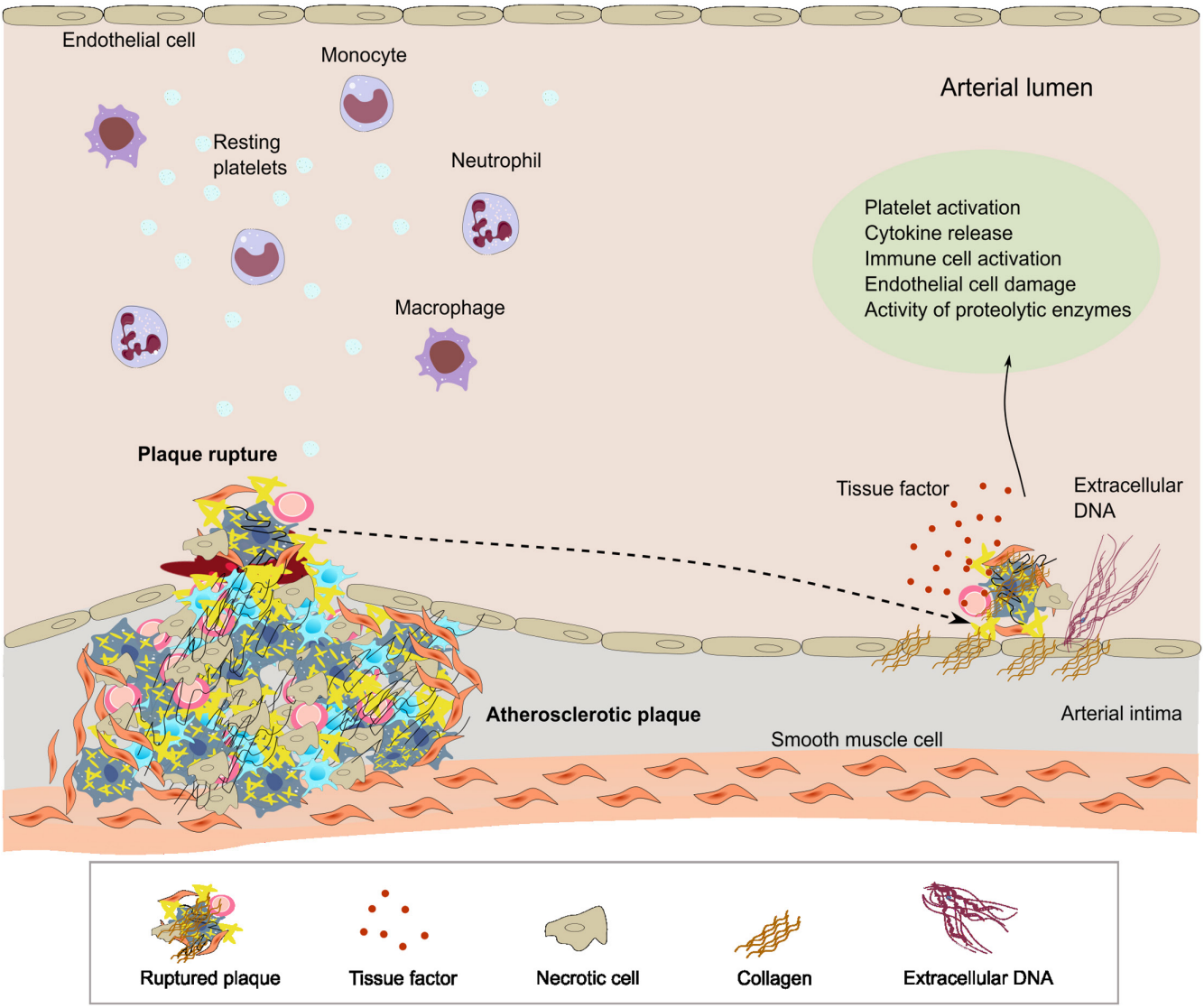

FIGURE 3 Ruptured vulnerable plaque-induced vascular occlusion. In the smaller arteries, the plaque not only mechanically restricts blood flow but also can damage vascular walls releasing tissue factor and extracellular DNA, following platelet activation and aggregation, immune cell activation, cytokine release, etc.

TUNEL positivity, an analytical method detecting breaks in double-stranded DNA.[62] However, the discovery of other forms of regulated necrosis clarified the unspecific nature of TUNEL positivity, which is now considered more a general marker of cell death. Currently, each form of regulated cell is defined by a unique signaling pathway. For example, apoptosis is characterized by cell death that occurs in the presence of caspase-3 cleavage and can be inhibited by caspase-3 inhibitors.[63] With the use of these analytical tools, it appears that apoptosis does not contribute significantly to ischemic cell necrosis. Indeed, apoptosis is a non-immunogenic form of homeostatic cell death, e.g., with major importance in the silent deletion of autoreactive immune cells or of cells with significant DNA damage to prevent malignant transformation.

4.3.3 | Netosis

In 2004, the group of Arturo Zychlinsky first described an alternative form of neutrophil bacterial killing, i.e., the release of NETs. NETosis has two distinct forms, suicidal and vital NETosis. NET release is frequently associated with neutrophil necrosis, driving necroinflammation in many pathological conditions, including ischemic tissue necrosis NETs mostly form inside the microvasculature, and primarily cause direct endothelial cell injury and intravascular thrombosis.[56,64] Activated platelets can directly induce NETosis, thereby amplifying necroinflammatory and thrombotic events.[65]

NETosis does not only imply the release of nuclear DNA and histones but also the release of toxic proteases from neutrophil granules. DNase I treatment has been proposed as an

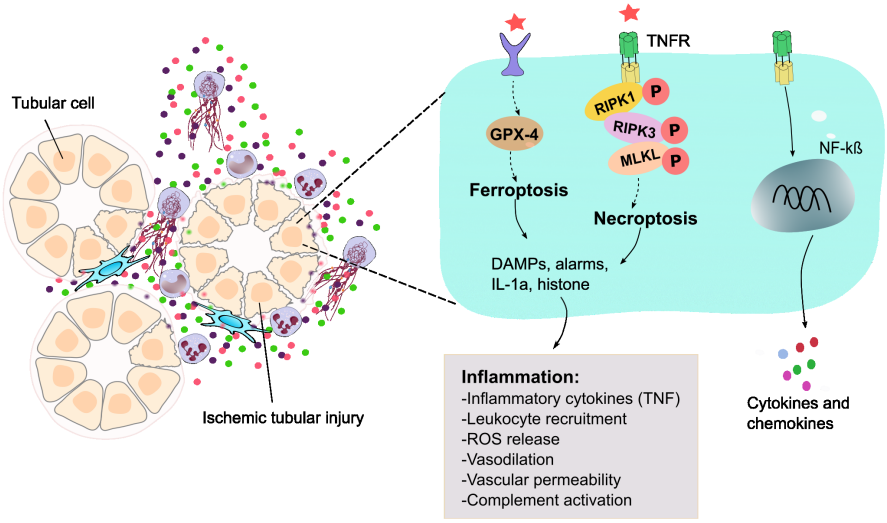

FIGURE 4 CC embolism-induced programmed cell death pathways in the kidney. Ischemia triggers primary tubular injury through different pathways, damaged tubular cells release DAMPs, IL-1α, histone, cytokine and chemokines, which lead to inflammatory responses, like leukocyte recruitment, complement activation, vasodilation and change the permeability of vascular endothelium. Most importantly, the inflammation in turn induces further tubule injury. DAMP: damage-associated molecular patterns, IL-1α: Interleukin 1 alpha, GPX-4: Glutathione peroxidase 4, RIPK: Receptor-interacting serine/threonine-protein kinase, MLKL: Mixed Lineage Kinase Domain Like Pseudokinase, NF-kβ: Nuclear Factor Kappa B.

effective tool to neutralize NETs but this treatment does not influence lytic enzymatic activities. Systemic administration of DNase I is potent to prevent ischemic tissue necrosis in CC embolism to dissolve the chromatin mesh contributing to intravascular thrombosis at the site of the crystal clot.[54] It has been proposed that perilesional neutrophil infiltrates in the kidney may be involved in NET formation. Therefore, we speculate that inhibiting NETosis may help to limit the size of tissue infarction in CC embolism.

Neutrophil infiltration and consequent tissue necrosis are regulated by the circadian rhythm, which was explained by the differential expression of adhesion molecules and cytokine on vascular endothelium and immune cells during CC embolism.[66] Indeed, the expression of adhesion molecules (Icam-1, Vcam-1) on endothelial cells is lower during the day than at night, the release of chemokine (CXCL1) from neutrophils cells is less than at night, the expression of CXCR2 is lower, while CXCR4 is high, which leads to more tissue necrosis during the night.[66]

4.3.4 | Necroinflammation

Ischemic necrosis is accompanied by rapid neutrophil recruitment followed by pro-inflammatory macrophages, which both contribute to perilesional inflammation. The auto-amplification loop between ischemic necrosis and perilesional inflammation also referred to as necroinflammation, involves damage-associated molecular patterns (DAMPs) and proinflammatory cytokines (interleukins), as well as cytotoxic DAMPs such as extracellular histones that trigger both cell death and inflammation.

CC-induced TMAs are a complex pathological process, involving thrombosis, tissue necrosis and inflammation. Thrombosis within the microvasculature leads to the release of cytokines and chemokines from activated platelets.[67] These mediators activate endothelial cells and attract leukocytes to the site of injury.[56,67]

Patients with metabolic syndromes often have a proinflammatory state, due to the accumulation of several disease conditions, such as atherosclerotic cardiovascular disease, insulin resistance and diabetes mellitus.[68–70] Recently, we showed that hyperglycemia aggravated TMA and glomerular filtration rate by increasing neutrophil recruitment, necroinflammation and infarct size during CC embolism.[55] In line with this, CC enhanced the prothrombotic response of hyperglycemic platelets.[55] Altogether, these studies indicate that a CC-induced prothrombotic environment can exacerbate necroinflammation. Of note, the long-term outcomes of renal TMA depend on the regenerative capacity of different cell types through intrinsic or extrinsic progenitor potential. In general, the suppression of necroinflammation can be achieved by blocking proinflammatory and prothrombotic mediators or pathways that regulate necrosis.[71] However, the efficacy of these treatments may vary depending on the degree of injury and the stage of the disease.

5 | TARGETED TREATMENTS OF CC EMBOLISM

The adhesion and activation of immune cells and platelets are tightly regulated at different steps of CC embolism, thereby influencing the inflammatory response, thrombus formation and ischemia in patients with CC embolism. Several signaling receptors and bioactive mediators from immune cells, platelets and vascular endothelium are involved in this process (Table 1). In this chapter, we discuss the potential of anti-inflammatory, anti-coagulant and fibrinolytic therapies at different steps of CC embolism.

5.1 | The site of CC emission

Statins are commonly used to reduce atherosclerotic lesions by effectively decreasing the levels of low-density lipoproteins (LDL) and stabilizing or regressing the established atherosclerotic plaque.[72] Furthermore, statins have pleiotropic effects, such as reducing inflammation and oxidative stress in the vascular system.[72] Statins also block cholesterol synthesis in the liver by inhibiting 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, thereby reducing the production of mevalonate, which is an essential metabolic product for cholesterol synthesis.[80] Other studies suggested that statins not only limit hyperlipidemia but also decrease oxidative stress, activating anti-inflammatory and anti-proliferative mechanisms on vascular endothelial and smooth muscle cells.[81] It was shown that atorvastatin and simvastatin exert a positive effect on nitric oxide (NO) levels by increasing the synthesis and activity of endothelial NO synthase 3 (eNOS) via the inhibition of miRNA-221 and miR-222. Increased levels of NO are associated with a low concentration of ROS, reduced vascular endothelium permeability and inflammatory response (Figure 5).[73] Statins also exert antiplatelet

TABLE 1 Possible therapeutic interventions in CC embolism syndrome.

Targeted Pathomechanism	Specific pathway	Pharmacological inhibitor/device	Ref.
Stabilization of vulnerable plaque			[72–78]
Arterial occlusion by crystal clots			[54]
Ischemic necrosis Necroinflammation	MPT-driven necrosis	Cyclosporin A NIM811	[79]
	Necroptosis	Necrostatins, phenytoin, ...	[60,61]
	Autophagy	3-methyladenine	[79]

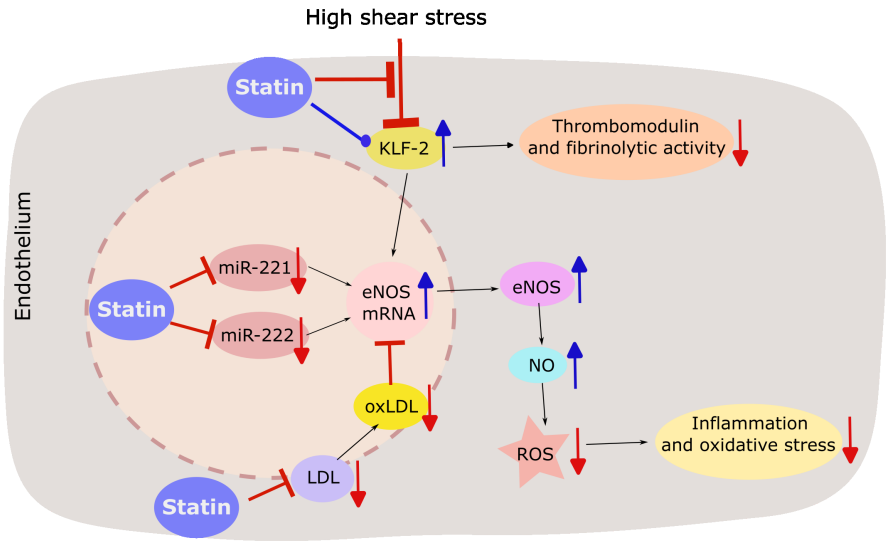

FIGURE 5 Targeted treatments at the site of CC emission. The mechanism of statin therapy on endothelial cells. On one hand, statin therapy can reduce inflammation and oxidative stress in the vascular system, because it regulates endothelial proinflammatory factors such as ROS release by inhibiting miR-222, and miR-221, and decreasing the levels of LDL. On the other hand, statin can also enhance the activity of KLF2 decreasing the thrombomodulin and fibrinolytic activity. ROS: Reactive oxygen species, KLF2: Kruppel-like transcription factor 2, LDL: low-density lipoprotein, NO: nitrogen oxide, eNOS: Endothelial nitric oxide synthase.

effects by inhibiting the release and expression of thromboxane A2, ox-LDL and CD36 receptors and enhancing the production of eNOS, which improves the production of platelet NO. In its turn, the NO inhibits platelet activation and aggregation, thereby suppressing blood clotting and coagulation by downregulating the tissue factor expression and activity.[74–78] In addition, statins can inhibit thrombosis by upregulating Krüppel-like Factor 2 (KLF2) levels, which promotes the expression of thrombomodulin and fibrinolytic activity.[82,83] Whether statins may have anti-inflammatory and anti-thrombotic effects in CC embolism awaits future investigation.

5.2 | The sites of peripheral occlusion and ischemic necrosis

The presence of the crystal component, which occupies only a small fraction of the vascular lumen, results in arterial thrombosis and vascular occlusion, thereby inducing acute kidney injury, which is followed by a sudden drop in glomerular filtration rate (GFR), and ischemic kidney infarction (Figure 6).[54] This thrombo-inflammatory process involves the complex interplay between platelets, neutrophils and vascular endothelium.[54] During CC embolism, the hemostatic balance is disturbed by the rupture of the endothelial layer, leading to the exposure of the vascular ECM. CC can enhance platelet attachment to the exposed collagen, further amplifying platelet reactivity by promoting the exposure of P selectin, the release of fibrinogen and ATP and the increasing the adhesion of circulating platelets to the CC-induced thrombi.[54] Consequently, the prothrombotic environment becomes abundant in FGN and thrombin, leading to the generation of a fibrin clot, which can further induce the recruitment of circulating platelets to the growing thrombus sites. Accordingly, preemptive injection of fibrinolytic drugs, anticoagulants, DNase I and antiplatelet agents was shown to completely prevent crystal clot formation, acute kidney injury and kidney

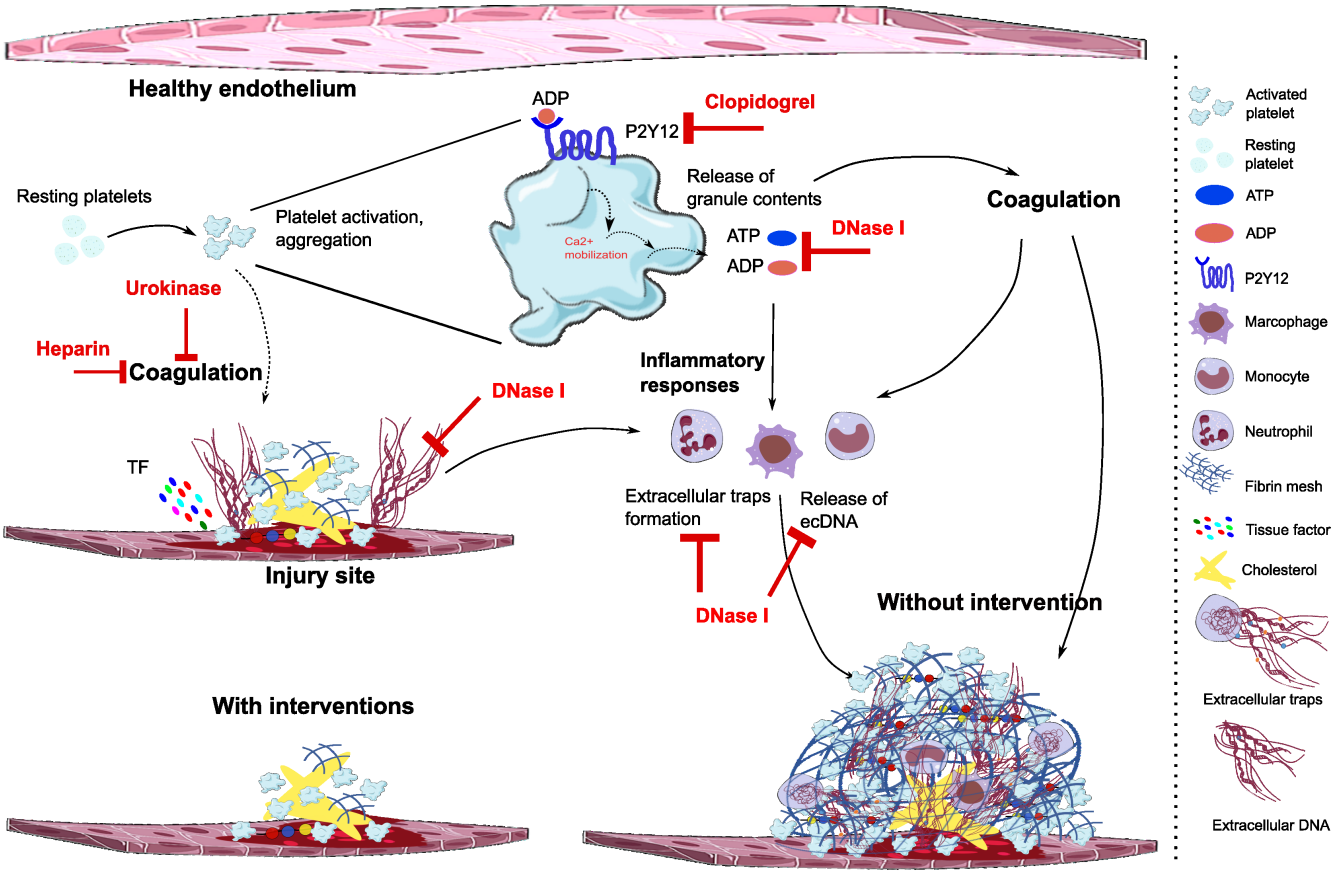

FIGURE 6 Targeted treatments at the peripheral vascular occlusion. Damaged endothelium release tissue factor (TF) and ecDNA activate platelets, meanwhile, the role of ecDNA as a DAMP can also trigger an inflammatory response. Activated platelets release granule contents, and cause blood coagulation. DNase I can degrade ecDNA released from damaged endothelial cells to prevent platelet activation and inflammation. DNase I prevents ecDNA mesh formation, platelet activation, and aggregation. Clopidogrel can be combined with P2Y12 to prevent platelet activation and aggregation, urokinase prevents coagulation via balancing the plasmin in the system, and heparin involves in various steps of coagulation.

infarction without affecting the crystal component inside the arteries.[54]

Ischemic necrosis is a common form of tissue injury, e.g., in stroke, myocardial infarction or limb ischemia but despite a myriad of experimental studies demonstrating tissue-protective effects from targeted interventions, the medical practice remains focused on revascularization or fibrinolytic and antithrombotic drugs. One of the reasons is that whenever ischemic necrosis is a consequence of vascular occlusion, tissue-protective interventions have limited potential as long as vascular occlusion and ischemia persist. For example, genetic or pharmacological inhibition of CC embolism-related ischemic necrosis of the kidney did not improve the early decline of kidney function as further proof that vascular obstruction is an upstream mechanism of CC embolism.[54] Therefore, the drug pipeline of necrosis inhibitors at best may help to increase the window of opportunity for revascularization strategies, e.g., with fibrinolytic or antithrombotic drugs, in established CC embolism.[84]

5.2.1 | Fibrinolysis

Fibrin-rich clot formation plays an important role in the process of hemostasis, serving as the main product of the coagulation cascade and also acting as the ultimate substrate for fibrinolysis. The process of fibrinolysis involves the conversion of plasminogen into plasmin by either tissue-type plasminogen activator (tPA) or urokinase-type plasminogen activator and the breakdown of fibrin degradation through the hydrolytic action of plasmin.[85] However, the intrinsic fibrinolytic system cannot effectively reduce thrombus growth and intravascular coagulation during CC embolism to restore blood flow and revascularization. Platelet-rich thrombi are resistant to tPA-induced thrombolysis, compared to red blood cell-rich clots.[86] This resistance is linked to the presence of the platelet-derived alpha subunit of factor XIII and protease inhibitors.[86] Therefore, fibrinolytic therapies are used in the treatment of thrombotic disease conditions, such as stroke, pulmonary embolism and deep vein thrombosis, thereby helping to dissolve blood clots and restoring blood flow and promoting tissue healing and revascularization. We showed that after 24 hours, urokinase significantly reduced the number of occlusions in the arteries, while the crystal component persisted. Urokinase treatment could improve GFR loss and significantly reduce the size of kidney infarcts, kidney injury, neutrophil infiltration, vascular injury, and kidney tubular cell death.

Although fibrinolytic drugs could completely prevent crystal clot formation, acute kidney injury and kidney infarction without affecting the crystal component inside the arteries.[54] However, these interventions can be associated with bleeding complications, thereby limiting their effectiveness in clinical settings. Recently, we showed that Glu-plasminogen extracts from human plasma injected intravenously 4 h after CC injection into the left kidney artery of mice attenuated thrombotic angiopathy, AKI, and cortical necrosis in a dose-dependent manner.[87] An intermediate dose had a transient effect, which renders Glu-plasminogen a well-controllable intervention although no bleeding complications occurred during operatory or postoperatory periods.[87] Therefore, Glu-plasminogen substitution could be a potential therapeutic approach in patients with CC embolism syndrome. Although Glu-plasminogen treatment can restore balance to local fibrinolysis in the injured kidney arteries, inhibiting fibrin-rich blood clot formation, it may probably require continuous anticoagulant therapy to prevent recurrent crystal clot formation.

5.2.2 | Anticoagulant therapies

Heparin is an anti-coagulant to prevent blood clotting during surgical interventions, kidney dialysis, and for the management of various diseases such as VTE, heart attacks, and angina. Heparin affects the blood coagulation pathway by inactivating thrombin.[88] Heparin induces a conformational change in antithrombin III, thereby facilitating a complex formation with thrombin. Although heparin is considered an anti-coagulant, it can also have anti-inflammatory and anti-proliferative and profibrinolytic effects.[88] Heparin has also been shown to protect against organ and tissue damage caused by histones, as well as decrease the levels of NETs in a mouse model of sepsis.[89] Similar to urokinase, heparin could also prevent crystal clot formation, improving GFR loss and reducing acute kidney injury and infraction during crystal clot embolism.[54] However, heparin treatment may cause adverse effects such as hemorrhagic diathesis, intra-organ bleeding, osteoporosis and thrombocytopenia. Heparin-induced thrombocytopenia is frequently caused by the production of antibodies against heparin-platelet factor 4 (PF4) complexes, which can also activate neutrophils and promote NET formation, thereby enhancing thrombosis.[90] Low-molecular-weight heparin (LMWH) offers several benefits compared to heparin, including decreased risks of HIT and osteoporosis, longer bioavailability and half-life, and predictable anticoagulant response. However, both LMWH and unfractionated heparin (UFH) can increase the risk of bleeding in patients with kidney disease, due to their accumulation in kidney tissue.[91]

In order to minimize the bleeding risk, various clinical therapies have been recommended, including reducing

the dosage, adjusting treatment based on anti-Xa heparin levels, and using lower doses of LMWH.[92] Nonetheless, the risk of bleeding may differ depending on the particular clinical circumstance. For example, patients with venous disease may experience lower bleeding risks with LMWH compared to UFH, whereas those with acute coronary syndrome may be at higher risk for bleeding with LMWH.[92]

5.2.3 | Anti-$P2Y_{12}$-based antiplatelet therapies

The platelet $P2Y_{12}$ receptor is a Gi-coupled ADP binding receptor that regulates thrombus stability by amplifying platelet aggregation and activation, granule secretion and procoagulant activity.[93] The $P2Y_{12}$ receptor is considered a prime candidate for antithrombotic medication, due to its central role in the purinergic signaling pathway.[94] Clopidogrel is a selective $P2Y_{12}$ receptor antagonist, which is used in the treatment and prevention of heart attacks, ischemic stroke and peripheral artery diseases.[94] In the CC embolism model, preventive injection of clopidogrel completely protected mice from intravascular obstructions, GFR loss, acute kidney injury and kidney infarction without affecting the crystal component inside the arteries. However, clopidogrel treatment could be associated with bleeding complications, which complicates their usage in clinical settings. In line with this, genetic or functional deficiency of the $P2Y_{12}$ receptor in mice or humans induces longer bleeding times and severe hemorrhage.[94,95] Mice treated with a high dose of clopidogrel displayed bleeding times that can last up to 30 minutes and even result in death from bleeding.[95] Hence, bleeding risks represent the major drawbacks when clopidogrel is administered, either alone or in combination with aspirin.[94,96] These challenges led to the development of new anti-$P2Y_{12}$ drugs, such as prasugrel, ticagrelor and cangrelor. More recently, in patients with myocardial infarction, selatogrel emerged as a new potent, selective and reversible $P2Y_{12}$ blocker, inducing a rapid and strong platelet inhibition without major bleeding complications. Further studies are needed to test the effect of selatogrel on CC-induced AKI and thromboinflammation.

5.2.4 | DNase I-based anti-thrombotic therapy

DNase I is an enzyme that degrades DNA, leading to the dissolution of extracellular DNA and NET formation. Although DNase I treatment has a strong impact on NETosis during immunothrombosis, it also prevents thrombus growth without the involvement of neutrophils.[54,97] Interestingly, platelet activation, P-selectin exposure, aggregation in response to collagen, collagen-related peptide, or thrombin were suppressed when washed platelets were preincubated with DNase I.[54] In addition, DNase I treatment strongly inhibited CC-induced fibrin production and ATP release from activated platelets.[54] In line with this, in vivo neutrophil depletion in peripheral blood did not affect the severity of disease in the mouse model of CC embolism, whereas DNase I treatment dramatically reduced the number of occluded arteries, ischemic organ failure and kidney infarction in vivo.[54]

Tissue-factor-expressing neutrophils contribute to thrombosis in the laser-induced arterial injury model.[98] DNase I treatment could also prevent thrombosis in this in vivo model.[97] The electron microscopy studies could not detect neutrophil-associated extracellular trap structures. DNase I treatment also affects platelet functions, probably reducing fibrin formation and increasing ATP/ADP hydrolysis.[97] DNase treatment also limits the venous thrombus growth in a mouse model of HIT. DNase I administrated systematically could potentially serve as an effective treatment for preventing ischemic tissue necrosis in CC embolism. However, its inhibitory effect is limited to the early CC embolism (i.e., the first 3 h).[54] Extracellular DNA also plays a significant role in the hemostatic system, as it activates factor XI and factor XII.[99] Furthermore, extracellular DNA can be incorporated into the fibrin clot thereby inhibiting the anticoagulant activities of drugs such as unfractionated heparin and enoxaparin.[100] Conversely, RE31 DNA aptamers have been shown to inhibit the generation of thrombin formation, thereby accelerating fibrinolysis and suppressing thrombosis.[101,102] Damaged endothelial cells can also release potent procoagulant molecules, TF, extracellular DNA and the endogenous fibrinolysis inhibitor (plasminogen activator inhibitor 1).[103] Extracellular DNA can activate platelet aggregation and contribute to thrombus formation.[54] Future studies are required to test the effects of DNA aptamers in CC-induced thrombosis and thromboinflammation.

5.2.5 | Other potential targets

CC embolism disrupts the hemostatic balance by rupturing kidney vasculature, exposing the subendothelial collagen matrix. This results in platelet adhesion and aggregation, causing abnormal fibrin clot formation, kidney ischemia, and perilesional inflammation. Activated platelets initiate a procoagulant surface, thereby triggering the recruitment of neutrophils and initiating thromboinflammation.[54] CC strongly increases platelet aggregation response to thrombin and GPVI-specific agonists (collagen,

collagen-related peptide). Additionally, a significant increase in P-selectin exposure and αIIbβ3 integrin activation was observed indicating that CC embolism activates PAR-Gq (protease-activated receptor) and GPVI-ITAM (GPVI-immunoreceptor tyrosine-based activation motif) signaling.[54] These findings suggest that CC-induced procoagulant and thromboinflammatory phenotypes may be regulated by multiple platelet activation pathways. Further investigations are necessary to elucidate the specific roles of platelet-derived molecules, including key adhesion receptors and secondary mediators, and to determine their therapeutic potential in modulating renal hemostasis, thrombosis, and thromboinflammation.

6 | CONCLUSIONS

CC embolism-related vascular occlusions and tissue ischemia are the consequences of CC-induced TMA. Endothelial injury, complement-mediated intravascular clotting involving platelet activation, NET release and formation of a fibrin and chromatin mesh as in other forms of TMA or arterial thrombosis. The main approach to CC embolism is primary prophylaxis in patients at risk by stabilizing atherosclerotic plaques and avoiding unnecessary catheter interventions. The broad use of platelet aggregation inhibitors and anticoagulants may reduce the risk of vascular occlusions and tissue ischemia in case CC embolism occurs, which probably explains the relatively low prevalence of clinical manifestations of CC embolism, which are frequently found in autopsy studies. Clinical manifestations of CC embolism have crystal clots as the primary therapeutic target and may respond to platelet aggregation inhibitors, anticoagulants, and fibrinolytic drugs.

In analogy to other forms of TMA, complement inhibitors may have a future in this context. Animal studies suggest that a single prophylactic dose of necrosis inhibitors can increase the window of opportunity for vascular interventions but if this could implement into the clinical practice of high-risk patients remains unknown. Nevertheless, the availability of an animal model of CC embolism-induced ischemic necrosis, tissue infarction, and organ failure improves our knowledge about CC embolism.

Like CC embolism syndrome, severe SARS-CoV-2 infection involves neutrophils, platelets, and complement activation in coagulation, organ injury, and immunothrombosis.[104] This process is triggered by the release of proinflammatory cytokines, and NET formation, thereby activating platelets and enhancing fibrin generation. Despite the viral etiology, the composition of the clot, including platelet and fibrin-rich thrombi, extracellular DNA, vascular obstruction, tissue damage, and organ injury, shows similarities with CC embolism.[104,105] Consistently, administrating therapeutic doses of anticoagulants was shown to reduce the occurrence of thrombotic events and mortality rates in patients with moderate SARS-CoV-2 infection.[79] Understanding these mechanisms and the impact of anti-inflammatory and antithrombotic drugs can help to interconnect treatment strategies and propose new modalities for preventing and treating immunothrombotic complications.

AUTHOR CONTRIBUTIONS

Hans-Joachim Anders drafted the outline of the manuscript and all authors wrote parts of it, edited the complete version, and approved the final version.

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DISCLOSURES

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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REFERENCES

1. Saric M, Kronzon I. Aortic atherosclerosis and embolic events. *Curr Cardiol Rep*. 2012;14(3):342-349. doi:10.1007/s11886-012-0261-2
2. Kronzon I, Saric M. Cholesterol embolization syndrome. *Circulation*. 2010;122(6):631-641. doi:10.1161/CIRCULATIONAHA.109.886465
3. Branco L. Atheromas of the thoracic aorta: clinical and therapeutic update. *Rev Port Cardiol*. 2000;19(6):745-746.
4. Dulíček P, Bártová J, Beránek M, Malý J, Pecka M. The purple toe syndrome in female with factor v Leiden mutation successfully treated with enoxaparin. *Clin Appl Thromb*. 2013;19(1):100-102. doi:10.1177/1076029612440033
5. Arroyo LH, Lee RT. Mechanisms of plaque rupture: mechanical and biologic interactions. *Cardiovasc Res*. 1999;41(2):369-375. doi:10.1016/s0008-6363(98)00308-3

6. Scolari F, Ravani P, Divisione S, Dialisi N. Atheroembolic renal disease. *Lancet*. 2010;375(9726):1650-1660. doi:10.1016/S0140-6736(09)62073-0
7. Fine MJ, Kapoor W, Falanga V. Cholesterol crystal embolization: a review of 221 cases in the English literature. *Angiology*. 1987;38(10):769-784. doi:10.1177/000331978703801007
8. Fukumoto Y, Tsutsui H, Tsuchihashi M, Masumoto A, Takeshita A. The incidence and risk factors of cholesterol embolization syndrome, a complication of cardiac catheterization: a prospective study. *J Am Coll Cardiol*. 2003;42(2):211-216. doi:10.1016/s0735-1097(03)00579-5
9. Scolari F, Bracchi M, Valzorio B, et al. Cholesterol atheromatous embolism: an increasingly recognized cause of acute renal failure. *Nephrol Dial Transplant*. 1996;11(8):1607-1612.
10. Tunick PA, Nayar AC, Goodkin GM, et al. Effect of treatment on the incidence of stroke and other emboli in 519 patients with severe thoracic aortic plaque. *Am J Cardiol*. 2002;90(12):1320-1325. doi:10.1016/s0002-9149(02)02870-9
11. Carroccio A, Olin JW, Ellozy SH, et al. The role of aortic stent grafting in the treatment of atheromatous embolization syndrome: results after a mean of 15 months follow-up. *J Vasc Surg*. 2004;40(3):424-429. doi:10.1016/j.jvs.2004.06.036
12. Moolenaar W. Cholesterol crystal embolization in The Netherlands. *Arch Intern Med*. 2011;156(6):653. doi:10.1001/archinte.1996.00440060081009
13. Yücel AE, Kart-Köseoglu H, Demirhan B, Ozdemir FN. Cholesterol crystal embolization mimicking vasculitis: success with corticosteroid and cyclophosphamide therapy in two cases. *Rheumatol Int*. 2006;26(5):454-460. doi:10.1007/s00296-005-0012-4
14. Yu E, Rimm E, Qi L, et al. Diet, lifestyle, biomarkers, genetic factors, and risk of cardiovascular disease in the Nurses' health studies. *Am J Public Health*. 2016;106(9):1616-1623. doi:10.2105/AJPH.2016.303316
15. Berger JS, Hochman J, Lobach I, Adelman MA, Riles TS, Rockman CB. Modifiable risk factor burden and the prevalence of peripheral artery disease in different vascular territories. *J Vasc Surg*. 2013;58(3):673-681.e1. doi:10.1016/j.jvs.2013.01.053
16. Perk J, De Backer G, Gohlke H, et al. European Guidelines on cardiovascular disease prevention in clinical practice (version 2012). The Fifth Joint Task Force of the European Society of Cardiology and Other Societies on Cardiovascular Disease Prevention in Clinical Practice (constituted by representatives of nine societies and by invited experts). *Eur Heart J*. 2012;33(13):1635-1701. doi:10.1093/eurheartj/ehs092
17. Galicia-Garcia U, Benito-Vicente A, Jebari S, et al. Pathophysiology of type 2 diabetes mellitus. *Int J Mol Sci*. 2020;21(17):6275. doi:10.3390/ijms21176275
18. Dawber TR, Meadors GF, Moore FEJ. Epidemiological approaches to heart disease: the Framingham study. *Am J Public Health Nations Health*. 1951;41(3):279-281. doi:10.2105/ajph.41.3.279
19. Kataoka Y, Puri R, Hammadah M, et al. Sex differences in nonculprit coronary plaque microstructures on frequency-domain optical coherence tomography in acute coronary syndromes and stable coronary artery disease. *Circ Cardiovasc Imaging*. 2016;9(8):1-9. doi:10.1161/CIRCIMAGING.116.004506
20. Wendorff C, Wendorff H, Pelisek J, et al. Carotid plaque morphology is significantly associated with sex, age, and history of neurological symptoms. *Stroke*. 2015;46(11):3213-3219. doi:10.1161/STROKEAHA.115.010558
21. Vrijenhoek JEP, Den Ruijter HM, De Borst GJ, et al. Sex is associated with the presence of atherosclerotic plaque hemorrhage and modifies the relation between plaque hemorrhage and cardiovascular outcome. *Stroke*. 2013;44(12):3318-3323. doi:10.1161/STROKEAHA.113.002633
22. Halvorsen DS, Johnsen SH, Mathiesen EB, Njølstad I. The association between inflammatory markers and carotid atherosclerosis is sex dependent: the Tromsø study. *Cerebrovasc Dis*. 2009;27(4):392-397. doi:10.1159/000207443
23. Bailey IR, Page KB, Jones RG, Payne RB, Little AJ. Mnemonic coding system for clinical data entry into laboratory computers: its effect on quality and efficiency. *J Clin Pathol*. 1991;44(12):1018-1021. doi:10.1136/jcp.44.12.1018
24. Pan B, Jin X, Jun L, Qiu S, Zheng Q, Pan M. The relationship between smoking and stroke: a meta-analysis. *Medicine*. 2019;98(12):e14872. doi:10.1097/MD.0000000000014872
25. Larsson SC, Burgess S, Michaëlsson K. Smoking and stroke: a mendelian randomization study. *Ann Neurol*. 2019;86(3):468-471. doi:10.1002/ana.25534
26. Thun MJ, Carter BD, Feskanich D, et al. 50-year trends in smoking-related mortality in the United States. *N Engl J Med*. 2013;368(4):351-364. doi:10.1056/NEJMsa1211127
27. Gambardella J, Sardu C, Sacra C, Del Giudice C, Santulli G. Quit smoking to outsmart atherogenesis: molecular mechanisms underlying clinical evidence. *Atherosclerosis*. 2017;257:242-245. doi:10.1016/j.atherosclerosis.2016.12.010
28. Cheezum MK, Kim A, Bittencourt MS, et al. Association of tobacco use and cessation with coronary atherosclerosis. *Atherosclerosis*. 2017;257:201-207. doi:10.1016/j.atherosclerosis.2016.11.016
29. Blauth CI, Cosgrove DM, Webb BW, et al. Atheroembolism from the ascending aorta. An emerging problem in cardiac surgery. *J Thorac Cardiovasc Surg*. 1992;103(6):1102-1104.
30. Scolari F, Ravani P, Pola A, et al. Predictors of renal and patient outcomes in atheroembolic renal disease: a prospective study. *J Am Soc Nephrol*. 2003;14(6):1584-1590. doi:10.1097/01.asn.0000069220.60954.f1
31. Hitti WA, Wali RK, Weinman EJ, Drachenberg C, Briglia A. Cholesterol embolization syndrome induced by thrombolytic therapy. *Am J Cardiovasc Drugs*. 2008;8(1):27-34. doi:10.2165/00129784-200808010-00004
32. Thadhani RI, Camargo CAJ, Xavier RJ, Fang LS, Bazari H. Atheroembolic renal failure after invasive procedures. Natural history based on 52 histologically proven cases. *Medicine*. 1995;74(6):350-358. doi:10.1097/00005792-199511000-00005
33. Blankenship JC, Butler M, Garbes A. Prospective assessment of cholesterol embolization in patients with acute myocardial infarction treated with thrombolytic vs conservative therapy. *Chest*. 1995;107(3):662-668. doi:10.1378/chest.107.3.662
34. Scolari F, Tardanico R, Zani R, et al. Cholesterol crystal embolism: a recognizable cause of renal disease. *Am J Kidney Dis off J Natl Kidney Found*. 2000;36(6):1089-1109. doi:10.1053/ajkd.2000.19809
35. Belenfant X, Meyrier A, Jacquot C. Supportive treatment improves survival in multivisceral cholesterol crystal embolism. *Am J Kidney Dis*. 1999;33(5):840-850. doi:10.1016/S0272-6386(99)70415-4

36. Li X, Bayliss G, Zhuang S. Cholesterol crystal embolism and chronic kidney disease. *Int J Mol Sci*. 2017;18:1120. doi:10.3390/ijms18061120
37. Vidt DG. Cholesterol emboli: a common cause of renal failure. *Annu Rev Med*. 1997;48:375-385. doi:10.1146/annurev.med.48.1.375
38. Graff-Radford J, Boes CJ, Brown RDJ. History of Hollenhorst plaques. *Stroke*. 2015;46(4):e82-e84. doi:10.1161/STROKEAHA.114.007771
39. Thurlbeck WM, Castleman B. Atheromatous emboli to the kidneys after aortic surgery. *N Engl J Med*. 1957;257(10):442-447. doi:10.1056/NEJM195709052571002
40. Rushovich AM. Perforation of the jejunum: a complication of atheromatous embolization. *Am J Gastroenterol*. 1983;78(2):77-82.
41. Saric M, Kronzon I. Cholesterol embolization syndrome. *Curr Opin Cardiol*. 2011;26(6):472-479. doi:10.1097/HCO.0b013e32834b7fdd
42. Flory CM. Arterial occlusions produced by emboli from eroded aortic atheromatous plaques. *Am J Pathol*. 1945;21(3):549-565.
43. Kealy WF. Atheroembolism. *J Clin Pathol*. 1978;31(10):984-989. doi:10.1136/jcp.31.10.984
44. Libby P. Mechanisms of acute coronary syndromes and their implications for therapy. *N Engl J Med*. 2013;368(21):2004-2013. doi:10.1056/NEJMra1216063
45. van der Wal AC, Becker AE, van der Loos CM, Das PK. Site of intimal rupture or erosion of thrombosed coronary atherosclerotic plaques is characterized by an inflammatory process irrespective of the dominant plaque morphology. *Circulation*. 1994;89(1):36-44. doi:10.1161/01.cir.89.1.36
46. Kolodgie FD, Burke AP, Farb A, et al. The thin-cap fibroatheroma: a type of vulnerable plaque: the major precursor lesion to acute coronary syndromes. *Curr Opin Cardiol*. 2001;16(5):285-292. doi:10.1097/00001573-200109000-00006
47. Galis ZS, Sukhova GK, Kranzhöfer R, Clark S, Libby P. Macrophage foam cells from experimental atheroma constitutively produce matrix-degrading proteinases. *Proc Natl Acad Sci U S A*. 1995;92(2):402-406. doi:10.1073/pnas.92.2.402
48. Muller JE, Stone PH, Turi ZG, et al. Circadian variation in the frequency of onset of acute myocardial infarction. *N Engl J Med*. 1985;313(21):1315-1322. doi:10.1056/NEJM198511213132103
49. Mittleman MA, Mostofsky E. Physical, psychological and chemical triggers of acute cardiovascular events: preventive strategies. *Circulation*. 2011;124(3):346-354. doi:10.1161/CIRCULATIONAHA.110.968776
50. Martin JF, Kristensen SD, Mathur A, Grove EL, Choudry FA. The causal role of megakaryocyte–platelet hyperactivity in acute coronary syndromes. *Nat Rev Cardiol*. 2012;9(11):658-670. doi:10.1038/nrcardio.2012.131
51. Soufi M, Sattler AM, Maisch B, Schaefer JR. Molecular mechanisms involved in atherosclerosis. *Herz*. 2002;27(7):637-648. doi:10.1007/s00059-002-2431-2
52. Scharf RE. Platelet signaling in primary Haemostasis and arterial thrombus formation: part 1. *Hamostaseologie*. 2018;38(4):203-210. doi:10.1055/s-0038-1675144
53. Mammadova-Bach E, Jaeken J, Gudermann T, Braun A. Platelets and defective N-glycosylation. *Int J Mol Sci*. 2020;21(16):1-16. doi:10.3390/ijms21165630
54. Shi C, Kim T, Steiger S, et al. Crystal clots as therapeutic target in cholesterol crystal embolism. *Circ Res*. 2020;126:e37-e52. doi:10.1161/CIRCRESAHA.119.315625
55. Yang L, Steiger S, Shi C, et al. Both hyperglycemia and hyperuricemia aggravate acute kidney injury during cholesterol embolism syndrome despite opposite effects on kidney infarct size. *Kidney Int*. 2023;104(1):139-150. doi:10.1016/j.kint.2023.03.016
56. Shi C, Yang L, Braun A, Anders HJ. Extracellular DNA—A danger signal triggering Immunothrombosis. *Front Immunol*. 2020;11:11. doi:10.3389/fimmu.2020.568513
57. Jackson SP, Darbousset R, Schoenwaelder SM. Thromboinflammation: challenges of therapeutically targeting coagulation and other host defense mechanisms. *Blood*. 2019;133(9):906-918. doi:10.1182/blood-2018-11-882993
58. Lisman T. Platelet-neutrophil interactions as drivers of inflammatory and thrombotic disease. *Cell Tissue Res*. 2018;371(3):567-576. doi:10.1007/s00441-017-2727-4
59. George JN, Nester CM. Syndromes of thrombotic microangiopathy. *N Engl J Med*. 2014;371(7):654-666. doi:10.1056/NEJMra1312353
60. Bertheloot D, Latz E, Franklin BS. Necroptosis, pyroptosis and apoptosis: an intricate game of cell death. *Cell Mol Immunol*. 2021;18(5):1106-1121. doi:10.1038/s41423-020-00630-3
61. Weinlich R, Oberst A, Beere HM, Green DR. Necroptosis in development, inflammation and disease. *Nat Rev Mol Cell Biol*. 2017;18(2):127-136. doi:10.1038/nrm.2016.149
62. Labat-Moleur F, Guillermet C, Lorimier P, et al. TUNEL apoptotic cell detection in tissue sections: critical evaluation and improvement. *J Histochem Cytochem off J Histochem Soc*. 1998;46(3):327-334. doi:10.1177/002215549804600306
63. Galluzzi L, Vitale I. Molecular mechanisms of cell death: recommendations of the nomenclature committee on cell death 2018. *Cell Death Differ*. 2018;25:486-541. doi:10.1038/s41418-017-0012-4
64. Sorvillo N, Cherpokova D, Martinod K, Wagner DD. Extracellular DNA NET-works with dire consequences for health. *Circ Res*. 2019;125(4):470-488. doi:10.1161/CIRCRESAHA.119.314581
65. Linkermann A. Death and fire-the concept of necroinflammation. *Cell Death Differ*. 2019;26(1):1-3. doi:10.1038/s41418-018-0218-0
66. Shi C, Zhao D, Lyubenov L, et al. Neutrophil circadian rhythm is associated with different outcomes of acute kidney injury due to cholesterol crystal embolism. *Front Cardiovasc Med*. 2022;9:974759. doi:10.3389/fcvm.2022.974759
67. Engelmann B, Massberg S. Thrombosis as an intravascular effector of innate immunity. *Nat Rev Immunol*. 2013;13(1):34-45. doi:10.1038/nri3345
68. Lee YH, Pratley RE. The evolving role of inflammation in obesity and the metabolic syndrome. *Curr Diab Rep*. 2005;5(1):70-75. doi:10.1007/s11892-005-0071-7
69. Silveira Rossi JL, Barbalho SM, Reverete de Araujo R, Bechara MD, Sloan KP, Sloan LA. Metabolic syndrome and cardiovascular diseases: going beyond traditional risk factors. *Diabetes Metab Res Rev*. 2022;38(3):e3502. doi:10.1002/dmrr.3502
70. Allam R, Darisipudi MN, Tschopp J, Anders HJ. Histones trigger sterile inflammation by activating the NLRP3 inflammasome. *Eur J Immunol*. 2013;43(12):3336-3342. doi:10.1002/eji.201243224
71. Mulay SR, Linkermann A, Anders HJ. Necroinflammation in kidney disease. *J Am Soc Nephrol*. 2016;27(1):27-39. doi:10.1681/ASN.2015040405
72. Koushki K, Shahbaz SK, Mashayekhi K, et al. Anti-inflammatory action of statins in cardiovascular disease:

the role of Inflammasome and toll-like receptor pathways. *Clin Rev Allergy Immunol*. 2021;60(2):175-199. doi:10.1007/s12016-020-08791-9

73. Cerda A, Fajardo CM, Basso RG, Hirata MH, Hirata RDC. Role of microRNAs 221/222 on statin induced nitric oxide release in human endothelial cells. *Arq Bras Cardiol*. 2015;104(3):195-201. doi:10.5935/abc.20140192
74. Notarbartolo A, Davì G, Averna M, et al. Inhibition of thromboxane biosynthesis and platelet function by simvastatin in type IIa hypercholesterolemia. *Arterioscler Thromb Vasc Biol*. 1995;15(2):247-251. doi:10.1161/01.atv.15.2.247
75. Puccetti L, Santilli F, Pasqui AL, et al. Effects of atorvastatin and rosuvastatin on thromboxane-dependent platelet activation and oxidative stress in hypercholesterolemia. *Atherosclerosis*. 2011;214(1):122-128. doi:10.1016/j.atherosclerosis.2010.10.006
76. Laufs U, Gertz K, Huang P, et al. Atorvastatin upregulates type III nitric oxide synthase in thrombocytes, decreases platelet activation, and protects from cerebral ischemia in normocholesterolemic mice. *Stroke*. 2000;31(10):2442-2449. doi:10.1161/01.str.31.10.2442
77. Owens AP 3rd, Mackman N. The antithrombotic effects of statins. *Annu Rev Med*. 2014;65:433-445. doi:10.1146/annurev-med-051812-145304
78. Haramaki N, Ikeda H, Takenaka K, et al. Fluvastatin alters platelet aggregability in patients with hypercholesterolemia: possible improvement of intraplatelet redox imbalance via HMG-CoA reductase. *Arterioscler Thromb Vasc Biol*. 2007;27(6):1471-1477. doi:10.1161/ATVBAHA.106.128793
79. Lawler PR, Goligher EC, Berger JS, et al. Therapeutic anticoagulation with heparin in noncritically ill patients with Covid-19. *N Engl J Med*. 2021;385(9):790-802. doi:10.1056/NEJMoa2105911
80. Liao JK. Effects of statins on 3-hydroxy-3-methylglutaryl coenzyme a reductase inhibition beyond low-density lipoprotein cholesterol. *Am J Cardiol*. 2005;96(5A):24F-33F. doi:10.1016/j.amjcard.2005.06.009
81. Margaritis M, Channon KM, Antoniades C. Statins as regulators of redox state in the vascular endothelium: beyond lipid lowering. *Antioxid Redox Signal*. 2014;20(8):1198-1215. doi:10.1089/ars.2013.5430
82. Parmar KM, Nambudiri V, Dai G, Larman HB, Gimbrone MAJ, García-Cardeña G. Statins exert endothelial atheroprotective effects via the KLF2 transcription factor. *J Biol Chem*. 2005;280(29):26714-26719. doi:10.1074/jbc.C500144200
83. Lin Z, Kumar A, SenBanerjee S, et al. Kruppel-like factor 2 (KLF2) regulates endothelial thrombotic function. *Circ Res*. 2005;96(5):e48-e57. doi:10.1161/01.RES.0000159707.05637.a1
84. Chen DQ, Guo Y, Li X, Zhang GQ, Li P. Small molecules as modulators of regulated cell death against ischemia/reperfusion injury. *Med Res Rev*. 2022;42(6):2067-2101. doi:10.1002/med.21917
85. Longstaff C, Kolev K. Basic mechanisms and regulation of fibrinolysis. *J Thromb Haemost*. 2015;13(Suppl 1):S98-S105. doi:10.1111/jth.12935
86. Komorowicz E, Kolev K, Léránt I, Machovich R. Flow rate-modulated dissolution of fibrin with clot-embedded and circulating proteases. *Circ Res*. 1998;82(10):1102-1108. doi:10.1161/01.res.82.10.1102
87. Lyubenov L, Shi C, Zhao D, et al. Intravenous Glu-plasminogen attenuates cholesterol crystal embolism-induced thrombotic angiopathy, acute kidney injury and kidney infarction. *Nephrol Dial Transplant*. 2023;38(1):93-105. doi:10.1093/ndt/gfac273
88. Onishi A, St Ange K, Dordick JS, Linhardt RJ. Heparin and anticoagulation. *Front Biosci*. 2016;21(7):1372-1392. doi:10.2741/4462
89. Jiang J, Mu S, Zhang F, et al. Effect of heparin pretreatment on the level of neutrophil extracellular traps of serum and lung tissue in septic mice. *Zhonghua Wei Zhong Bing Ji Jiu Yi Xue*. 2017;29(4):337-341. doi:10.3760/cma.j.issn.2095-4352.2017.04.010
90. Perdomo J, Leung HHL, Ahmadi Z, et al. Neutrophil activation and NETosis are the major drivers of thrombosis in heparin-induced thrombocytopenia. *Nat Commun*. 2019;10(1):1322. doi:10.1038/s41467-019-09160-7
91. Thorevska N, Amoateng-Adjepong Y, Sabahi R, et al. Anticoagulation in hospitalized patients with renal insufficiency: a comparison of bleeding rates with unfractionated heparin vs enoxaparin. *Chest*. 2004;125(3):856-863. doi:10.1378/chest.125.3.856
92. Crowther MA, Warkentin TE. Bleeding risk and the management of bleeding complications in patients undergoing anticoagulant therapy: focus on new anticoagulant agents. *Blood*. 2008;111(10):4871-4879. doi:10.1182/blood-2007-10-120543
93. Gachet C. P2Y(12) receptors in platelets and other hematopoietic and non-hematopoietic cells. *Purinergic Signal*. 2012;8(3):609-619. doi:10.1007/s11302-012-9303-x
94. Gachet C. Antiplatelet drugs: which targets for which treatments? *J Thromb Haemost*. 2015;13(Suppl 1):S313-S322. doi:10.1111/jth.12947
95. Cattaneo M. The platelet $P2Y_{12}$ receptor for adenosine diphosphate: congenital and drug-induced defects. *Blood*. 2011;117(7):2102-2112. doi:10.1182/blood-2010-08-263111
96. Wiviott SD, Braunwald E, McCabe CH, et al. Prasugrel versus clopidogrel in patients with acute coronary syndromes. *N Engl J Med*. 2007;357(20):2001-2015. doi:10.1056/NEJMoa0706482
97. Carminita E, Crescence L, Brouilly N, Altié A, Panicot-Dubois L, Dubois C. DNAse-dependent, NET-independent pathway of thrombus formation in vivo. *Proc Natl Acad Sci U S A*. 2021;118(28):1-12. doi:10.1073/pnas.2100561118
98. Darbousset R, Thomas GM, Mezouar S, et al. Tissue factor-positive neutrophils bind to injured endothelial wall and initiate thrombus formation. *Blood*. 2012;120(10):2133-2143. doi:10.1182/blood-2012-06-437772
99. Kannemeier C, Shibamiya A, Nakazawa F, et al. Extracellular RNA constitutes a natural procoagulant cofactor in blood coagulation. *Proc Natl Acad Sci*. 2007;104(15):6388-6393. doi:10.1073/pnas.0608647104
100. Ammollo CT, Semeraro N, Carratu MR, Colucci M, Semeraro F. Histones differentially modulate the anticoagulant and Profibrinolytic activities of heparin, heparin derivatives, and dabigatran. *J Pharmacol Exp Ther*. 2016;356(2):305-313. doi:10.1124/jpet.115.229823
101. Spiridonova VA, Novikova TM, Sizov VA, et al. DNA aptamers to thrombin exosite I. Structure-function relationships and antithrombotic effects. *Biochemistry (Mosc)*. 2019;84(12):1521-1528. doi:10.1134/S0006297919120113
102. Mazurov AV, Titaeva EV, Khaspekova SG, et al. Characteristics of a new DNA aptamer, direct inhibitor of thrombin. *Bull Exp Biol Med*. 2011;150(4):422-425. doi:10.1007/s10517-011-1158-6

103. Bevilacqua MP, Schleef RR, Gimbrone MAJ, Loskutoff DJ. Regulation of the fibrinolytic system of cultured human vascular endothelium by interleukin 1. *J Clin Invest*. 1986;78(2):587-591. doi:10.1172/JCI112613
104. Conway EM, Mackman N, Warren RQ, et al. Understanding COVID-19-associated coagulopathy. *Nat Rev Immunol*. 2022;22(10):639-649. doi:10.1038/s41577-022-00762-9
105. Ackermann M, Anders HJ, Bilyy R, et al. Patients with COVID-19: in the dark-NETs of neutrophils. *Cell Death Differ*. 2021;28(11):3125-3139. doi:10.1038/s41418-021-00805-z

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