

Neutrophil extracellular traps in cardiovascular disease: Mechanisms and therapeutic implications (Review)

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Abstract. Neutrophil extracellular traps (NETs) are web-like structures composed of decondensed chromatin and granular proteins. Initially identified as an antimicrobial defense mechanism, NETs have now been demonstrated to be profoundly involved in the processes of sterile inflammation, thrombosis and fibrosis in cardiovascular diseases. The present review systematically delineated the interaction networks between NETs and macrophages, monocytes, endothelial cells, fibroblasts and platelets, untangling NETs as a central hub that integrates the pathological triad of inflammation, thrombosis and fibrosis. Building on this foundation, the specific mechanisms of NETs in cardiac diseases, including acute myocardial infarction, atrial fibrillation and heart failure and in vascular diseases, such as atherosclerosis, hypertension and abdominal aortic aneurysm, are elaborated. Finally, from four dimensions, NETs degradation, targeting of NETs components, inhibition of NETosis formation and indirect intervention through inflammatory metabolic pathways, the preclinical evidence and translational bottlenecks of strategies such as DNase I,

histone-neutralizing agents and PAD4 inhibitors were systematically evaluated. The present review aimed to promote the transition of NETs from a fundamental pathological hub into a targetable node for cardiovascular therapy.

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1. Introduction

Cardiovascular diseases (CVDs) are the leading cause of death worldwide. In 2023, the global prevalence of CVDs reached 626 million, representing a 19.7% increase compared with 2019, while cardiovascular deaths totaled 19.2 million and accounted for 32% of global mortality (1). If this trend persists, annual CVD-related deaths are projected to approach 23.3 million by 2030 (2). A growing body of evidence indicates that inflammation is a shared pathological basis for numerous CVDs (3-8). Among innate immune cells, neutrophils are among the earliest first-line responders recruited to sites of cardiovascular injury. They arrive within hours of tissue damage and establish the conditions for subsequent inflammatory cell infiltration and tissue remodeling (9). Although neutrophils were historically regarded as short-lived effector cells with limited functions, they are now recognized as active

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participants in cardiovascular pathological processes through multiple mechanisms, among which the release of neutrophil extracellular traps (NETs) is particularly important (10). NETs are web-like structures composed of decondensed chromatin and granular proteins and were initially identified as an antimicrobial defense mechanism capable of capturing and killing pathogens. However, accumulating evidence over the past decade has demonstrated that NETs can also form under sterile inflammatory conditions and play critical roles in cardiovascular pathophysiology by promoting inflammation, thrombosis and fibrosis. Aberrant NET release has been implicated in nearly all major CVDs; nevertheless, the cellular interactions, molecular mechanisms and therapeutic potential associated with NETs remain incompletely understood.

Therefore, the present review systematically integrated the interaction networks between NETs and multiple cell types, including macrophages, monocytes, endothelial cells, fibroblasts and platelets. It further examined the specific mechanisms through which NETs mediate pathological injury in different CVDs and evaluates the clinical translational potential of NET-targeted therapeutic strategies within a 'target-drug-translational status' framework. The present review aims to deepen understanding of the roles of NETs in CVDs, provide further insights into the mechanisms underlying CVD development and progression and identify potential avenues for translating therapeutic strategies into clinical practice.

2. NETs: An overview

Definition and composition of NETs. In 2004, Brinkmann *et al* (11) first demonstrated the existence of NETs and showed that they contain neutrophil elastase (NE), cathepsin G, myeloperoxidase (MPO), lactoferrin, gelatinase, histones H1, H2A, H2B, H3 and H4 and H2A-H2B-DNA complexes. Subsequent research has generated extensive proteomic data on NETs. Dwyer *et al* (12) found that NETs induced by both nonmucoid and mucoid *Pseudomonas aeruginosa* strains contain a conserved group of proteins, irrespective of the inducing factor, which they termed the 'core NET signature'. This conserved protein set includes nucleosome-associated proteins (such as histones), structural proteins (such as coronin-1, β -actin and α -actinin), enzymes (such as NE, transaldolase, thymidine kinase, glyceraldehyde-3-phosphate dehydrogenase and α -enolase) and antimicrobial proteins (such as MPO, lysozyme C, lactoferrin and azurocidin).

However, NETs are not structurally uniform and their protein composition varies according to the inducing stimulus and tissue microenvironment. Proteomic analyses have identified stimulus-specific NET signatures. For example, phorbol 12-myristate 13-acetate (PMA)-induced NETs are enriched in heat shock proteins, whereas lipopolysaccharide (LPS)-induced NETs contain higher levels of lysosomal proteins, such as LAMP2 (13).

Mechanisms of NET formation. NETs are released by activated neutrophils through a process termed NETosis. The currently recognized pathways of NET formation are broadly classified as NADPH oxidase (NOX)-dependent NETosis, also known

as suicidal NETosis and NOX-independent NETosis, also known as vital NETosis. Vital NETosis can be further divided into nuclear DNA-derived and mitochondrial DNA-derived NET (mtNET) subtypes. The specific mechanisms are shown in Fig. 1.

i) NOX-dependent NETosis (classical pathway). Also known as suicidal NETosis, this process typically occurs within 2-4 h and culminates in the complete rupture of the neutrophil nuclear envelope and plasma membrane, accompanied by cell death. It can be initiated by high concentrations of LPS, pathogens such as *Staphylococcus aureus*, PMA, high concentrations of complement component C5a, immune complexes and inflammatory mediators such as tumor necrosis factor (TNF)- α and interleukin (IL)-8. These stimuli activate neutrophil Toll-like receptors (TLRs), Fc γ receptors and complement receptors (14). Following receptor activation, calcium is initially released from endoplasmic reticulum stores, followed by substantial extracellular Ca²⁺ influx through store-operated calcium channels. The resulting increase in cytosolic calcium activates protein kinase C (PKC), which subsequently initiates the Raf-MEK-ERK signaling pathway (15). This pathway phosphorylates and activates the NOX complex, resulting in the sustained generation of large amounts of reactive oxygen species (ROS), which further increase intracellular calcium levels (16). Calcium-dependent peptidylarginine deiminase 4 (PAD4) is subsequently activated and catalyzes the citrullination of arginine residues in the tails of histones H1, H2A, H3 and H4. Citrullination neutralizes the positive charges of histones, weakens their electrostatic interactions with DNA and initiates chromatin decondensation (17). Concurrently, NOX-derived ROS promote the release of MPO and NE from azurophilic granules into the cytoplasm, after which these enzymes translocate to the nucleus. NE cleaves histones through its proteolytic activity, whereas MPO enhances oxidative signaling and facilitates chromatin relaxation; together, these processes promote complete chromatin decondensation. Downstream ROS and calcium signaling activate gasdermin D (GSDMD), which oligomerizes and forms membrane pores. The nuclear envelope subsequently disintegrates, allowing decondensed chromatin to mix with granule proteins. Finally, the plasma membrane ruptures, releasing large quantities of NETs into the extracellular space (18).

ii) NOX-independent NETosis. Also referred to as vital NETosis, this pathway occurs rapidly, typically within 15-60 min. The plasma membrane remains intact throughout the process, allowing neutrophils to survive and retain their phagocytic and chemotactic functions. This pathway does not depend on plasma membrane-associated NOX-derived ROS, although certain subtypes require mitochondrial ROS (mtROS). It can be divided into two principal categories: i) Nuclear DNA-derived vital NETs: Classical inducers include the calcium ionophore A23187, low concentrations of C5a, *S. aureus* and low-dose LPS presented by activated platelets. Following stimulation, rapid and substantial extracellular Ca²⁺ influx directly activates PAD4, which catalyzes histone citrullination and promotes nuclear chromatin decondensation (14). Decondensed chromatin and granule proteins are packaged into vesicles that bud from the nuclear envelope and

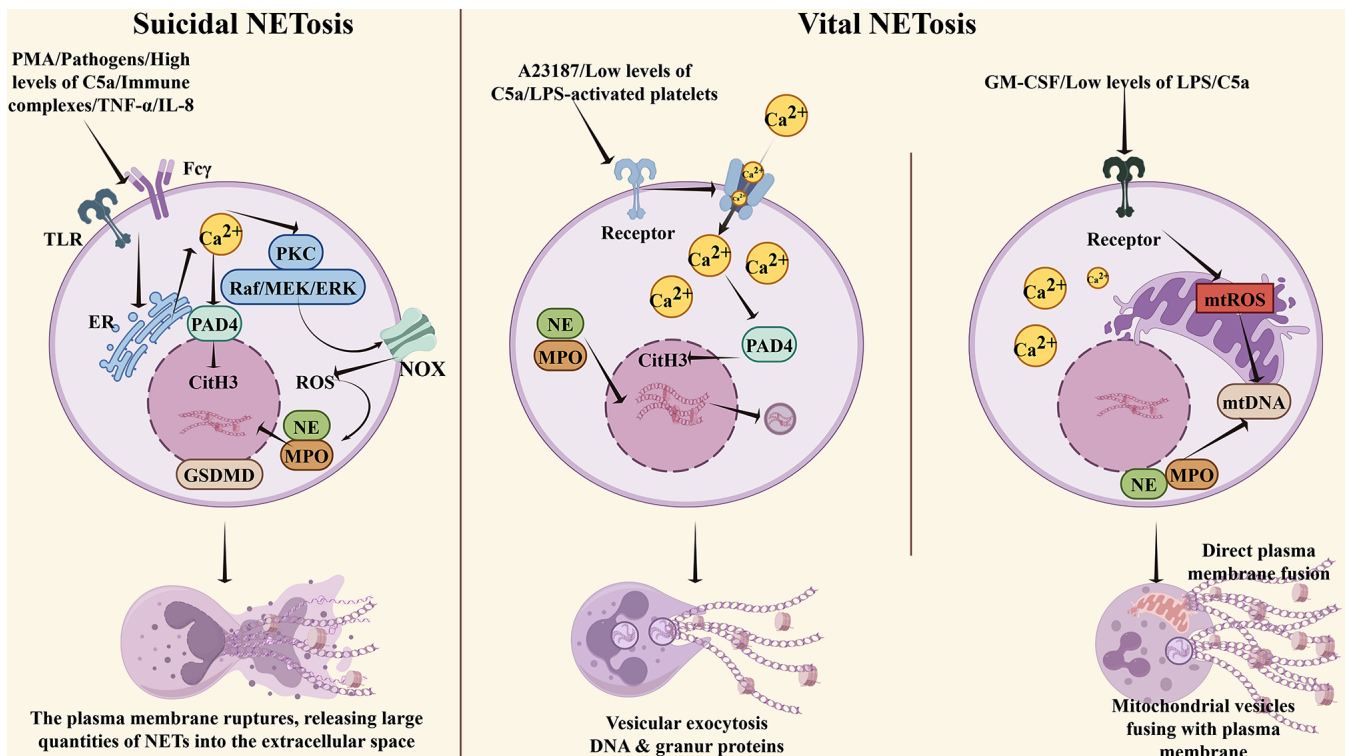


Figure 1. The two major pathways of neutrophil extracellular trap formation. i) Suicidal NETosis: High concentrations of LPS, PMA, pathogens and inflammatory mediators stimulate TLRs, Fc γ and complement receptors on the cell membrane, triggering endoplasmic reticulum calcium release and extracellular Ca²⁺ influx through store-operated calcium channels. This activates PKC, which subsequently phosphorylates the NOX complex via the Raf/MEK/ERK pathway, resulting in the generation of large amounts of membrane-derived ROS. Elevated intracellular calcium activates PAD4, which catalyzes histone arginine citrullination and initiates chromatin decondensation. Concurrently, NOX-derived ROS induce degranulation of azurophilic granules, releasing MPO and NE. NE cleaves histones, while MPO synergistically amplifies oxidative signaling, completing full chromatin decondensation. Downstream calcium signaling and ROS activate GSDMD, which forms pores in the plasma membrane. The nuclear envelope disintegrates, decondensed chromatin mixes with granular proteins and the cell ultimately lyses, releasing NETs. ii) Vital NETosis: Upon stimulation with A23187, low concentrations of C5a, *Staphylococcus aureus*, or low-dose LPS presented by activated platelets, rapid extracellular Ca²⁺ influx directly activates PAD4, driving nuclear chromatin decondensation. The nuclear envelope buds to form vesicles that release nuclear DNA-derived vital NETs via exocytosis. Alternatively, under stimulation with GM-CSF and low-dose LPS, calcium signaling induces opening of the mitochondrial permeability transition pore, generating mtROS and releasing mtDNA. After oxidative modification, mtDNA associates with granular proteins such as MPO and NE and is released as mtDNA-type NETs through vesicle secretion or fusion of mitochondria with the plasma membrane. NETs, neutrophil extracellular traps; LPS, lipopolysaccharide; PMA, phorbol 12-myristate 13-acetate; TLRs, Toll-like receptors; TNF, tumor necrosis factor; IL, interleukin; PKC, protein kinase C; PAD4, peptidylarginine deiminase 4; NOX, NADPH oxidase; Raf, rapidly accelerated fibrosarcoma; MEK, mitogen-activated protein kinase kinase; ERK, extracellular signal-regulated kinase; ROS, reactive oxygen species; MPO, myeloperoxidase; NE, neutrophil elastase; GSDMD, gasdermin D; mtROS, mitochondrial ROS; GM-CSF, granulocyte-macrophage colony-stimulating factor; mtDNA, mitochondrial DNA. Image created with Figdraw.com, with permission.

are released into the extracellular space through exocytosis. Once outside the cell, granule proteins bind to DNA to form web-like NETs. The resulting enucleated neutrophils retain phagocytic vacuoles and remain capable of phagocytosis (19). Notably, stimulation with *Candida albicans* can induce PAD4-independent vital NETosis, in which chromatin decondensation is mediated solely by NE-dependent histone cleavage without requiring histone citrullination (20). ii) Mitochondrial DNA (mtDNA)-derived vital NETs: When neutrophils are primed with granulocyte-macrophage colony-stimulating factor (GM-CSF) and subsequently stimulated with low-dose LPS or C5a, increased calcium signaling induces opening of the mitochondrial permeability transition pore, resulting in mitochondrial damage, mtROS production and the release of mtDNA (14). After oxidative modification, mtDNA associates with granule proteins, including MPO and NE, to form complexes that are released through mitochondrial vesicle secretion or local fusion of mitochondria with the plasma membrane (21). This process generates mtNETs with mtDNA

as their structural backbone, while the neutrophil remains intact and does not undergo lysis.

NETs in the context of cardiovascular disease. Although NETs were initially identified in the context of microbial infection, numerous stimuli relevant to cardiovascular pathology can also induce NETosis. Within the cardiovascular system, NET-inducing stimuli predominantly include lipid metabolites, damage-associated molecular patterns (DAMPs) and intercellular signaling, rather than direct stimulation by exogenous pathogens. First, among lipid-related triggers, oxidized low-density lipoprotein (oxLDL) and endogenous cholesterol crystals can engage TLR4 and the receptor for advanced glycation end products on the neutrophil surface, thereby promoting sustained neutrophil activation and acting as major chemical initiators of NETosis in lipid-associated CVDs (22,23). Metabolic abnormalities, including hyperglycemia and elevated levels of free fatty acids, can also function as persistent stimuli and induce sustained NET release from

perivascular neutrophils (14,24). Second, DAMPs released following tissue injury contribute substantially to NET formation. After myocardial infarction, ischemic stroke, or organ ischemia-reperfusion injury, necrotic endothelial and parenchymal cells release high-mobility group box 1 (HMGB1), cell-free mtDNA and extracellular histones (25). These molecules can establish a positive-feedback loop that continuously stimulates circulating neutrophils to generate additional NETs. Third, intercellular signaling associated with vascular injury can induce NETosis. P-selectin expressed on activated platelets binds to P-selectin glycoprotein ligand-1 (PSGL-1) on neutrophils, thereby promoting NET formation (26). The complement activation product C5a regulates NETosis in a concentration-dependent manner: Low concentrations of C5a primarily induce calcium influx and mtNET formation, whereas high concentrations activate the NOX complex and promote suicidal NETosis (14). Furthermore, cardiovascular risk factors, including monosodium urate crystals, tobacco smoke and fine particulate matter with an aerodynamic diameter of $\leq 2.5 \mu\text{m}$ (PM_{2.5}), can chronically stimulate neutrophils within the vascular wall, thereby sustaining persistent, low-grade NET release (14).

NETs induced in the cardiovascular milieu are enriched with a variety of disease-specific functional molecules that form the molecular basis of their prothrombotic and vascular-damaging effects. First, NETs contain procoagulant proteins: NET fibers display abundant tissue factor (TF) and von Willebrand factor (vWF) on their surfaces. TF directly activates the extrinsic coagulation pathway, whereas vWF captures circulating platelets; moreover, NET-bound NE can degrade tissue factor pathway inhibitor, thereby disrupting the anticoagulant balance and promoting immunothrombosis (27,28). Second, NETs contain matrix-degrading proteases: matrix metalloproteinase (MMP)-9 is abundantly associated with plaque-derived NETs and degrades collagen within the arterial fibrous cap, resulting in cap thinning and increased plaque vulnerability to rupture, thereby precipitating acute cardiovascular and cerebrovascular events (29). Third, NETs contain inflammatory chemotactic mediators: NET-associated chemokines such as IL-8 continuously recruit peripheral neutrophils to vascular lesions, thereby perpetuating chronic inflammation (30). At the clinical level, plasma MPO-DNA complexes, circulating citrullinated histone H3 (CitH3) and cell-free mtDNA are specific biomarkers of NET formation in cardiovascular disease; their levels are markedly elevated in patients with coronary artery disease, ischemic stroke and deep vein thrombosis and can be used to assess disease severity (31). However, direct comparative proteomic analyses of NETs across different CVDs remain scarce. Defining disease-specific NET signatures will not only deepen mechanistic understanding but also facilitate the development of targeted diagnostic biomarkers and selective therapeutic strategies.

3. NETs as a universal cellular interaction network in cardiovascular pathology: Inflammation, thrombosis and fibrosis

NETs are not merely antimicrobial structures; they also function as multifunctional signaling platforms that coordinate

crosstalk among diverse cell types in the cardiovascular system. Through their interactions with macrophages, monocytes, endothelial cells, fibroblasts and platelets, NETs establish an integrated cellular network that drives three interrelated pathological processes: inflammation, thrombosis and fibrosis.

NETs and inflammation. At sites of cardiovascular injury, NETs shape and amplify the local inflammatory micro-environment by acting on macrophages, monocytes and endothelial cells. These effects occur at three interconnected levels: Induction of pro-inflammatory macrophage polarization, chemotactic recruitment of monocytes and maintenance of their pro-inflammatory phenotype and pro-inflammatory activation of endothelial cells (Fig. 2).

NETs drive pro-inflammatory macrophage polarization and inflammation amplification. Macrophages are heterogeneous innate myeloid cells that perform diverse functions in host defense, regulation of inflammation, tissue repair and maintenance of homeostasis (32–34). They are often broadly classified into pro-inflammatory M1 and anti-inflammatory M2 phenotypes and play central roles in both the initiation and resolution of inflammatory responses (35). The pro-inflammatory interaction between NETs and macrophages is bidirectional and forms a positive-feedback loop. A recent study has shown that pro-inflammatory M1 macrophages can directly induce NETosis through the transcription factor ARID3A (36). Following its nuclear translocation in M1 macrophages under inflammatory conditions, ARID3A binds directly to the THBS1 promoter and activates its transcription, thereby promoting substantial THBS1 secretion. Secreted THBS1 then engages the CD47 receptor on neutrophils, activates the p38 MAPK signaling pathway and specifically induces NET release (36). These findings indicate that NET-activated macrophages can, in turn, promote further NET formation, creating a self-reinforcing inflammatory amplification circuit. NETs complete this positive-feedback loop by driving pro-inflammatory macrophage polarization and amplifying inflammation through several synergistic pathways. At the molecular level, NET-associated DNA is recognized by TLR9 in macrophages, activating the NF- κ B signaling cascade and inducing the transcription of pro-inflammatory cytokines, including IL-1 β , IL-8 and TNF- α (22,30). Following internalization by macrophages, NET-associated NE further activates the intracellular Rab5a-NF- κ B pathway, thereby sustaining NF- κ B-dependent inflammatory gene expression (37). These mechanisms establish two positive-feedback circuits. First, NET-induced IL-1 β secretion increases T cell-derived IL-17 production, which induces C-X-C motif chemokine ligand (CXCL) 1 and CXCL2 expression, recruits additional neutrophils and sustains a self-reinforcing cycle of NET release and macrophage activation (22). Second, macrophage-derived IL-8 induces further NET release through C-X-C motif chemokine receptor (CXCR) 2 on neutrophils; the resulting NETs then stimulate additional IL-8 production, forming a bidirectional IL-8-CXCR2 inflammatory amplification loop (30). Moreover, NETs inhibit autophagosome formation in macrophages through the EGFR-Beclin-1 pathway. The resulting impairment of autophagy further enhances inflammasome activity and promotes IL-1 β maturation, thereby reinforcing the pro-inflammatory response at the level of post-translational processing (38).

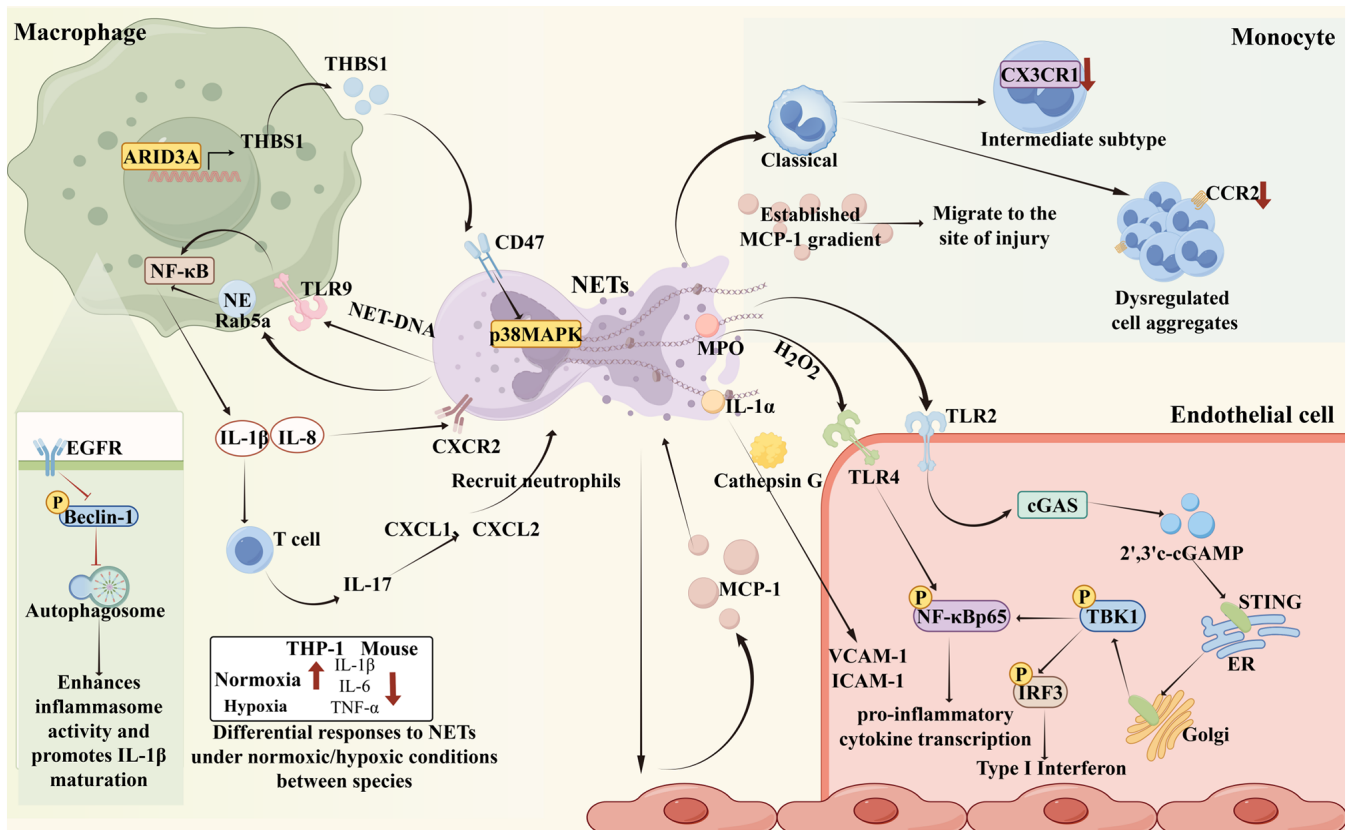


Figure 2. Mechanisms by which NETs establish and amplify the inflammatory microenvironment at sites of cardiovascular injury. NETs form a multicellular pro-inflammatory network through interactions with macrophages, monocytes and endothelial cells. i) NETs drive pro-inflammatory macrophage polarization via the TLR9/NF-κB and NE/Rab5a-NF-κB pathways and establish inflammatory amplification loops through IL-1β/IL-17 and IL-8/CXCR2. M1 macrophages, in turn, induce NETosis through the ARID3A/THBS1/CD47/p38 MAPK axis, creating a bidirectional positive-feedback loop. ii) NETs upregulate endothelial MCP-1, which promotes monocyte chemotaxis and maintains their pro-inflammatory phenotype; MCP-1 reciprocally enhances NET release, establishing a NETs-MCP-1 loop (3). NETs carry IL-1α and activate endothelial cells via the TLR4/NF-κB and cGAS-STING pathways, upregulating adhesion molecules and releasing pro-inflammatory cytokines, thereby collectively amplifying local inflammation. NETs, neutrophil extracellular traps; TLR, Toll-like receptor; IL, interleukin; CXCL, C-X-C motif chemokine ligand; CXCR, C-X-C motif chemokine receptor; ARID3A, AT-rich interaction domain 3A; THBS1, thrombospondin 1; CD47, cluster of differentiation 47; p38 MAPK, p38 mitogen-activated protein kinase; NE, neutrophil elastase; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; EGFR, epidermal growth factor receptor; TNF, tumor necrosis factor; MPO, myeloperoxidase; CCR, C-C chemokine receptor; ER, endoplasmic reticulum; TBK1, TANK-binding kinase 1; IRF3, interferon regulatory factor 3; VCAM, vascular cell adhesion molecule; ICAM, intercellular adhesion molecule; MCP, monocyte chemoattractant protein; cGAMP, cyclic GMP-AMP; cGAS-STING, cyclic GMP-AMP synthase-stimulator of interferon genes. Image created with Figdraw.com, with permission.

Notably, the functional consequences of NET-macrophage interactions are not uniformly pro-inflammatory but are modulated by the tissue microenvironment. For example, the effects of NETs vary between human and murine macrophages and according to oxygen availability. Under normoxic conditions, NETs markedly upregulate the pro-inflammatory cytokines IL-1β, TNF-α and IL-6 in human THP-1-derived macrophages (39). By contrast, NETs strongly suppress pro-inflammatory cytokine expression in murine macrophages under both normoxic and hypoxic conditions (39). The mechanistic basis for these species- and context-dependent effects remains incompletely understood and may reflect differences in macrophage origin, differentiation state, or oxygen-sensing pathways.

NETs regulate monocyte chemotactic recruitment and maintenance of pro-inflammatory phenotype. Monocytes are highly plastic innate immune cells. The monocyte chemoattractant protein (MCP)-1/CCR2 chemokine axis is a central mechanism governing monocyte recruitment to inflammatory sites. NETs regulate monocyte recruitment and phenotype through several interconnected mechanisms.

First, NETs stimulate vascular endothelial cells to markedly increase MCP-1 transcription and secretion, establishing a local chemotactic gradient that directs circulating monocytes toward sites of injury. In turn, MCP-1 can prime neutrophils, promoting robust NET release upon secondary stimulation. This establishes a bidirectional NET-MCP-1 inflammatory amplification loop that sustains local inflammatory infiltration (40). Second, NETs drive the transition of monocytes from the classical to the intermediate subset while downregulating the repair-associated molecule CX3CR1. These effects maintain monocytes in a pro-inflammatory state, impair their tissue-reparative capacity and prolong local inflammatory injury (41). Furthermore, NETs can downregulate CCR2 expression on the monocyte surface, thereby inhibiting MCP-1-mediated directional migration. This disrupts the homeostatic regulation of immune-cell recruitment and shifts inflammatory infiltration from regulated chemotaxis toward dysregulated aggregation (40).

NETs activate a pro-inflammatory endothelial cell phenotype. Endothelial cells form the innermost barrier of the

vessel wall and endothelial dysfunction is a critical step in the initiation of inflammation. NETs can carry mature IL-1 α and, together with cathepsin G, activate endothelial cells, markedly increasing the expression of leukocyte adhesion molecules such as vascular cell adhesion molecule (VCAM)-1 and intercellular adhesion molecule (ICAM)-1 and thereby facilitating leukocyte adhesion and transendothelial infiltration (42). NETs can also activate the endothelial TLR4/NF- κ B signaling axis through an MPO/H₂O₂-dependent pathway, thereby driving endothelial cells toward a broadly pro-inflammatory phenotype (43). At the level of intracellular DNA sensing, the cyclic GMP-AMP synthase (cGAS)-stimulator of interferon genes (STING) pathway serves as a central axis through which NETs amplify endothelial inflammation. NETs activate cGAS-STING signaling through TLR2 on the endothelial cell surface. Sequence-independent binding of double-stranded DNA activates cGAS, which catalyzes the synthesis of the second messenger 2',3'-cyclic GMP-AMP (cGAMP). cGAMP subsequently binds to endoplasmic reticulum-localized STING, inducing its oligomerization and trafficking to the Golgi apparatus, where STING recruits and activates TBK1 kinase (44-46). Activated TBK1 phosphorylates both IRF3 and the NF- κ B p65 subunit (44,45). IRF3 signaling drives robust type I interferon secretion, whereas NF- κ B activation induces multiple pro-inflammatory cytokines; together, these responses amplify the local inflammatory response (44,45).

NETs and thrombosis. NETs drive immunothrombosis by inducing procoagulant activity in endothelial cells and activating platelets, thereby forming a key interface at which inflammation and thrombosis reinforce each other in cardiovascular disease. In this pathological sequence, NETs first shift endothelial cells from an anticoagulant to a procoagulant phenotype, providing the initiating signal for subsequent platelet adhesion and activation. Activated platelets and NETs then establish a bidirectional amplification loop that markedly augments the initial thrombotic signal and ultimately promotes the formation of stable immunothrombi.

NETs induce a procoagulant endothelial cell phenotype. Under homeostatic conditions, endothelial cells possess intrinsic anticoagulant properties; however, endothelial injury markedly increases the risk of local thrombosis (47). NETs act synergistically with IL-1 α and cathepsin G to stimulate endothelial cells, increase TF transcription and procoagulant activity and thereby directly enhance the local propensity for coagulation (42). The cGAS-STING pathway also mediates endothelial procoagulant activity. In addition to inducing pro-inflammatory cytokines, activated NF- κ B promotes TF transcription and downregulates endothelial anticoagulant molecules. Together, these effects establish a procoagulant endothelial phenotype (47).

NETs-platelet bidirectional amplification loop. Platelets are central mediators at the interface between inflammation and thrombosis and their bidirectional interactions with NETs continuously amplify the local immunothrombotic microenvironment. Platelet-driven NETosis occurs through several mechanisms. P-selectin on the platelet surface induces NET release by binding to PSGL-1 on neutrophils (48). TLR4-activated platelets can adhere directly to neutrophils and induce NETosis, whereas the interaction between platelet

integrin α IIB β 3 and neutrophil SLC44A2 triggers intracellular calcium mobilization and promotes NOX-dependent NET release. Soluble mediators released from degranulating platelets, including thromboxane, PF4, vWF and HMGB1, can also act as upstream triggers of NETosis (49,50).

A mutually reinforcing positive-feedback loop operates between platelets and NETs. S100A8/A9 can induce platelet pyroptosis and the release of oxidized mitochondrial DNA, which further promotes NET formation. In turn, S100A8/A9 released from NETs can induce additional platelet pyroptosis, perpetuating the local inflammatory-thrombotic response (51). One study has further delineated this bidirectional mechanism, showing that double-stranded DNA (dsDNA), a structural component of NETs, is a central effector of platelet hyperactivation (52). A functional platelet-intrinsic cGAS-STING signaling axis specifically recognizes intracellular dsDNA and initiates a distinct signaling cascade that enhances thrombotic potential. Following uptake by platelets, NET-derived dsDNA activates the cytosolic DNA sensor cGAS, which catalyzes the production of the second messenger cGAMP. cGAMP binds to endoplasmic reticulum-localized STING and induces its oligomerization and trafficking, leading to activation of the downstream NLRP3 inflammasome. Activated NLRP3 promotes caspase-1 cleavage and the maturation and release of IL-1 β . IL-1 β subsequently modulates outside-in signaling through platelet integrin α IIB β 3, increasing the phosphorylation of Src, Syk and PLC γ 2 and promoting intracellular calcium influx. These events ultimately enhance platelet aggregation, granule release, spreading and clot retraction (52).

NETs and fibrosis. NETs promote pathological fibrosis in cardiovascular tissues through three principal mechanisms: Direct activation of fibroblasts, induction of monocyte-to-fibroblast-like transdifferentiation and promotion of endothelial-to-mesenchymal transition (EndoMT). The pro-fibrotic matrix deposition and tissue-invasive capacity conferred on monocytes by NETs provide an important link between inflammation and fibrosis.

NETs directly activate fibroblasts. Fibroblasts are the principal stromal cells responsible for maintaining tissue homeostasis. Under physiological conditions, they synthesize and secrete extracellular matrix (ECM) and contribute to tissue development and normal structural repair. Following tissue injury, local stress signals and the inflammatory microenvironment activate quiescent fibroblasts, inducing proliferation, directed migration and extensive synthesis of matrix components and thereby initiating the tissue-repair program (53). Transforming growth factor- β (TGF- β) is a central regulator of matrix remodeling. Downstream TGF- β signaling can activate several cascades, including SMAD, MAPK and RhoA, which regulate the transcription and modification of type I and type III collagens. Persistent overactivation of this pathway causes abnormal collagen accumulation and promotes pathological tissue fibrosis (54-57). Studies have shown that NETs can directly promote the pro-fibrotic activation of fibroblasts by targeting the TGF- β signaling axis. Under hypoxic conditions, NETs markedly increase TGF- β transcript levels in fibroblasts. Concurrently, NETs directly induce fibroblast-to-myofibroblast transition, enhance fibroblast migration and increase the levels of type I collagen, phosphorylated SMAD2, phosphorylated

p38 and RhoA. These changes collectively activate the SMAD, MAPK and RhoA pro-fibrotic pathways and promote excessive matrix deposition (58,59). In addition to TGF- β signaling, NET-associated histones and MPO can induce substantial ROS production, further stimulating fibroblast activation and proliferation and exacerbating dysregulated ECM remodeling (60).

As well as directly activating the fibroblast fibrotic program, NETs can indirectly amplify matrix remodeling by reshaping the local inflammatory microenvironment. NET release is accompanied by the release of various pro-inflammatory mediators, including TNF- α and IL-6, which coordinately regulate fibroblast function. TNF- α primarily promotes fibroblast proliferation, whereas IL-6 both induces cell proliferation and increases ECM synthesis (61-64). A recent study has also shown that NETs can promote fibrosis by inducing pyroptosis in cardiac fibroblasts (65). NETs directly increase the levels of NLRP3, caspase-1 and GSDMD-N, triggering canonical pyroptosis characterized by plasma membrane rupture and the release of intracellular contents. Simultaneously, NETs upregulate type I collagen and α -SMA expression. This pyroptotic response is accompanied by substantial secretion of IL-1 β and IL-6 (65). However, the specific upstream and downstream molecular pathways mediating this process remain unclear.

NETs induce monocyte-to-fibroblast-like transdifferentiation and tissue invasion. NETs not only promote fibrosis indirectly through inflammatory signaling but also directly regulate monocyte differentiation and effector functions, converting monocytes into active participants in ECM degradation and deposition. NETs induce the transdifferentiation of monocytes into fibroblast-like cells through TLR4 signaling, leading to extensive collagen deposition and directly contributing to pathological fibrosis (40). During tissue invasion, NET-derived DNA is recognized by monocytes and upregulates the alarmin S100A9. S100A9 then activates TLR4 signaling in monocytes in an autocrine manner and induces substantial MMP-9 synthesis, thereby enhancing monocyte tissue invasiveness and exacerbating ECM degradation and disruption of tissue architecture (66). This mechanism directly links the inflammatory regulatory functions of NETs to subsequent matrix remodeling and provides an important cellular basis for their role in bridging early inflammation and later fibrosis. Monocytes thus function as both responders to inflammatory signals and architects of the fibrotic microenvironment.

NETs drive EndoMT. Endothelial cells can undergo EndoMT, during which they lose endothelial-specific markers such as VE-cadherin and concomitantly upregulate mesenchymal markers, including α -smooth muscle actin, vimentin and type I collagen. These changes confer myofibroblast-like properties and enable the synthesis and secretion of large amounts of matrix proteins, thereby promoting abnormal tissue fibrosis (67). Low levels of NETs can be rapidly cleared by endothelial cells through the receptor for advanced glycation end products without inducing pathological changes. However, when NETs accumulate extensively in the microenvironment, NET-bound elastase cleaves VE-cadherin and disrupts the structural integrity of the endothelial monolayer while simultaneously activating β -catenin signaling. Together, these effects induce a sustained EndoMT phenotype and promote excessive ECM deposition (68).

An integrative perspective: NETs as a central hub of the inflammation-thrombosis-fibrosis axis. Growing evidence indicates that inflammation, thrombosis and fibrosis are not isolated pathological events in cardiovascular disease but instead form an interconnected network of mutually reinforcing and sequential processes. NETs serve as a central integrative node within this triad.

NETs establish a bidirectional amplification loop between inflammation and thrombosis. NET-associated IL-1 α and cathepsin G, together with the activation of endothelial TLR2 and downstream cGAS-STING signaling, synergistically upregulate TF and adhesion molecules, shifting the endothelium toward a pro-inflammatory and procoagulant phenotype (46,60). Activated platelets, in turn, potentially induce NETosis through multiple pathways, including P-selectin/PSGL-1, TLR4 and α IIB β 3-SLC44A2. Conversely, NET components such as S100A8/A9 and dsDNA activate platelets; S100A8/A9 induces platelet pyroptosis, whereas dsDNA elicits cGAS-STING-dependent platelet activation. Together, these reciprocal interactions form a bidirectional platelet-NET amplification loop that continuously reinforces the local immunothrombotic microenvironment (48-51). During acute events such as myocardial infarction and plaque erosion, this inflammation-thrombosis axis is particularly prominent. NET-rich thrombi not only exacerbate ischemic tissue injury but also promote further recruitment of inflammatory cells.

NETs also link early inflammation to subsequent fibrosis. NET-recruited monocytes can transdifferentiate into fibroblast-like cells and directly contribute to ECM production (40). At the endothelial level, NET-associated elastase cleaves VE-cadherin and activates β -catenin signaling, inducing EndoMT and the acquisition of a myofibroblast-like phenotype (68). Furthermore, NETs directly activate cardiac fibroblasts through the TGF- β /SMAD, MAPK and RhoA signaling axes, promoting collagen deposition and myofibroblast differentiation (58,59).

A bidirectional relationship also exists between thrombosis and fibrosis. The thrombotic network formed by NETs and fibrin provides a structural scaffold for fibroblast migration and matrix deposition (69,70). Meanwhile, thrombin and other coagulation proteases generated through the NET-driven coagulation cascade can activate protease-activated receptors on the fibroblast surface, further reinforcing the fibrotic response and promoting the gradual progression of thrombotic lesions toward fibrosis (71).

Collectively, these findings support a unified model in which NETs act as a central hub that integrates and propagates the inflammation-thrombosis-fibrosis cascade in cardiovascular pathology. This framework has potential therapeutic implications: targeting NETs may attenuate all three arms of the cascade and therefore offer broader clinical benefits than inhibiting a single pathological component.

4. NETs in cardiac diseases

NETs contribute to a broad spectrum of CVDs and participate in the pathogenesis of multiple cardiac conditions. Building on the general inflammation-thrombosis-fibrosis framework aforementioned, this section examines the roles of NETs in

acute myocardial infarction (AMI), atrial fibrillation (AF), viral myocarditis (VMC), endocarditis and heart failure (HF), with an emphasis on disease-specific mechanisms.

AMI. In AMI, NETs are not merely markers of sterile inflammation but also constitute an important mechanistic link between the initial inflammatory burst and subsequent reparative fibrosis. Clinical studies have consistently demonstrated that coronary thrombi and infarcted myocardial tissue from patients with ST-segment elevation myocardial infarction (STEMI) are enriched in NETs and that a greater NET burden is closely associated with microvascular obstruction, adverse left ventricular remodeling and worse clinical outcomes (72-75).

The intense sterile inflammatory response triggered by myocardial infarction makes NET-monocyte interactions a major determinant of repair quality. NETs stimulate vascular endothelial cells to secrete large amounts of MCP-1. High local concentrations of MCP-1, in turn, prime neutrophils for NETosis, establishing a positive-feedback loop between NETs and MCP-1 in the infarct zone that directs monocyte infiltration (40). Following NET stimulation, infiltrating monocytes transdifferentiate into fibroblast-like cells through TLR4 signaling, directly participate in collagen deposition and become an important cellular source of post-infarction fibrosis (75). These findings suggest that NETs function as a molecular switch in AMI, redirecting the acute defensive response toward pro-fibrotic remodeling.

Studies have further clarified the upstream triggers and downstream amplification mechanisms of NETosis. Zhang *et al* (76) found that post-infarction hypoxia induces high S100A12 expression in neutrophils through HIF1 α . S100A12 then binds to ANXA5, triggering calcium influx and activating the NOX4-ROS-ERK pathway to drive 'suicidal' NETosis, thereby aggravating cardiac dysfunction. In parallel, dsDNA released from NETs activates the cGAS-STING-NLRP3 pathway in platelets, enhancing platelet activation and thrombus formation and further worsening myocardial ischemia (52). Together, these studies delineate a pathogenic sequence in which the S100A12-ANXA5-Ca²⁺ axis initiates NETosis, whereas the dsDNA-cGAS-platelet pathway amplifies thrombotic injury following NET release. These mechanisms operate sequentially as upstream and downstream components of the same pathogenic cascade. Notably, during the later stages of infarct healing, moderate NET levels may facilitate the clearance of necrotic tissue by promoting Mertk+ reparative macrophage polarization (77), suggesting that NETs exert stage-dependent effects in AMI. Therefore, transient suppression of excessive NETosis during the early phase, while preserving the physiological functions of NETs during tissue repair, may represent a more rational therapeutic strategy. However, the longitudinal dynamics of NET activity in humans following myocardial infarction remain poorly characterized. Identifying the signals that terminate NETosis and define the repair window is therefore an important priority for translational research.

AF. AF is the most common sustained tachyarrhythmia and atrial remodeling is central to its initiation and maintenance. Early histological studies showed that approximately two-thirds of patients with 'lone AF' exhibited localized

atrial inflammation that met the criteria for myocarditis (78). NETosis has been shown to participate in the inflammatory response associated with AF (79), making NETs an emerging focus of AF pathophysiology research.

Emerging evidence indicates that NETs are not merely an inflammatory epiphenomenon in AF but instead function as a central hub driving atrial remodeling. He *et al* (80) found that elevated angiotensin II (Ang II) levels in AF increase AKT phosphorylation in neutrophils through the AT1 receptor. However, because the mTOR pathway is simultaneously inhibited, Ang II does not induce autophagy and is insufficient to initiate NETosis alone; instead, it synergizes with inflammatory stimuli to enhance NET release. Meanwhile, mtDNA and HMGB1 released following rapid pacing-induced mitochondrial damage in cardiomyocytes can act directly as DAMPs and trigger NETosis. Meng *et al* (81) further showed that chronic stress induces high S100A8/A9 expression in atrial tissue. S100A8/A9 recruits neutrophils and activates the TLR4-NLRP3 inflammatory pathway, providing another upstream trigger of NETosis. Downstream, NETs exert distinct effects on cardiomyocytes and fibroblasts. In cardiomyocytes, NETs induce autophagic apoptosis by upregulating p62/Beclin-1/LC3B signaling and cause irreversible mitochondrial damage through mitochondrial membrane depolarization and a burst of mtROS. In fibroblasts, NETs activate Smad/p-p38 MAPK signaling, promote fibroblast-to-myofibroblast transition and exacerbate atrial fibrosis and electrical remodeling (80). Collectively, these findings delineate a self-sustaining pathogenic circuit in AF: Ang II-mediated synergistic signaling and the S100A8/A9-TLR4 axis initiate NETosis; DAMPs such as mtDNA and HMGB1 sustain NET formation; and NET-induced mitochondrial damage promotes the release of additional mtDNA. These three components establish a positive-feedback loop that can persist independently of the initial stressor, thereby driving the perpetuation and recurrence of AF.

However, some gaps remain in AF research. Although NETs induce mitochondrial damage and mtDNA release, direct experimental evidence that the classical mtDNA-cGAS-STING cytosolic DNA-sensing pathway is activated in AF and amplifies the inflammatory cycle is currently lacking.

VMC. VMC is myocardial inflammation caused by viral infection and may progress to dilated cardiomyopathy or HF. In a mouse model of coxsackievirus B3 (CVB3)-induced VMC, NETs were detected in both serum and myocardial tissue and NET degradation markedly attenuated the severity of inflammation (82). The attenuation of inflammation following NET degradation provides causal evidence that NETs participate in the inflammatory pathology of VMC.

Recent research using the CVB3 myocarditis model has identified a previously unrecognized mechanism through which CVB3 infection regulates NETosis. CVB3 infection markedly upregulates calpain small subunit 1 (CAPNS1) expression in neutrophils. CAPNS1 binds to the catalytic subunit calpain-1, which then directly cleaves the nuclear envelope protein nesprin-1. This disrupts nuclear envelope integrity, permits chromatin release and thereby drives NETosis (83). However, the specific mechanisms through which NETs contribute to VMC-associated inflammation remain unexplored.

Subsequent studies showed that CVB3 infection induces the mitochondrial accumulation of calpain-1, leading sequentially to ATP5A1 cleavage, excessive mitochondrial ROS production, mitochondrial dysfunction, NLRP3 inflammasome activation and cardiomyocyte pyroptosis (84). These findings raise the possibility that NETs promote VMC-associated inflammation by disrupting mitochondrial function and activating NLRP3, a hypothesis that warrants further investigation.

Importantly, NETs may exert context-dependent protective and pathological effects in VMC. NETs can restrict the intercellular spread of viruses; therefore, indiscriminate NET depletion may increase the viral load and promote tissue dissemination. This trade-off represents a broader challenge for NET-targeted anti-inflammatory strategies in infectious diseases. Future interventions should selectively suppress the pathological effects of NETs without compromising host defense.

Endocarditis. Endocarditis is inflammation of the inner lining of the heart and its valves and may present acutely or chronically. It is broadly categorized as infective endocarditis (IE) or non-IE. IE is a severe, potentially fatal cardiac disease characterized by direct pathogen invasion of the heart valves, resulting in valvular damage, vegetation formation and inflammation. Li *et al* (85) detected NETs in the peripheral blood of patients with IE and found that elevated NET levels were markedly associated with poor prognosis. During IE pathogenesis, NETs do not function as host bactericidal effectors; instead, pathogens ‘hijack’ them to create a central platform for vegetation formation and immune evasion. This process may occur through both classical and nonclassical pathways. In the classical pathway, *Streptococcus mutans* bridges to platelet Fc γ receptors via specific IgG, thereby upregulating platelet P-selectin expression through the Src family kinase/Syk/PI3K/p38 MAPK signaling pathway (86). Activated platelets subsequently bind to PSGL-1 on the neutrophil surface via P-selectin, providing one of the key signals that trigger NETosis. Simultaneously, bacteria stimulate neutrophil ROS production and induce histone H3 citrullination via TLR2/Fc γ R signaling. Both signals are indispensable and act together to drive NET release (86). The nonclassical pathway is mediated directly by EsxA, an effector protein secreted through the type VII secretion system of *Streptococcus gordonii*. EsxA can induce NETosis at a distance and in a dose-dependent manner, independently of platelets, thereby enabling bacteria to evade phagocytosis during the early stages of bloodstream infection (87). Through their DNA scaffolds and histone components, the released NETs physically entrap and immobilize bacteria-platelet aggregates, promoting the formation of multilayered, biofilm-like vegetations. They also activate the coagulation cascade by promoting thrombin generation and enhance platelet aggregation, thereby establishing a self-amplifying bacteria-platelet-NET feedback loop that drives continued vegetation growth (88). Notably, in the aforementioned studies, NETs exhibited no direct bactericidal activity against the pathogens tested. Instead, they encased the bacteria and consolidated free-floating organisms into biofilm-like vegetations that were difficult to eradicate.

Regarding non-IE, a case report involving patients with systemic lupus erythematosus suggested that Libman-Sacks endocarditis may be associated with NETs (89). However,

mechanistic evidence supporting this association remains extremely limited and currently consists only of isolated case observations. Systematic studies are therefore needed to determine whether NETs have a causal role in non-IE.

HF. HF is a clinical syndrome resulting from structural and functional abnormalities of the heart and is among the leading causes of death worldwide (90). Persistent low-grade inflammation is recognized as a central mechanism underlying the pathogenesis and progression of HF (91) and NETs are emerging as important mediators linking immune-mediated inflammation to myocardial energy failure. Clinical studies have shown that serum NET levels are markedly elevated in patients with HF and are associated with poor prognosis (92,93).

Studies have identified NETs as active drivers of HF progression rather than merely inflammatory biomarkers. Tang *et al* (94) found that elevated Ang II levels in HF downregulate the transcription factor KLF2 in neutrophils and relieve suppression of the HIF1 α pathway, thereby promoting neutrophil infiltration into the heart and NET release. Zhang *et al* (95) further showed that HMGB1 released following pressure overload-induced myocardial injury acts as a DAMP, directly recruiting neutrophils and inducing NETosis. Furthermore, Mang *et al* (96) demonstrated that vWF/ADAMTS13 imbalance in HF results in excessive vWF accumulation, which activates NET formation through SLC44A2 on the neutrophil surface. Zhao *et al* (97) found that downregulation of the endogenous anti-inflammatory protein DEL-1 relieves inhibition of p38 signaling in neutrophils, providing another upstream mechanism for initiating NETosis. Downstream, NET-associated NE activates TLR4-p38 signaling in cardiomyocytes, suppresses peroxisome proliferator-activated receptor gamma coactivator 1- α (PGC-1 α)-mediated mitochondrial biogenesis and causes mitochondrial respiratory dysfunction and cardiomyocyte apoptosis (96). NETs also serve as procoagulant scaffolds that induce immunothrombosis in the myocardial microvasculature, resulting in episodes of ischemia, hypoxia and microcirculatory dysfunction (94). In addition, NETs promote macrophage infiltration and exacerbate cardiac inflammation (95,97). Together, these pathways define the mechanistic framework through which NETs contribute to HF. Neurohormonal activation mediated by Ang II, the release of damage-associated signals such as HMGB1 and coagulation-factor imbalance involving VWF initiate NETosis. NET-induced mitochondrial injury and microthrombosis then promote further DAMP release and aggravate ischemia. These mechanisms establish a self-sustaining positive-feedback loop that persists independently of the initial stressor and drives the progression and deterioration of HF. Notably, empagliflozin can disrupt the HMGB1-NET axis, suggesting that the clinical benefits of SGLT2 inhibitors may be mediated partly through NET-related pathways. This possibility warrants further validation (Fig. 3).

5. NETs in vascular diseases

As well as cardiac diseases, NETs also contribute substantially to vascular pathologies. Building on the general cellular mechanisms linking inflammation, thrombosis and fibrosis aforementioned, this section examines the disease-specific

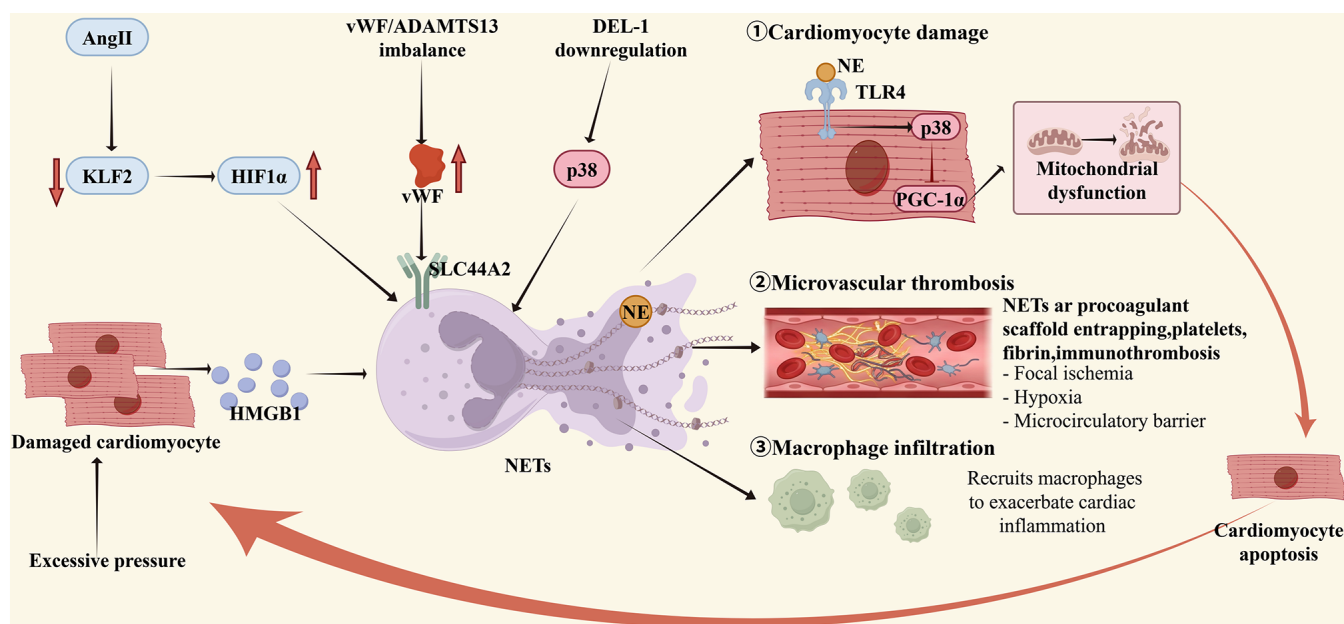


Figure 3. Mechanisms by which NETs drive the progression of heart failure. i) Upstream initiation: Ang II downregulates KLF2, thereby relieving the suppression of HIF1 α ; myocardial injury releases HMGB1; an imbalance in vWF/ADAMTS13 activates NETosis via SLC44A2; and downregulation of DEL-1 relieves the inhibition of p38 signaling. Together, these pathways induce NET formation. ii) Downstream effects: The NET component NE inhibits PGC-1 α through the TLR4-p38 pathway, impairing mitochondrial biogenesis and function in cardiomyocytes and leading to myocardial apoptosis. NETs also serve as a procoagulant scaffold, triggering microvascular immunothrombosis and causing microcirculatory disturbance. Meanwhile, they promote macrophage infiltration, aggravating cardiac inflammation (3). Positive-feedback loop: Mitochondrial damage leads to cardiomyocyte apoptosis and the release of DAMPs, while microthrombosis exacerbates ischemia. These events, in turn, reactivate the upstream NETosis pathways, establishing a self-sustaining pro-inflammatory cycle that drives the self-perpetuation and deterioration of heart failure. NETs, neutrophil extracellular traps; Ang II, angiotensin II; KLF2, Krüppel-like factor 2; HIF1 α , hypoxia-inducible factor 1- α ; ADAMTS13, a disintegrin and metalloproteinase with thrombospondin type 1 motif, member 13; SLC44A2, solute carrier family 44 member 2; DEL-1, developmental endothelial locus-1; HMGB1, high-mobility group box 1; vWF, von Willebrand factor; NE, neutrophil elastase; PGC-1 α ; TLR, Toll-like receptor. Image created with Figdraw.com, with permission.

roles of NETs in atherosclerosis (AS), abdominal aortic aneurysm (AAA), hypertension and aortic valve stenosis.

AS. AS is the most common underlying pathology leading to myocardial infarction, stroke and peripheral vascular disease and is fundamentally a lipid-driven chronic inflammatory disease affecting the intima of large- and medium-sized arteries (98). In 2012, Megens *et al* (99) first directly visualized NET deposition in human and murine AS lesions. Subsequent studies have shown that serum NET levels in patients with coronary artery disease are positively correlated with the severity of coronary stenosis (100). Evidence accumulated in recent years indicates that the mechanisms through which NETs contribute to AS can be organized into three stages: NETosis induction, impaired NET clearance and downstream effector activity (Fig. 4).

The initiating signals for NETosis are highly dependent on the pathological context. At the hemodynamic level, low shear stress at sites such as vascular bifurcations down-regulates the mechanosensitive ion channel Piezo1, thereby reducing Ca²⁺ influx and upregulating histone deacetylase 2. These changes promote NOX-mediated ROS generation and directly induce NETosis (101), helping to explain the predilection of AS for vascular bifurcations. At the metabolic level, oxLDL can induce NETosis through several pathways. First, oxLDL-stimulated macrophages release exosomes enriched in miR-146a that induce NETosis at a distance by targeting and suppressing neutrophil SOD2, resulting in a burst of mitochondrial ROS (102). Second, oxLDL stimulates vascular

endothelial cells to secrete exosomes enriched in miR-505. Following uptake by neutrophils, these exosomes target and suppress the mitochondrial deacetylase SIRT3, causing a burst of mitochondrial ROS and NET release (103). Finally, oxLDL downregulates the cystic fibrosis transmembrane conductance regulator in neutrophils, increasing intracellular Cl⁻ concentrations and activating the serum and glucocorticoid inducible protein kinase 1 (SGK1)-ROS pathway to promote NETosis (104). Aging represents another important contributing factor. Myeloid cells from aged mice exhibit markedly greater mitochondrial oxidative stress (mitoOS) and 7-ketocholesterol induces NET release more potently in aged neutrophils, whereas mitoOS scavenging effectively inhibits this process (105). Thus, NETosis in AS arises from the convergence of mechanical, metabolic and age-related stimuli.

The pathological effects of NETs depend not only on their rate of formation but, more importantly, on the efficiency of their clearance. Under homeostatic conditions, deoxyribonuclease (DNase) I and DNase1L3 rapidly degrade NETs and restore homeostasis. However, AS-associated metabolic abnormalities can severely impair this clearance system. Hypercholesterolemia systemically suppresses the NET-induced DNase feedback response. In ApoE^{-/-} mice, severe hypercholesterolemia impairs the secretory responses of hepatic DNase I and intestinal DNase1L3, delaying NET clearance and preventing the resolution of inflammation (106). Within plaques, macrophages are the principal source of DNase. However, lipid loading-induced endoplasmic reticulum stress

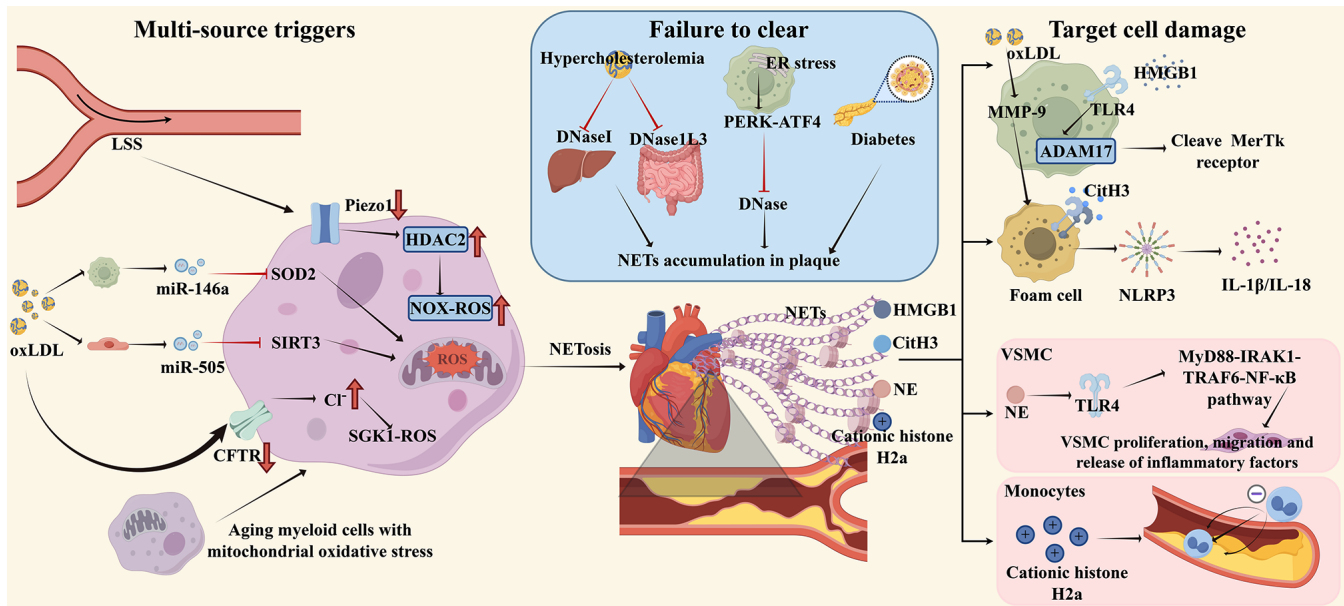


Figure 4. The 'trigger-clearance-effector' mechanism by which NETs drive the progression of atherosclerosis. i) Trigger: Low shear stress downregulates Piezo1 and upregulates HDAC2, thereby promoting NOX-dependent ROS generation; oxLDL induces NETosis via exosomal miR-146a/miR-505 and the CFTR/SGK1 pathway; and aging-related mitochondrial oxidative stress further enhances NET release. ii) Clearance: Hypercholesterolemia, endoplasmic reticulum stress (PERK-ATF4) in plaque macrophages and diabetes synergistically suppress DNase I/DNase1L3-mediated NET degradation, resulting in persistent NET accumulation within plaques. iii) Effector: NETs enhance macrophage oxLDL uptake and MMP-9 expression, impair efferocytosis through HMGB1/TLR4/ADAM17-mediated MerTK cleavage and expand the necrotic core; NE cleaves TLR4 to drive VSMC proliferation and migration; histone H2A captures monocytes; and CitH3 activates the NLRP3 inflammasome, collectively exacerbating plaque inflammation and instability. NETs, neutrophil extracellular traps; HDAC2, histone deacetylase 2; NOX, NADPH oxidase; ROS, reactive oxygen species; ox-LDL, oxidized low-density lipoprotein; miR, micro RNA; CFTR, cystic fibrosis transmembrane conductance regulator; SGK1, serum and glucocorticoid inducible protein kinase 1; MMP, matrix metalloproteinase; HMGB1, high-mobility group box 1; TLR, Toll-like receptor; NE, neutrophil elastase; CitH3, citrullinated histone H3; LSS, low shear stress; DNase, deoxyribonuclease; ER, endoplasmic reticulum; VSMC, vascular smooth muscle cell; SOD2, histone deacetylase 2; SIRT3, sirtuin 3; PERK, protein kinase RNA-like endoplasmic reticulum kinase; ATF4, activating transcription factor 4; ADAM17, a disintegrin and metalloproteinase domain-containing protein 17; NLRP3, NLR family pyrin domain containing 3; IL, interleukin; MyD88, myeloid differentiation primary response 88; IRAK1, interleukin-1 receptor-associated kinase 1; TRAF6, TNF receptor-associated factor 6; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells. Image created with Figdraw.com, with permission.

activates the PERK-ATF4 signaling axis, which markedly inhibits macrophage DNase secretion and promotes persistent NET accumulation (107). Diabetes further aggravates this clearance defect. In STZ-induced diabetic *Ldlr*^{-/-} mice, NETs persist within plaques and fail to regress spontaneously following lipid lowering, unlike those in nondiabetic mice, thereby sustaining the inflammatory microenvironment (108). Importantly, these clearance defects do not occur in isolation. Hypercholesterolemia frequently coexists with diabetes and advanced age and their synergistic effects can lead to widespread disruption of the NET clearance system.

Once NETs persist within plaques, they disrupt the functions of multiple cell types and establish a self-reinforcing pathological cycle. Their effects on macrophages are multifaceted. NETs directly increase macrophage uptake of oxLDL and promote MMP-9 expression and secretion, thereby exacerbating plaque instability (39). A recent study has further shown that NET-associated HMGB1 activates ADAM17 through TLR4. ADAM17 then cleaves the efferocytosis receptor MerTK on the macrophage surface, impairing apoptotic-cell clearance and consequently promoting expansion of the necrotic core (107). In vascular smooth muscle cells (VSMCs), NE cleaves TLR4 and activates the downstream MyD88-IRAK1-TRAF6-NF-κB pathway, promoting VSMC proliferation, migration and inflammatory cytokine release.

These effects drive neointimal hyperplasia and contribute to plaque inflammation, growth and instability (109). In infection-associated AS, cationic histone H2A on the NET surface captures negatively charged monocytes through receptor-independent electrostatic attraction, facilitating their infiltration into plaques and markedly accelerating plaque progression (110). In foam cells, NET-associated CitH3 directly binds to and activates the NLRP3 inflammasome, promoting IL-1β and IL-18 release (111). Collectively, these findings demonstrate that NETs affect virtually all major cell types within plaques through diverse molecular mechanisms.

Overall, NET-driven AS can be conceptualized as a systemic pathological process involving multisource induction, multistep failure of clearance and multicellular effector activity. However, most existing studies rely on endpoint analyses and the specific contributions of NETs to plaque initiation, progression, regression and rupture have not been longitudinally characterized. Furthermore, the functional differences among NETs induced by distinct stimuli have not been systematically compared. LSS-induced NETosis may primarily determine lesion localization, whereas metabolic and aging-related factors may be more important in sustaining plaque progression. Defining these spatiotemporal regulatory differences will be essential for developing stage-specific NET-targeted interventions.

Hypertension. Hypertension is one of the most common chronic diseases and is characterized by persistently elevated arterial blood pressure. Its pathogenesis involves inflammation-driven oxidative stress, endothelial dysfunction, VSMC phenotypic switching and ECM remodeling (6). Serum levels of the NET markers cfDNA and MPO are markedly elevated in patients with hypertension (112), whereas the Ang II-induced increase in blood pressure is markedly attenuated in NET-deficient mice (113), providing functional evidence for the involvement of NETs in hypertension.

Studies have delineated a pathogenic cascade extending from upstream stimuli and NET release to downstream effects and target-organ damage. Under hypertensive conditions, vascular mechanical stretch and neurohumoral activation constitute the two principal triggers of NETosis. Krishnan *et al* (113) demonstrated that cyclic mechanical stretch of the vessel wall activates the mechanosensitive calcium channel TRPV4 on the neutrophil membrane, triggering calcium influx and robust NET release. A stretch magnitude of 10%, simulating hypertension, induced markedly greater NETosis than 5% stretch, which simulated normotension. Concurrently, Chrysanthopoulou *et al* (114) showed that Ang II directly induces NET formation through the AT1 receptor in a process requiring NOX-derived ROS production, autophagy activation and PAD4-mediated histone citrullination. These pathways do not operate independently but converge on ROS as a common mediator. In addition to causing direct cellular damage, ROS generate isolevuglandins (isoLGs). IsoLGs covalently modify lysine residues in histones and disrupt higher-order nucleosome structures, thereby promoting chromatin decondensation and providing the structural basis for NET release (115).

Following NET release, NET-associated histones, particularly CitH3 and TF increase blood pressure through two distinct mechanisms: Vascular functional dysregulation and structural remodeling. At the functional level, CitH3 directly activates endothelial cells, upregulates adhesion molecules such as ICAM-1, VCAM-1 and E-selectin and impairs acetylcholine-induced endothelium-dependent vasodilation (113). At the vascular structural level, Fang *et al* (116) showed that NETs activate PI3K-Akt signaling in VSMCs, leading to downregulation of the cyclin-dependent kinase inhibitor CDKN1b. This relieves G₁/S checkpoint arrest and promotes cell-cycle progression. The response is accompanied by marked upregulation of thymidine kinase 1 (TK1), a rate-limiting enzyme in DNA synthesis that directly promotes VSMC proliferation. Moreover, NET-stimulated VSMCs can transmit proliferative signals horizontally to neighboring VSMCs not directly exposed to NETs by releasing TK1-containing exosomes, thereby extending the spatial range of vascular remodeling (116).

The pathological effects of NETs ultimately converge on fibrosis and functional decompensation in hypertensive target organs. At the cardiac level, Tu *et al* (117) first linked NETs to ferroptosis, demonstrating that NETs activate NF- κ B signaling, upregulate the pro-ferroptotic protein ACSL4 and simultaneously downregulate ferritin heavy chain FTH1 and the antioxidant enzyme GPX4. These changes induce iron-dependent lipid peroxidation and ferroptotic cardiomyocyte death. This process not only directly promotes

cardiomyocyte loss but also upregulates pro-fibrotic markers, including α -smooth muscle actin, fibronectin and collagen II, through pro-inflammatory and pro-fibrotic signaling, thereby driving myocardial interstitial fibrosis and cardiac dysfunction.

AAA. AAA is a degenerative vascular disease characterized by chronic vessel-wall inflammation, ECM degradation and disrupted vascular smooth muscle cell homeostasis (118). Multiple clinical studies have shown that circulating and aortic tissue levels of NET-specific markers, including CitH3, cell-free DNA, nucleosomes, MPO and NE, are markedly higher in patients with AAA than in healthy controls. The concentrations of these markers are positively correlated with aneurysm diameter, expansion rate and rupture risk, suggesting that NET burden may reflect AAA severity and adverse prognosis (119,120).

NET formation is tightly regulated by upstream signaling mechanisms. Phosphoinositide 3-kinase γ promotes noncanonical pyroptosis, specifically activation of the caspase-11/GSDMD pathway, through cAMP/PKA signaling, thereby enhancing NET release and aggravating AAA (121). Studies have identified another important upstream mechanism involving the gut microbiota. Patients with AAA exhibit marked gut dysbiosis characterized by a reduced abundance of the butyrate-producing bacterium *Roseburia intestinalis* and a concomitant decrease in butyrate levels (122). Reduced butyrate availability diminishes its inhibitory effect on neutrophil NOX2 expression, resulting in excessive NET release. Supplementation with *R. intestinalis* or butyrate markedly reduces neutrophil infiltration and NET deposition in the aortic wall and attenuates AAA progression (122). These findings link the gut microbiota-metabolite axis to NET formation and provide a rationale for microbiota-targeted interventions in AAA.

NETs promote AAA formation and progression through multiple convergent mechanisms. First, NETs directly induce ferroptosis, a form of programmed cell death driven by iron-dependent lipid peroxidation, in VSMCs. NETs inhibit PI3K/AKT signaling in VSMCs, downregulating SLC7A11 and GPX4 while upregulating ACSL4 and TFR1, thereby triggering lipid peroxidation and ferroptosis (123). Additionally, NETs also destabilize the dimeric form of the mitochondrial glutathione transporter SLC25A11, leading to mitochondrial glutathione (mitoGSH) depletion and further inducing ferroptosis in SMCs (124). Second, NETs suppress Hippo signaling through TLR9, thereby increasing YAP nuclear translocation and driving VSMC phenotypic switching from a contractile state to a synthetic and pro-inflammatory phenotype. This transition is characterized by downregulation of contractile marker genes, including ACTA2, MYH11 and CNN1 and upregulation of pro-inflammatory and matrix metalloproteinase genes, including MMP2, MMP9, IL-1 β and TNF- α , thereby exacerbating arterial-wall inflammation and matrix degradation (125). Third, NETs activate p38/JNK signaling and induce VSMC apoptosis, aggravating medial VSMC depletion and elastic fiber fragmentation and promoting AAA rupture (126). Furthermore, the DNA component of NETs forms complexes with cathelicidin-related antimicrobial peptide (CRAMP/LL-37), which recruit and activate

plasmacytoid dendritic cells (pDCs), induce robust type I interferon production and subsequently activate T cells to amplify the inflammatory cascade within the aortic wall (127).

In summary, NETs act through multiple mechanisms to drive AAA formation and progression, including the induction of SMC ferroptosis, phenotypic switching and apoptosis and the activation of pDC-mediated immune amplification. Potential therapeutic approaches therefore include inhibiting upstream NET formation, modulating gut microbial metabolites to reduce NET generation and targeting downstream effector mechanisms, such as ferroptosis, YAP signaling and pDC activation. These strategies may have translational potential for the treatment of AAA.

Aortic valve stenosis. Aortic valve stenosis is the most common valvular heart disease, with a prevalence exceeding 2% among individuals older than 60 years and a two-year mortality rate of ~50% among those with severe stenosis (128). The disease is primarily driven by progressive inflammatory and fibrocalcific processes involving interactions among circulating inflammatory mediators, valvular endothelial cells and valvular interstitial cells (129,130).

At the tissue level, abundant NET-specific markers, including CitH3 and cfDNA, have been detected in human stenotic aortic valve tissue (131,132), confirming the presence of NETs in valvular pathology. oxLDL is considered an important mediator of inflammation in aortic valve stenosis (133) and Awasthi *et al* (134) further showed that oxLDL induces NET formation through the TLR-PKC-IRAK-MAPK pathway and NADPH oxidase activation, establishing a molecular basis for the functional relationship between oxLDL and NETs. Building on these observations, evidence increasingly implicates NF- κ B as a key downstream effector of NETs. NF- κ B is a well-established pro-inflammatory signaling hub and contributes to the calcification associated with aortic valve stenosis (135). As NETs can activate NF- κ B through multiple pathways, the present study proposed that NETs within stenotic aortic valves may convert the initial inflammatory signal induced by oxLDL into an osteogenic differentiation program in valvular interstitial cells through NF- κ B signaling. NETs may simultaneously promote ECM remodeling through NET-associated elastase and cathepsins, thereby providing a collagen scaffold for calcific nodule formation. However, this hypothesis currently lacks direct functional validation. No studies have yet used NET inhibitors or PAD4-deficient animals in aortic valve stenosis models to evaluate changes in valvular NF- κ B activity, calcification markers and valve function.

6. Potential NETs-targeted therapeutic strategies in cardiovascular disease

Given the central role of NETs in cardiovascular disease, therapeutic targeting of NETs has attracted increasing attention. Investigated approaches span multiple intervention points, including inhibition of upstream NETosis signaling, enzymatic degradation of preformed NETs and neutralization of downstream NET components. This section categorizes current NET-targeted interventions according

to their mechanisms of action and evaluates the available preclinical evidence, clinical translational status and inherent limitations (Table I).

NETs degradation strategies. DNA forms the structural backbone of NETs, making enzymatic degradation the most direct approach for dismantling preformed NET structures.

DNase I is the most extensively investigated enzyme for NET degradation. Across multiple cardiovascular disease models, DNase I attenuates NET-mediated pathological changes. For example, in myocardial infarction models, DNase I dissolves NET-rich microthrombi, thereby improving microvascular perfusion and reducing infarct size (73). Recombinant human DNase I is clinically approved for cystic fibrosis, in which it reduces sputum viscosity when administered by aerosol inhalation, bronchoscopic instillation, or other routes (136,137). However, no relevant clinical studies have evaluated DNase I in cardiovascular disease and its translation to cardiovascular indications faces several challenges. First, NETs consist of a DNA backbone decorated with histones and granular proteins. DNase I cleaves DNA but does not neutralize histones. DNA degradation may therefore release substantial quantities of positively charged histones into the circulation. Extracellular histones are highly cytotoxic and can directly damage endothelial cells, activate platelets, promote microthrombus formation and trigger further sterile inflammation (138). Second, blood contains G-actin, a high-affinity inhibitor of DNase I. Plasma G-actin concentrations are sufficient to eliminate most of the activity of administered DNase I within minutes. Although modified DNase I variants, including actin-resistant mutants, are under investigation, none have entered cardiovascular clinical trials (139). Third, NETs in cardiovascular disease are predominantly localized within vascular-wall plaques, thrombi and the microcirculation. Neither intravenous nor intra-arterial administration achieves efficient lesion-specific accumulation of DNase I. Instead, DNase I nonspecifically degrades circulating NET structures, potentially disrupting the physiological roles of NETs in host defense and vascular homeostasis. Consequently, the translational potential of DNase I monotherapy in cardiovascular disease remains limited. Potential strategies for overcoming these limitations include combining DNase I with histone-neutralizing agents, developing actin-resistant DNase I variants and using nano-carriers for lesion-targeted delivery.

Other nucleases, including three-prime repair exonuclease 1 (TREX1), contribute to the physiological clearance of NETs by macrophages (140). TREX1-deficient mice develop inflammatory myocarditis that gradually progresses to dilated cardiomyopathy and circulatory failure, possibly because of impaired NET degradation (141). However, the therapeutic potential of pharmacologically enhancing TREX1 activity remains unexplored and is currently theoretical.

Targeting NETs components

Histone neutralization. Histones are the most abundant protein components of NETs and exert direct cytotoxic effects on endothelial cells, cardiomyocytes and other cell types (138). Several strategies have therefore been developed to sequester or neutralize extracellular histones.

Table I. Therapeutic interventions targeting NETs.

Authors, year	Intervention strategy	Target	Compound	Mechanism	Model	Key therapeutic outcomes	Evidence level	Translational status	Key limitations	(Refs.)
Mangold <i>et al</i> , 2022	NETs degradation	NETs-DNA backbone	DNase-I	Enzymatic cleavage of DNA matrix.	ApoE ^{-/-} mouse model of atherosclerosis (high-fat diet).	Reduced atherosclerotic plaque size by ~3-fold; decreased plasma IL-1 β and IL-6 levels.	Preclinical (<i>in vivo</i>).	Approved (Cystic fibrosis).	(1) Releases toxic histones; (2) Inactivated by plasma G-actin; (3) Poor lesion specificity.	(73)
Morita <i>et al</i> , 2004		NETs-DNA backbone	TREX1	Intracellular degradation of internalized NETs via TREX1 (DNase III) in macrophages; DNase 1L3 mediates extracellular NET degradation in DCs.	Primary human monocyte-derived macrophages (HMDMs) and dendritic cells (MDDCs); NETs from primary human neutrophils.	TREX1 silencing led to accumulation of undigested NETs in macrophages; DC-secreted DNase 1L3 degraded NETs extracellularly.	Preclinical (<i>in vivo</i>).	Preclinical (Theoretical target).	No pharmacological agonist available; pure theoretical target.	(141)
Targeting NETs components										
Iba <i>et al</i> , 2015; Wildhagen <i>et al</i> , 2014	Histone neutralization	Histones	Unfractionated/LMWH/Non-anticoagulant heparin	Charge-dependent electrostatic binding to neutralize histone cytotoxicity.	Mouse (C57BL/6) models of ConA-induced hepatitis, CLP and LPS challenge; Rat (Wistar) models of histone H3-induced organ injury; EA.hy926 cells and primary endothelial/leukocytes.	Attenuates histone-mediated cytotoxicity and organ damage.	Preclinical (<i>in vivo</i>).	Approved (Anticoagulant, repurposing).	The effective dose is far beyond the anticoagulation threshold; broad charge effects; poor tissue penetration; stimulus-specific efficacy.	(142, 143)
Osada <i>et al</i> , 2017			TM alfa	Promotes APC generation to proteolytically degrade histones.	EA.hy926 endothelial cells.	Reduces histone-induced thrombin generation and endothelial death.	<i>In vitro</i> .	Approved (DIC).	Limited cardiovascular-specific data.	(145)

Table I. Continued.

Authors, year	Intervention strategy	Target	Compound	Mechanism	Model	Key therapeutic outcomes	Evidence level	Translational status	Key limitations	(Refs.)
Abrams <i>et al</i> , 2013			CRP	Binds directly to histones, blocking their integration into cell membranes and subsequent Ca ²⁺ influx; competes with phospholipids for histone binding.	Mouse models; EA.hy926 cells; Serum from trauma/sepsis patients.	Reduces histone-mediated endothelial injury.	Preclinical and <i>Ex vivo</i> (Patient serum).	Biomarker/Drug candidate.	CRP has context-dependent pro-/anti-inflammatory effects.	(146)
Li <i>et al</i> , 2025	Neutrophil elastase inhibition	NE	Sivelestat	Selective NE inhibition	LPS-induced sepsis mouse; MI/R models	Preserves endothelial glycocalyx; reduces myocardial injury	Preclinical (<i>in vivo</i>).	Approved (ARDS)	Protease redundancy may (PR3, CatG, MMPs) compensate; PK challenges.	(147)
Bidouard <i>et al</i> , 2003			SSR69071	NE inhibition	Rabbit coronary ischemia-reperfusion model	Reduces infarct area	Preclinical (<i>in vivo</i>).	Preclinical	Intravenous delivery required; limited cardiovascular data.	(149)
Delbosc <i>et al</i> , 2016			AZD9668	NE inhibition	Elastase-induced AAA rat model (with P. gingivalis enhancement)	Suppressed Pg-enhanced AAA diameter growth; promoted healing process (smooth muscle cell colonization and collagen deposition) within the intraluminal thrombus; reduced calcification, MPO and cell-free DNA release.	Preclinical (<i>in vivo</i>).	Phase II (Respiratory diseases)	Repurposing potential but cardiovascular trials lacking.	(150)

Inhibition of
NETosis

Table I. Continued.

Authors year	Intervention strategy	Target	Compound	Mechanism	Model	Key therapeutic outcomes	Evidence level	Translational status	Key limitations	(Refs.)
Knight <i>et al</i> , 2014	PAD4 inhibition	PAD4	Cl-amidine	Irreversible pan-PAD inhibition	ApoE ^{-/-} mouse carotid thrombosis; AS models	Reduces NETosis, atherosclerosis and arterial thrombosis	Preclinical (<i>in vivo</i>).	Preclinical (Tool compound)	Narrow therapeutic time window; interferes with physiological NETosis; no reliable pharmacodynamic biomarker.	(151)
Du <i>et al</i> , 2020			GSK484	Reversible selective PAD4 inhibition	Mouse MI model (LAD ligation)	Reduces infarct size, neutrophil infiltration, CitH3 expression and cardiomyocyte apoptosis	Preclinical (<i>in vivo</i>).	Preclinical	Same as aforementioned; optimal dosing window undefined.	(153)
Heger <i>et al</i> , 2023			JB1-589	Oral selective PAD4 inhibition	Collagen-induced arthritis (DBA/1J mice)	Preserves LV diastolic function; reduces CitH3 and thrombotic inflammation	Preclinical (<i>in vivo</i>).	Preclinical	Long-term safety unknown; cardiovascular data limited.	(154)
Li <i>et al</i> , 2024	ROS inhibition	NADPH oxidase/ROS	Colchicine	Inhibits ROS production and Ca ²⁺ influx; suppresses NETosis	Mouse model of permanent LAD ligation-induced AMI.	Improves cardiac function and remodeling; reduces post-AMI inflammation	Preclinical (<i>in vivo</i>).	Approved (CVDs: CAD and post-MI),	NETs-specific benefit not demonstrated in clinical trials; anti-NETs effect inferred	(155)
Shirakawa <i>et al</i> , 2022			HOCl (hypochlorous acid)/Hydroxyl radicals (•OH)	H ₂	Neutralizes HOCl produced during oxidative burst, reducing DNA damage (γ-H2AX); suppresses CXCR4 ⁺ aged neutrophil subset and neutrophil aggregation; may also inhibit PAD4 activity in NOX-independent NETosis.	Scavenges hydroxyl radicals and HOCl; suppresses ROS. Inflammation	Inhibits PMA-induced NETosis; reduces DNA damage in neutrophils	Preclinical (<i>in vivo</i>).	Medical gas. Molecular target unclear; no direct CVDs patient evidence	(157)

Table I. Continued.

Authors, year	Intervention strategy	Target	Compound	Mechanism	Model	Key therapeutic outcomes	Evidence level	Translational status	Key limitations	(Refs.)
Khan <i>et al</i> , 2019	Others	DNA/Chromatin (intercalation)	Anthracyclines (e.g., doxorubicin, daunorubicin)	Intercalates into DNA, blocking chromatin decondensation and transcriptional firing; inhibits both Nox-dependent and Nox-independent NETosis without affecting ROS production or inducing apoptosis.	Primary human neutrophils.	Dose-dependent NETosis inhibition	<i>In vitro</i> .	Approved (Chemotherapy)	Intrinsic cardiotoxicity prevents repurposing; requires cardio-safe derivatives.	(158)
Kindberg <i>et al</i> , 2024	Indirect inhibition via inflammation/metabolism Cytokine targeting	IL-6 receptor	Tocilizumab	Reduces circulating NET markers (dsDNA, MPO-DNA, H3Cit) in STEMI patients; mechanism may involve downregulation of NETosis-related genes (histones, HDAC) and reduced neutrophil mobilization; exact mechanism remains unclear.	STEMI patients (ASSAIL-MI trial).	Reduced NET markers at 24h and 3-7 days; NET reduction partially mediated tocilizumab's beneficial effect on myocardial salvage index (MSI); higher NET levels associated with larger infarct size and more microvascular obstruction.	Clinical.	Approved (Rheumatoid arthritis, CRS).	Mechanism of NETs inhibition unclear; lacks robust cardiovascular outcome data.	(159)

Table I. Continued.

Authors, year	Intervention strategy	Target	Compound	Mechanism	Model	Key therapeutic outcomes	Evidence level	Translational status	Key limitations	(Refs.)
Awasthi <i>et al</i> , 2019	Immunometabolic Intervention	Lactate dehydrogenase	sodium oxamate dehydrogenase	Inhibits lactate LPS-induced reducing lactate accumulation; lactate itself acts as a signaling molecule promoting NETosis via Warburg effect (PKM2 dimerization).	Mouse model of formation. sepsis (C57BL/6, 10 mg/kg LPS, i.p.); primary human polymorphonuclear neutrophils (PMNs).	Suppresses NET (<i>in vivo</i>).	Preclinical	Preclinical. may disrupt cardiac	Broad LDH inhibition (160) lactate metabolism; off-target risks unassessed.	
Burczyk <i>et al</i> , 2022	Hif-1α/Nrf2 pathway	4-OI	Downregulates Hif-1α; upregulates Nrf2/HO-1 pathway.	Bone marrow neutrophils (C57BL/6J).	Suppresses LPS-induced NETosis with minimal effect on basal metabolism.	<i>In vitro</i> .	Preclinical.	Non-specific alkylation of metabolic enzymes (e.g., GAPDH); long-term cardiac effects unknown.		(161)

AAA, abdominal aortic aneurysm; AMI, acute myocardial infarction; APC, activated protein C; ARDS, acute respiratory distress syndrome; AS, atherosclerosis; CAD, coronary artery disease; CatG, cathepsin G; CitH3, citrullinated histone H3; CLP, cecal ligation and puncture; ConA, concanavalin A; CRP, C-reactive protein; CRS, cytokine release syndrome; CVDs, cardiovascular diseases; CXCR4, C-X-C chemokine receptor type 4; DCs, dendritic cells; DJC, disseminated intravascular coagulation; DNA, deoxyribonucleic acid; DNase-I, deoxyribonuclease 1-like 3; dsDNA, double-stranded DNA; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; γ-H2AX, gamma-H2AX (H2A histone family member X); HDAC, histone deacetylase; Hif-1α, hypoxia-inducible factor 1-α; HMDMs, human monocyte-derived macrophages; HO-1, heme oxygenase-1; HOC1, hypochlorous acid; IL, interleukin; i.p., intraperitoneal; LAD, left anterior descending artery; LDH, lactate dehydrogenase; LMWH, low molecular weight heparin; LPS, lipopolysaccharide; LV, left ventricular; MDDCs, monocyte-derived dendritic cells; MI, myocardial infarction; MI/R, myocardial ischemia/reperfusion; MMPs, matrix metalloproteinases; MPO, myeloperoxidase; MSI, myocardial salvage index; NADPH, nicotinamide adenine dinucleotide phosphate; NE, neutrophil elastase; NETs, neutrophil extracellular traps; NOX, NADPH oxidase; Nrf2, nuclear factor erythroid 2-related factor 2; PAD4, peptidyl arginine deiminase 4; Pg, Porphyromonas gingivalis; PK, pharmacokinetics; PKM2, pyruvate kinase M2; PMA, phorbol 12-myristate 13-acetate; PMNs, polymorphonuclear neutrophils; PR3, proteinase 3; ROS, reactive oxygen species; STEMI, ST-elevation myocardial infarction; TM, thrombomodulin; TREX1, three prime repair exonuclease 1.

Unfractionated heparin, low-molecular-weight heparin and their non-anticoagulant derivatives neutralize histones through electrostatic interactions mediated by their negative charge, thereby mitigating cytotoxicity and organ injury. However, the effective doses required substantially exceed those used for clinical anticoagulation, representing a major barrier to clinical translation (142,143). Although non-anticoagulant heparin derivatives may reduce bleeding risk, their efficacy in bacterial sepsis is markedly lower than that in sterile inflammation, suggesting that stimulus-dependent activity limits their generalizability. Moreover, the broad, charge-based binding of heparin may interfere with host-defense peptides, whereas its macromolecular structure limits penetration into the vessel wall and the neutralization of tissue-bound histones. Heparin also does not target other harmful NET components, including the DNA backbone and elastase (144). Targeting histones alone may carry the additional immunological risk of suppressing beneficial DAMP signaling and the therapeutic window appears narrow: Prophylactic administration is effective, whereas delayed treatment provides minimal benefit. Thus, heparin-based histone neutralization alone has limited translational potential. Further development should focus on histone-specific antibodies, tissue-penetrant small-molecule inhibitors and biomarker-guided patient stratification according to the underlying inflammatory stimulus.

Thrombomodulin alpha promotes the generation of activated protein C (APC), which proteolytically degrades extracellular histones and attenuates histone-induced toxicity (145). This mechanism may contribute to the protective effects of thrombomodulin signaling in sepsis-associated organ injury, although cardiovascular-specific evidence remains limited.

C-reactive protein (CRP) can form complexes with histones, preventing their incorporation into cell membranes and reducing histone-induced calcium influx and cellular injury (146). However, CRP has both pro-inflammatory and anti-inflammatory properties and its net effect on NET-mediated pathology remains context-dependent.

NE inhibitors. NE is a key mediator of NET formation and a major effector of NET-mediated tissue injury. Sivelestat is a selective NE inhibitor used clinically to treat acute respiratory distress syndrome (147). Preclinical studies have demonstrated that sivelestat protects against myocardial ischemia-reperfusion injury and preserves endothelial glycocalyx integrity (148). However, large-scale clinical trials in cardiovascular disease remain lacking. Second-generation NE inhibitors, including SSR69071 and AZD9668, have shown efficacy in preclinical models of myocardial ischemia-reperfusion injury and AAA (149,150). Their clinical development has been constrained by pharmacokinetic limitations and the need for intravenous administration.

A major limitation of NE inhibition is functional redundancy. NETs contain multiple proteases, including proteinase 3, cathepsin G and MMPs, that may compensate for NE blockade and thereby limit the efficacy of single-target therapy.

Inhibition of NETosis

PAD4 inhibitors. PAD4-mediated histone citrullination is a key step in chromatin decondensation during NETosis,

making PAD4 a major therapeutic target. Cl-amidine is a first-generation broad-spectrum PAD inhibitor. It has been extensively used in cardiovascular animal models, including models of AS and myocardial infarction, to suppress NET formation and attenuate tissue injury (151). It is also widely used as a reference compound for investigating the PAD4-NET pathway (152). GSK484 is a more selective PAD4 inhibitor that reduces neutrophil infiltration, inflammatory cytokine release and NET formation and attenuates cardiomyocyte apoptosis in myocardial infarction models (153). JBI-589 is another selective, orally bioavailable PAD4 inhibitor that reduces myocardial NET deposition and pro-fibrotic cytokine levels (154).

The principal translational challenge facing PAD4 inhibitors is uncertainty regarding the therapeutic window. NETs are not uniformly detrimental throughout the course of cardiovascular disease. Inhibition of excessive NETosis is particularly important during the early phase of AMI-associated ischemia-reperfusion injury, spanning hours to days. During later stages of infarct healing, however, moderate NET levels may contribute to necrotic tissue clearance and repair. Current evidence does not permit precise determination of the optimal timing or duration of PAD4 inhibition during cardiovascular events and pharmacodynamic biomarkers to guide treatment duration are lacking. Furthermore, long-term systemic PAD4 inhibition may compromise physiological NETosis-mediated host defense. Future clinical trials could consider circulating CitH3 as a surrogate endpoint when designing time-sequenced dosing regimens.

ROS inhibitors. Given the central role of ROS in classical NETosis, targeting oxidative stress represents another potential therapeutic strategy. Colchicine, a widely used anti-inflammatory drug, has been mechanistically shown to reduce NADPH oxidase-derived ROS production and calcium influx, thereby inhibiting NET formation (155). Supported by evidence from landmark randomized controlled trials, including COLCOT and LoDoCo2, low-dose colchicine (0.5 mg/day) administered as an adjunct to standard therapy markedly reduces the risk of cardiovascular events in patients with chronic coronary artery disease and those with AMI, with an overall favorable tolerability profile (156). Colchicine is therefore the only NET-related anti-inflammatory drug to date with clinical evidence of cardiovascular benefit, providing proof of principle for the clinical feasibility of NET-targeted strategies. However, clinical trials have not established that its cardiovascular benefits are attributable to NET inhibition.

Molecular hydrogen (H_2) is a safe antioxidant that inhibits PMA-induced NET formation by reducing ROS levels (157). However, its precise molecular targets in neutrophils remain unclear and direct evidence of NET inhibition in patients with cardiovascular disease is lacking.

Others. Anthracyclines, including doxorubicin and epirubicin, are widely used chemotherapeutic agents that inhibit NETosis while preserving ROS production and neutrophil viability (158). However, their inherent cardiotoxicity precludes their repurposing for cardiovascular indications. The cardioprotective agent dexrazoxane does not interfere with anthracycline-mediated NET inhibition, suggesting that the anti-NET and cardiotoxic effects of anthracyclines may be

separable (158). Future studies could integrate high-resolution mass spectrometry, nuclear magnetic resonance spectroscopy and X-ray crystallography to characterize the atomic-level interactions of doxorubicin with two key molecular targets: Topoisomerase II β , which mediates cardiotoxicity and the as-yet-unidentified target responsible for NET inhibition. Comparing their molecular interaction fingerprints may identify a toxicophore that is essential for cardiotoxicity but dispensable for NET inhibition. Structure-based drug design using bioisosteric replacement, the introduction of metabolic soft spots, or steric modifications could then selectively eliminate this toxicophore and potentially yield a first generation of anthracycline-derived NET inhibitors with reduced cardiotoxicity.

Indirect inhibition of NETs via inflammatory and metabolic pathways

Cytokine-targeted therapy. Tocilizumab is a monoclonal antibody targeting the IL-6 receptor. In a clinical study, tocilizumab reduced circulating levels of NET markers, including CitH3 and dsDNA, in patients with STEMI and attenuated myocardial injury (159). However, the mechanism underlying this effect remains unexplored. More robust clinical evidence and mechanistic studies are therefore required before tocilizumab can be considered a NET-targeted therapy for cardiovascular disease.

Immunometabolic interventions. Metabolic pathways are increasingly recognized as important regulators of NET formation. Glycolysis plays a central role in both NOX-dependent and NOX-independent NETosis. The lactate dehydrogenase inhibitor sodium oxamate reduces LPS-induced NET formation and lactate accumulation, highlighting the importance of glycolytic metabolism in NETosis (160). Furthermore, 4-octyl itaconate (4-OI), a cell-permeable derivative of the immunometabolite itaconate, markedly inhibits LPS-induced NET formation by downregulating HIF-1 α and, in part, through the Nrf2/HO-1 pathway (161). These findings identify 4-OI as an emerging immunometabolic modulator with NET-targeting potential.

Although sodium oxamate and 4-OI reduce NET formation in experimental models, their clinical translation raises potential concerns regarding off-target effects. As a broad-spectrum LDH inhibitor, sodium oxamate may disrupt systemic lactate metabolism, including that of the myocardium; however, this possibility has not been evaluated in cardiovascular models. 4-OI can nonspecifically alkylate metabolic enzymes such as GAPDH and its long-term effects on mitochondria-rich tissues, including the myocardium, remain unclear (161). Moreover, no direct evidence is available regarding the effects of either agent on the acid-base balance of the plaque microenvironment. Future studies should prioritize evaluating the metabolic toxicity of these inhibitors in cardiovascular cells and developing targeted delivery strategies to minimize off-target effects.

7. Conclusion

In summary, the present review synthesized the multidimensional regulatory network of NETs in cardiovascular disease. Initially characterized as antimicrobial defense structures,

NETs are now recognized as a central hub connecting inflammation, thrombosis and fibrosis. In major CVDs, including AMI, AF, HF, AS, hypertension and AAA, NETs drive disease initiation and progression through diverse effector mechanisms. These include disrupting mitochondrial function, inducing cellular phenotypic switching, activating the coagulation cascade, releasing matrix-degrading proteases and triggering ferroptosis and pyroptosis. Cardiovascular micro-environmental stimuli, including oxLDL, mechanical stretch, Ang II and DAMPs, interact with regulatory mechanisms such as gut microbiota-associated metabolic dysregulation, cGAS-STING-mediated cytosolic DNA sensing and the S100A8/A9 positive-feedback loop. Together, these factors establish disease-specific networks that initiate and sustain NETosis. Accordingly, NET-targeted strategies now encompass DNase I-mediated degradation, histone neutralization, NE inhibition, blockade of PAD4 and ROS signaling, immunometabolic modulation and cytokine-targeted interventions. Although low-dose colchicine has entered cardiovascular clinical practice, most NET-directed approaches remain limited by the modest efficacy of single-target interventions, poorly defined therapeutic windows, off-target effects and insufficient cardiovascular-specific clinical evidence.

Despite substantial advances, several important questions must be resolved before NET-targeted therapies can be translated into clinical practice. First, NET structure and function are highly heterogeneous. Single-cell multi-omics and spatial proteomics should be used to characterize NET molecular signatures across CVDs, disease stages and tissue regions. Such studies may distinguish pathogenic NET components from relatively benign or reparative signals and provide a molecular basis for selective neutralization. Second, NETosis exerts stage- and context-dependent pathogenic and reparative effects. During the inflammatory clearance and proliferative repair phases of AMI, NETs may perform opposing functions. This duality is even more pronounced in infectious conditions such as IE and VMC, in which indiscriminate NET inhibition may facilitate pathogen dissemination. Future studies should establish conditional animal models that permit temporal control of NETosis, characterize the kinetics of NET formation and degradation and define the transition from global NET clearance to precise temporal regulation. Interventions should also be stratified according to sterile or infectious etiology, with priority given to targets that suppress pathological NET activity without compromising host defense. Third, the release of toxic histones following DNase I-mediated degradation, functional redundancy among NET-associated proteases and the off-target effects of metabolic modulators indicate that single-node interventions may be insufficient because of the diverse activities and compensatory mechanisms of NET components. Multitarget combination strategies, such as DNase I combined with histone-specific antibodies, PAD4 inhibitors combined with SGLT2 inhibitors, or simultaneous inhibition of the upstream S100A8/A9-TLR4 axis and downstream cGAS-STING pathway, may provide synergistic efficacy. Lesion-targeted delivery using nanocarriers, antibody-drug conjugates, or localized perfusion may further reduce the risks of infection and impaired immune surveillance associated

with prolonged systemic NETosis inhibition. However, most NET-targeted interventions remain at the preclinical stage and cardiovascular-specific evidence is limited. Clinical trials incorporating NET-specific markers, such as MPO-DNA and CitH3, as pharmacodynamic surrogate endpoints are needed to evaluate the cardiovascular benefits of PAD4 inhibitors, DNase I and immunometabolic modulators and to define dose-biomarker-outcome relationships. Finally, identification of the microbiome-metabolite axis, exemplified by the *Roseburia intestinalis*-butyrate-NOX2 pathway, has expanded the therapeutic landscape of NET regulation. Integrating AI-assisted multimodal data analysis with pharmacogenetic approaches may enable precision cardiovascular therapy based on individualized NET molecular signatures and risk stratification. Such advances could ultimately translate NETs from mechanistic mediators identified in basic research into clinically actionable therapeutic targets that can be monitored and modulated.

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Authors' contributions

ZW was responsible for writing and revising the manuscript, creating the figures and constructing the table. MC contributed to the figure editing and revision process. WS contributed to the figure editing and revision process. YJ contributed to literature search and study screening. XS contributed to constructing the table. XC contributed to literature search and study screening. YY and JZ offered funding support and played a role in the conception, design, editing and supervision of the manuscript. Data authentication is not applicable. All authors read and approved the final manuscript.

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Not applicable.

Competing interests

The authors declare that they have no competing interests.

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