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Role of Neutrophil Extracellular Traps in Hypertension and Their Impact on Target Organs

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ABSTRACT

Hypertension is the predominant cause of cardiovascular diseases (CVDs) globally, and essential hypertension (EH) represents a significant public health challenge due to its multifactorial etiology involving complex interactions between genetic and environmental factors. However, the pathogenesis of EH is still unclear. Hypertension is a dysregulation in the renin–angiotensin–aldosterone system and sympathetic nervous system, both regulating saline homeostasis and cardiovascular function. However, current therapeutic interventions targeting these systems have limited efficacy in approximately 40% of cases, suggesting the involvement of alternative mechanisms. Inflammation is associated with the occurrence and progression of hypertension, but the underlying mechanism remains elusive, while chronic inflammation leads to tissue damage, fibrosis, and irreversible organ dysfunction. The development and maintenance of EH are caused by endothelial dysfunction, oxidative stress, and chronic inflammation. Neutrophils are involved in both acute and chronic inflammation since they represent the primary line of defense against inflammatory insults once recruited to the inflamed site where they remove harmful impurities. The process involving the formation of neutrophil extracellular traps (NETs) is called NETosis are involved in the pathogenesis and progression of CVDs, including coronary artery disease, acute myocardial infarction, peripheral arterial disease, heart failure, and atrial fibrillation. Recent investigations demonstrated that NETs facilitate the development of hypertension; however, the precise role of NETs in hypertension remains largely elusive. Therefore, this review aims to provide an overview of the current understanding regarding the involvement of NETosis in hypertension and explore the potential therapies targeting NETs for future interventions.

1 | Neutrophil and Neutrophil Extracellular Traps (NETs)

1.1 | NET Formation

Neutrophils are the primary guardians of the innate immune system and involved in host defense against microorganisms. They respond to infectious injury through a variety of defense mechanisms, including phagocytosis of microbes and toxic degranulation of cytoplasmic granule proteins that are microbicidal [2–5]. The production of neutrophils in the bone marrow is regulated by a colony-stimulating factor [6], while their maturation is orchestrated by transcription factors including PU-1, C/EBP, and GATA-1 [7]. Neutrophils use different strategies to eradicate

intracellular and extracellular pathogens. Neutrophils engulf microorganisms through phagocytosis once they encounter them and sequester them in the phagosomes. Besides phagocytosis and degranulation, neutrophils stimulated by specific sterile and nonsterile stimuli undergo a distinct form of cell death characterized by the extrusion of granular proteins bound to a meshwork of chromatin and other nuclear material [3]. These complexes of intermixed nuclear and cytoplasmic neutrophil contents are released into the extracellular space called NETs, and the process of NET formation as a new form of cell death is called NETosis [8]. Classical NETosis critically depends on the activation of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase. Excessive generation of reactive oxygen species (ROS) induces neutrophil degranulation resulting in the release of a complex protein mixture termed azurophilic granule. The formation of these NETs has been the subject of several theories, primarily centered around the regulation of intracellular ROS and calcium. Activation of NADPH oxidase (Nox2), triggered by the MAPK/ERK pathway signaling cascade, induces ROS production, which in turn facilitates the release and transport of myeloperoxidase (MPO) and neutrophil elastase (NE) from azurophilic granules to the nucleus. Once in the nucleus, they contribute to chromatin decondensation and subsequent breakdown of nuclear and cytoplasmic membranes, ultimately resulting in extracellular release of NETs [9]. An increase in intracellular calcium levels triggers an alternative pathway for the generation of NETs. Peptidylarginine deiminase 4 (PADI4), which is activated by calcium, catalyzes the conversion of histone arginine residues to citrulline. This enzymatic modification effectively alters the charge of the residue and induces chromatin condensation, subsequently leading to DNA strand release. Inhibition of either pathway impairs chromatin decondensation, ultimately resulting in neutrophil apoptosis [5]. Several recent studies identified caspase and gasdermin D as pivotal regulators in the formation of NETs. Moreover, mitochondrial DNA is also released under specific conditions, such as priming with granulocyte-macrophage colony-stimulating factor followed by the treatment with lipopolysaccharide Toll-like receptor 4 (TLR4) [10].

1.2 | NET Composition

NETs are composed of DNA, histones, and antimicrobial proteins released by neutrophils in response to pathogens but are also recognized for their involvement in a range of pathological processes, including autoimmune diseases, cancer, and cardiovascular diseases (CVDs), The DNA molecule serves as a structural scaffold for the assembly of other components, thereby imparting robustness to the network. Moreover, DNA itself possesses antimicrobial activity by its ability to sequester surface-bound cations, disrupt membrane integrity, and induce lysis in bacterial cells, particularly histones that are known to be released [11]. Cytotoxic MPO, NE, and histone G are the primary proteins derived from neutrophil granules. MPO facilitates pathogen degradation by generating ROS, hypochlorous acid, and nitrogen. NE is involved in NET release through histone cleavage (resulting in chromatin decondensation) as well as microbial protein degradation. Other proteases like cathepsin G also aid in neutralizing microbial components, breaking down extracellular matrix (ECM) proteins, and recruiting immune cells to infection/inflammation sites [11]. NETs may also contain other types of molecules, such as lactoferrin and pentanes, that additionally regulate the immune response. These granules comprise serine proteases including NE, cathepsin G, and azurocidin, alongside S100 family proteins like calprotectin and MPO. Inflammation induces neutrophils to release decondensed chromatin DNA structures along with histones and granule proteins such as MPO and NE into the extracellular environment, which together with proteolytic enzymes, and ROS-producing proteins form NETs [7, 12]. Serine proteases and MPO degrade lamins and histones, promoting the depolymerization and destruction of chromatin in the nuclear envelope. Highly activated neutrophils eliminate extracellular microbes by releasing NETs, which are able of capturing and killing extracellular pathogens [13, 14]. Histones and nucleic acids themselves possess bactericidal activity, while other antimicrobial peptides associated with NETs include granular, cytoplasmic, and cytoskeletal proteins, as well as metabolic enzymes derived from neutrophils [11, 15]. In addition, NETs contain effector molecules or components released by neighboring cells. Aggregated NET structures effectively trap and degrade proinflammatory mediators in vitro and in vivo [16].

1.3 | NET Function

Neutrophils, which are the most abundant leukocytes in the human body, are involved in the innate immune response, serving as the primary defense against microbial pathogens and actively participating in inflammatory reactions. NETs also trigger TLR4-mediated differentiation of monocytes into fibroblasts, which exert their effects on the myocardium, resulting in sterile inflammation and adverse remodeling. Furthermore, NETs activate and impair endothelial cells through an interferon response, thereby promoting endothelial dysfunction and vascular inflammation by recruiting other immune cells, predominantly macrophages. Consequently, this process contributes to the initiation and progression of atherosclerotic plaques [10]. NETs exacerbate plaque instability by inducing the release and local accumulation of proinflammatory cytokines and matrix metalloproteinases (MMPs), which degrade ECM proteins including collagen, thereby increasing the risk of plaque erosion and rupture. Recent studies revealed that NETs exert a negative regulatory effect on macrophage autophagy, enhancing the inflammatory activity of NLRP3 and promoting the secretion of interleukin-1β (IL-1β) and IL-18. This leads to increased lipid accumulation, foam cell formation, and further exacerbation of inflammation and plaque instability [17, 18]. NETs have been initially associated with the control of infectious pathogens, but recent research has discovered the involvement of NETs in the pathogenesis of various diseases, including lupus erythematosus, atherosclerosis, and hypertension (Figure 1). Neutrophil oxidative burst leads to the extrusion of NETs, and DNA and histones, as well as granular proteins initiate the inflammatory process (Figure 2). MPO catalyzes the production of hypochlorous acid in the presence of hydrogen peroxide and halides, and this acid generates long-acting oxidants like chloramine and prolongs oxidative stress. MPO is associated with oxidative stress, inflammation, and endothelial dysfunction, the latter caused by the sequestration and depolymerization of vascular endothelial cells [19, 20]. Therefore, the coordinated activation of Nox2 and MPO causes NET-induced endothelial dysfunction and vascular injury. NETs are involved in the pathogenesis of CVDs by increasing thrombosis, activation of endothelial

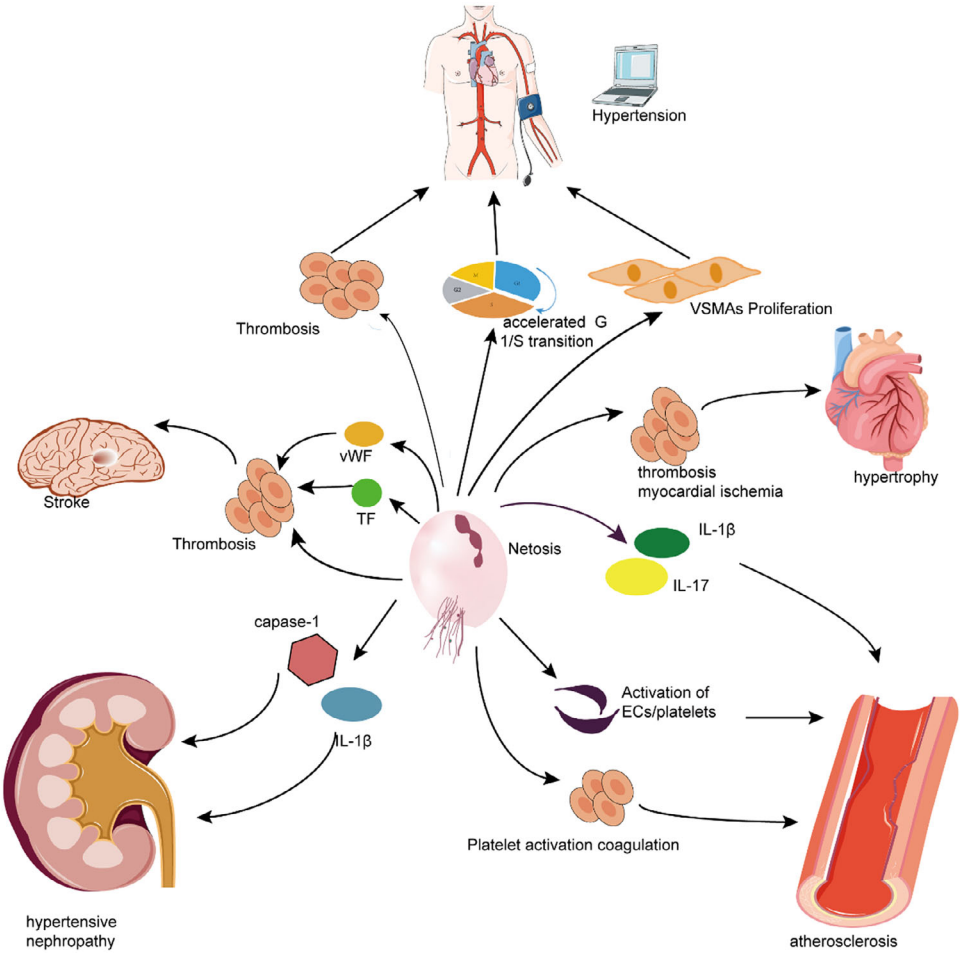

FIGURE 1 | Mechanisms associated with NETs and hypertension and their target organs. This figure mainly outlines the mechanism of NETs related to hypertension and their target organs. NETs induce hypertension by promoting thrombosis, accelerating cell cycle, and promoting smooth muscle cell proliferation. In addition, in the process of hypertension-related target organ damage, NETs can lead to myocardial hypertrophy through mechanisms such as myocardial ischemia and thrombosis. It activates endothelial cells and platelet aggregation by mediating inflammatory factors IL-1β and IL-17, leading to atherosclerosis, and induces hypertensive nephropathy through inflammatory factors IL-β and Capase-1. Finally, Nets can promote thrombosis by mediating TFs and coagulation factors, thus causing stroke events. EC indicates endothelial cell; IL-1β, interleukin—1β; IL-17, interleukin—17; TF, tissue factor; VSMA, vascular smooth muscle cell; vWF, von Willebrand Factor.

cells, direct injury to endothelial cells, and sterile inflammation. Moreover, when neutrophils are activated, components of Nox2 generate aggregated ROS, which are involved in various inflammatory diseases including rheumatoid arthritis, atherosclerosis, diabetes mellitus, kidney disease, and hypertension [21]. Moreover, NETs contribute to arterial and venous thrombosis.

2 | Relationship Between Neutrophils, NETs, and Hypertension

The extensive research on hypertension did not clarify completely the exact etiology of this disease in most patients. However, like many other chronic diseases, hypertension is characterized by inflammation and low-grade inflammation determines its development and organ damage. Inflammation is involved in various processes leading to an increase in blood pressure; for instance, a positive correlation exists among hypertension and increased levels of white blood cells, C-reactive protein, and IL-6. In addition, an increased neutrophil count is associated with an increased risk of developing essential hypertension (EH) and renal insufficiency [1, 22]. Neutrophils secrete mediators responsible for the inflammatory response such as elastase, MPO, ROS, and various hydrolases. These factors induce tissue damage and atherosclerotic plaque rupture, which in turn increases the risk of hypertension [23, 24]. Notably, three major intrinsic functions of neutrophils such as phagocytosis, degranulation, and NETs, are potential mechanisms underlying this switch along with externally related effects involving other innate and adaptive immune cells [25]. Neutrophils and their function play a pivotal role in the pathogenesis of hypertension, since an increased neutrophil count is associated with an increased risk of developing hypertension. Neutrophils secrete inflammatory mediators [26], including elastase [23], MPO, oxygen free radicals, and various hydrolases [24], all contributing to tissue damage and atherosclerotic plaque destruction, thereby increasing the risk of hypertension. Moreover, the neutrophil/lymphocyte ratio is linked to an increasing risk of hypertension. Specifically, hypertensive patients show increased superoxide secretion by neutrophils compared to healthy individuals. An association exists between NETs and hypertension, with

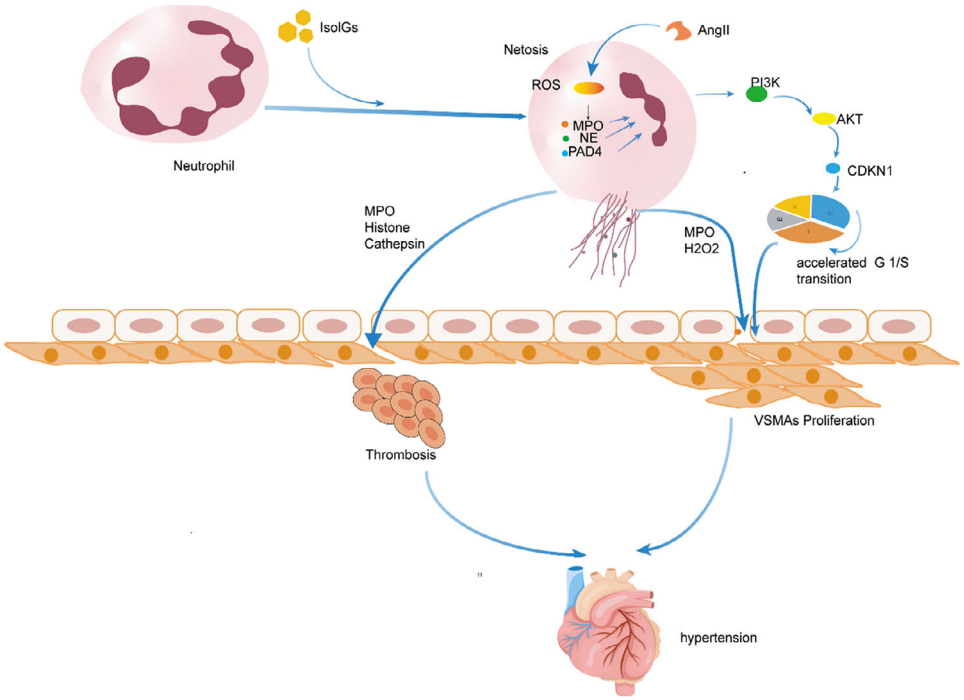

FIGURE 2 | Regulatory mechanisms of NETs in hypertension. The role of NETs in hypertension is that IsoLGs promote neutrophil traps, and AngII further acts on ROS to promote the release of NETs. On the one hand, Nets can promote smooth muscle cell proliferation, while on the other hand, they act on the PI3K/Akt/CDKN1b signaling axis to accelerate G1/S conversion and promote VSMC proliferation, thereby promoting blood pressure elevation. On the other hand, NETs induce endothelial dysfunction and vascular damage by releasing substances such as MPO, increasing thrombosis and leading to hypertension. AKT indicates protein kinase B; Ang II, angiotensinogen II; CDKN1b, cyclin dependent kinase inhibitor1b; H_2O_2, hydrogen peroxide; IsoLGs, isolevuglandins; MPO, myeloperoxidase; NE, neutrophil elastase; NETs, neutrophil extracellular traps; PAD4, peptidyl arginine deiminase4; PI3K, Phosphatidylinositol-3-hydroxykinase; ROS, reactive oxygen species; VSMC, vascular smooth muscle cell.

the former accumulating in the kidney and aorta of hypertensive patients. NETs are present in human vascular tissues and are associated with vessel wall lesions, such as atherosclerosis. Additionally, NETs contribute to the development of pulmonary hypertension by promoting angiogenesis. Proteins involved in the formation of NETs, including peptidyl-arginine deiminase 4 (PAD4), induce fibrosis, which is a pathological alteration of vascular remodeling linked to the progression and advancement of hypertension [27]. Furthermore, NETs stimulate a transforming growth factor-β (TGF-β)-dependent proliferation, invasion, migration, and epithelial–mesenchymal transition of gastric cancer cells [28]. TNF-α upregulates NE in vascular smooth muscle cell (VSMC), facilitating their migration, proliferation, inflammation, and mediating NET formation [29]. VSMC proliferation induces the development of vascular lesions associated with hypertension. NETs-induce the proliferation of VSMCs, which is due to an increased cell cycle progression or inhibition of apoptosis (Figure 1). Indeed, NETs decrease the proportion of cells in G1 phase while they increase those in S phase, thereby promoting VSMC proliferation, suggesting an association between NETs and hypertension (Figure 2). NET promotion of VSMC proliferation due to the increase in G1/S transition is promoted by TK1 through the regulation of the PI3K/Akt/CDKN1b signaling axis (Figure 2, Table 1) [30]. Li et al. [31] identified a positive correlation among the levels of NETs and both systolic blood pressure and diastolic blood pressure in patients with EH. Moreover, neutrophils not only induce direct oxidative reactions but also exert vasoactive effects through the release of various mediators, including cytokines, adenosine, proteases, and phospholipases, and their interaction with adhesion molecules activates endothelial signaling pathways. Hofbauer et al. reported a positive association between blood pressure and NET levels, with angiotensin II and Nox2-derived ROS being two pro-hypertensive factors crucial for stimulating NET formation. Furthermore, a positive correlation exists between fluctuations in blood pressure and neutrophil counts [32]. The study conducted by Jaya et al. revealed that the primary mechanism used by which NETs to induce endothelial cell dysfunction subsequently leading to hypertension is predominantly mediated by PAD4 and TRPV4. Angiotensin II triggers the release of NETs while also contributing to thrombosis in hypertension. Indeed, one study on angiotensin II-induced hypertensive mice identified an autonomous function for chromatin expansion during NETosis in promoting isolevuglandin accumulation known for driving inflammation the heart and aorta under hypertension [33]. These findings collectively indicate a correlation between NETs and increased blood pressure, as well as vascular impairment in individuals with hypertension.

The association between NETs and hypertension is further confirmed by studies on EH population. Li et al. [31] discovered that plasma levels of NETs are increased in patients with moderate to severe EH compared to those in healthy controls or patients with mild EH. Moreover, inflammatory cytokines stimulate the production of NETs, and a positive correlation exists among plasma levels of NETs, fibrinogen, TAT complex, and D-dimer in patients with moderate and severe EH. This indicate an

TABLE 1 | Summary of the included studies of NETs in hypertension.

Study	Models	Main conclusion
Reus-Chavarría [37]	15 healthy people and 15 HTN patients	We have shown an α-ENaC overexpression in platelets from hypertensive patients compared to platelets from normotensive subjects, suggesting it makes a contribution to the activation state of platelets and the physiopathology of hypertension.
Li [31]	168 patients with untreated hypertension were found for the first time	Our study reveals that EH drives a systemic inflammatory environment, which, in turn, drives neutrophils to prime and NET releasing, and found a link between hypercoagulability and NET levels in moderate to severe EH patients.
Fang [30]	Spontaneously hypertensive rat (SHR) and Wistar–Kyoto (WKY) (male, 6 weeks old)	Nets promote VSMC proliferation through AKT/CDKN1b/TK1 and are associated with the development of hypertension.
Chrysanthopoulou et al. [34]	Five untreated adult patients with newly diagnosed EH and 26 healthy, sex-matched individuals	This study describes the pathogenic role of Ang II in EH, which links neutrophils to thrombotic inflammatory tissue damage in the EH environment, activated neutrophils expose active TFS through NETs.
Krishnan [33]	C57BI/6 mice 15 healthy research subject	This study is the first to demonstrate that neutrophil and NETosis increase with IsoLGs formation and are reduced by IsoLGs scavenger.

Abbreviations: α-ENaC, α-epithelial sodium channel; AKT, protein Kinase B; Ang II, angiotensinogen II; CDKN1b, cyclin-dependent kinase inhibitor 1b; EH, essential hypertension; HTN, hypertension; NETs, neutrophil extracellular traps; TK1, thymidine kinase 1; VSMC, vascular smooth muscle cell.

association between increased levels of NETs and a hypercoagulable state in EH, suggesting the role of NETs as regulators of the hypercoagulable state in EH, although the pathogenesis underlying their involvement remains unclear. The production of NETS is triggered by several infectious and non-infectious factors. Chronic inflammation disrupts normal immune homeostasis leading to an increased thrombotic risk, particularly among obese patients with EH. Chrysanthopoulou et al. [34] demonstrated the role of angiotensin II in EH connecting neutrophils/NETs to thrombotic inflammatory tissue damage by the promotion of collagen production through the activation of endothelial cells, vascular injury, and interstitial renal fibrosis (Table 1). The renin–angiotensin system is involved in the pathogenesis of hypertension; therefore, classical antihypertensive therapies target angiotensin II for an effective regulation of blood pressure. This study discovered that angiotensin II acts as a stimulator in the formation of NET, and patients with EH have a significantly reduced ability to release NETs in the peripheral blood after the treatment with angiotensin receptor blockers. Li et al. [31] also observed that EH patients with hyperhomocysteinemia (HHcy) have a significantly increased level of NET markers in their bloodstream compared to EH patients without HHcY (Table 1). Furthermore, homocysteine induces the release of neutrophil-derived NETs in vitro in a concentration and time-dependent manner, and these NETs from both EH and HHcY patients enhance the procoagulant activity [35]. However, the mechanisms underlying the hypercoagulable state in EH and HHcY patients have not been extensively investigated. Increased levels of NETs exert detrimental effects on noncommunicable diseases such as acute myocardial infarction and venous thrombosis, and a close correlation between levels of NET markers, thrombin-antithrombin complex, and D-dimer levels. These findings suggest that NETs modulate the hypercoagulable state in patients with EH and HHcY, which is in agreement with previous reports indicating that NETs activate coagulation [36]. Erika et al. [37] investigated a cohort of 15 hypertensive patients and 15 healthy individuals, revealing that neutrophils possess high α-ENaC expression (Table 1). Furthermore, they identified the actin cytoskeleton as a crucial regulator of ENaC, which in turn reorganizes actin cytoskeleton during polarization and directional migration. The activation of ENaC facilitates Na+ influx and Ca^{2+} increase, thereby intensifying the active state of neutrophils and promoting oxidative stress as well as endothelial cell damage. These findings collectively indicate a correlation between NETs and increased blood pressure, as well as vascular impairment in individuals with hypertension.

3 | NETs and Complications Associated With Hypertension

3.1 | NETs and Atherosclerosis

Atherosclerosis is a significant contributor to mortality and morbidity associated with coronary artery disease, stroke, and peripheral arterial stenotic disease. Its pathophysiology is characterized by hyperlipidemia and inflammation that are closely intertwined [38]. Hypercholesterolemia increases the number of circulating monocytes, thereby enhancing their propensity to migrate toward atherosclerotic lesions. Consequently, extensive research has been performed on the involvement of monocytes in the development and progression of atherosclerosis. Immune cells such as T lymphocytes, mast cells, dendritic cells, and platelets are also involved in atherosclerosis [39]. However, little attention has been paid to the role of neutrophils in the pathophysiology of this condition, although it has been recently discovered that neutrophils are present and have a function in early and established atherosclerotic lesions in humans and mice. In brief, neutrophils exacerbate endothelial

dysfunction by attracting leukocytes (especially monocytes) into the atherosclerotic lesions while promoting foam cell formation. Advanced plaques become exposed to ROS and to proteases derived from neutrophils, leading to plaque instability [40, 41]. NETs are present in atherosclerotic plaques of both mice and humans, as revealed by immunohistochemical staining by Megens et al. [42] (Figure 1). Studies performed on Apoe−/− mouse models demonstrated that neutrophils contribute to the progression of atherosclerosis by releasing NETs [43], although studies on human plaque samples revealed that unstable plaques and ruptured plaque are those primarily containing neutrophils and NETs [44, 45], being the predominant leukocyte populations present in atherosclerotic thrombi [46]. Another study investigating the relationship between extracellular DNA formation and atherosclerosis discovered that patients with severe coronary atherosclerosis possess levels of circulating DNA and nucleosomes in the serum significantly increase from 50.09 to 69.59 ng/mL and from 1.32 to 2.02 ng/mL, respectively, suggesting an involvement of NETs in the development of atherosclerosis [47]. Cholesterol induces the formation of NETs, leading to IL-1β production and upregulation of IL-17, consequently promoting immune cell accumulation into the atherosclerotic lesions, contributing to atherosclerosis development (Figure 1) [48]. Oxidized low-density lipoproteins interact with Toll-like receptors 2 and 6 in neutrophils to induce the formation of NETs by generating ROS [49]. NETs induce atherosclerotic plaque development, contributing to endothelial cell injury and plaque erosion. A reduced accumulation of isolevuglandins adducts in the atherosclerotic aorta and stable atherosclerotic plaques are present in atherogenic Ldlr-/- mice treated with 2-hydroxybenzylamine. Furthermore, MPO present in neutrophil granules and NETs oxidizes high-density lipoprotein in the atherosclerotic lesions and impairs cholesterol efflux mediated by apolipoprotein A-I 45 [36]. Treatment with 2-hydroxybenzylamine restored high-density lipoprotein cholesterol efflux capacity, further suggesting that neutrophils and NETs directly contribute to isolevuglandin-mediated atherosclerosis and endothelial cell damage [50]. NETs also promote mesenchymal cell differentiation into collagen-producing myofibroblasts in vitro, while both NETs and tissue factor (TF)/thrombin signaling increase the fibrotic response associated with systemic autoimmune disorders. Experimental studies in patients with hypertension revealed that angiotensin II promotes vascular fibrosis either by directly affecting VSMCs or inducing TGF-β and the expression of the connective tissue growth factor CCN2 [51, 52]. A cohort study in patients with coronary artery disease revealed a significant association among increased levels of NETs in plasma, thrombin production and major adverse cardiovascular events during the follow-up period [47]. The study found that markers related to NETs are increased in patients with coronary atherosclerosis, with extracellular double-stranded DNA in coronary artery disease being 69.59 ng/mL compared to 50.09 ng/mL in healthy controls [53].

3.2 | NETs and Cardiac Hypertrophy

Cardiac hypertrophy is an increase in the volume and mass of cardiac muscle cells, typically resulting from an increase in hemodynamic load. It can manifest as a beneficial adaptive response known as physiological cardiac hypertrophy or as a detrimental pathological response in patients with chronic hypertension, valvular heart disease, myocardial infarction, or sarcomere protein mutations. Pathological cardiac hypertrophy leads to heart failure, arrhythmia, and mortality [54]. Numerous factors influence cardiomyocyte hypertrophy including molecular mediators (such as angiotensin II, endothelin, and catecholamines), activation of mammalian target of rapamycin (mTOR) signaling transduction pathway, regulation mechanisms for natriuretic peptide release, involvement of mechanosensors and modulation of myogenin expression by epigenetic regulatory factors and non-coding RNA molecules. Various cell types (e.g., cardiomyocytes themselves along with endothelial cells fibroblasts and immune cells) are also involved, contributing to the pathogenesis of this condition. However, the underlying mechanisms leading to cardiac hypertrophy and subsequent failure are still incomplete, thus limiting certain therapeutic approaches. Currently, significant advancements have been made in understanding the distinct roles and impacts of macrophages and various subsets of T cells in cardiac hypertrophy. Increased levels of pro-inflammatory cytokines, including TNF-α, and members of the IL-1 and IL-6 families are found in patients suffering from heart failure. Furthermore, the overexpression of these cytokines in experimental models demonstrated the development of cardiac hypertrophy and subsequent heart failure [55]. Moreover, the depletion of neutrophils mitigates cardiac hypertrophy and dysfunction induced by pressure overload in mice subjected to transverse aortic constriction. Tang et al. [56] discovered that cellular hypertrophy is promoted by angiotensin II-induced formation of NETs coupled with KLF2 deficiency. Additionally, their findings suggest that angiotensin II-mediated activation of neutrophils contributes to cardiac hypertrophy through the KLF2/NETosis/thrombosis pathway. Activated neutrophils adhere to vessel walls and release NETs, resulting in the thrombotic occlusion of small vessels and impairment of myocardial microcirculation (Figure 1). Chronic microthrombosis leads to reduced capillary density, further exacerbating myocardial hypoxia.

3.3 | NETs and Hypertensive Nephropathy

Hypertensive nephropathy is a significant late complication of hypertension and is typically directly associated with the progression of chronic kidney disease, which ranks second as the leading cause of mortality in individuals suffering from it worldwide. The long-term impact of hypertension on renal function causes renal interstitial fibrosis, characterized by excessive production of extracellular interstitial proteins by abnormally activated renal fibroblasts. Proteinuria, tubular hypertrophy, oxidative stress, activation of the renin–angiotensin–aldosterone system, collagen turnover, chronic inflammation, and vasoactive substances collectively contribute to the pathogenesis of hypertensive renal fibrosis. Furthermore, inflammation is involved in both hypertension and its comorbidities. Hypertension-induced renal injury includes renal fibrosis, tubular hypertrophy, and glomerular changes. Dysregulation of renal homeostasis leads to fibrotic scarring and consequent loss of renal function [57]. Hypertensive renal fibrosis involves several pathological mechanisms associated with scar formation, including disorders in the assembly, anchoring or degradation of ECM, the generation of inflammatory factors, impaired regeneration of renal tubular epithelial cells, microvascular rarefaction, and activation of fibroblasts [58]. The renal microcirculation system is composed

of glomeruli and peritubular capillaries, which represent the initial barrier for blood circulation and the frontline defense against neutrophil response to injury [59, 60]. Chrysanthopoulou et al. discovered that TF-modified NETosis neutrophil/remnants are present in sites with significant interstitial fibrosis. This study also revealed that TF-carrying NETs promote the differentiation of resident renal fibroblasts into myofibroblasts and increase their potential for fibrosis. Chen et al. discovered a positive correlation between blood urea nitrogen and creatinine levels with neutrophil-to-lymphocyte ratio (NLR) in hypertensive patients, suggesting that NLR is a sensitive predictor for renal dysfunction in the general hypertensive population. Another study conducted by Tonyali et al. revealed that the average NLR level among 46 healthy controls is 2.1419, while it is 3.5219 in patients with chronic kidney disease patients [61]. Wang et al. [62] demonstrated that inhibition of NETs results in a reduced damage to glomerular and peritubular capillary endothelial cells, along with a remarkable decrease in apoptotic cell count in the kidneys in contrast-induced acute kidney injury mice, while reduction of NETs leads to a decreased caspase-1 and IL-1β expression, indicating their potential therapeutic effect on mitigating kidney injury. Peptidyl arginine deiminase 4 is an enzyme involved in classical NETosis involved in various kidney-related diseases. Indeed, some selective inhibitors targeting this protein such as CI-MI, YW3-56, or GSK484 significantly attenuate neutrophil infiltration, secretion of inflammatory factors, and formation of NETs in animal models exhibiting renal ischemia-reperfusion injury [63, 64].

3.4 | NETs and Hypertension-Related Stroke

Stroke is a hazardous cerebrovascular disorder caused by thrombosis, with high morbidity and mortality rates [65]. The pathogenesis of stroke is intricate and involves several pathological processes including energy metabolism, cell membrane depolarization, excitotoxicity, oxidative stress, and inflammatory response [66]. Inflammation is also involved in the onset of stroke. Neutrophils exert immune defense by phagocyting the invading microorganisms or killing them by the release of antimicrobial peptides. TNF-α, IL-1β, IL-8, and intercellular adhesion molecule 1 are the primary driving forces during the neutrophil infiltration process leading to ROS release that exacerbates blood-brain barrier damage and further aggravates the risk of stroke [67]. Hypertension is an important risk factor for ischemic stroke. EH refers to elevated blood pressure with exclusion of secondary causes, representing a prothrombotic state associated with more life-threatening thrombotic complications rather than bleeding incidents. NETs are linked to thrombosis risk under various conditions including myocardial infarction and acute stroke [68]. EH is also characterized by the activation of neutrophils. However, the role of neutrophils in the development of a prothrombotic state in EH has received limited attention. However, neutrophils and NETs are involved in inflammation-related thrombotic disorders [69]. NETs participate in stroke pathophysiology through various mechanisms, including their promotion by activated platelets and induction of platelet activation, thereby contributing to thrombosis (Figure 1). Furthermore, NET structures contain TF, von Willebrand factor, and histones, which works as scaffolds for platelet adhesion, activation, and aggregation, thus contributing to the coagulation cascade [70]. NETs activate and aggregate platelets to facilitate thrombosis, while inducing an inflammatory response that leads to stroke [71]. Additionally, NETs are involved in the pathological progression of atherosclerosis by promoting endothelial cell apoptosis and exacerbating atherosclerosis [72].

4 | Therapies Targeting NETs

Approaches targeting NETs represent promising therapeutic approaches for the management of CVDs, as they offer potential strategies to mitigate vascular inflammation, thrombosis, and tissue damage associated with various cardiovascular conditions. Several therapeutic interventions have been developed to either prevent NET formation ("upstream NET targeting") or degrade and inactivate NET components ("downstream NET targeting"), aiming to reduce disease progression and enhance patient clinical outcomes. Pharmacological and biological interventions are employed to inhibit the formation of NETs. In a study, targeted knockdown of peptidylarginine deiminase 4 (PADI4) in mice resulted in the most significant prevention of NET formation, while small molecule inhibitors targeting key molecules involved in NET formation, such as NE, Nox2, and PADI4, demonstrated promising outcomes in preclinical investigations [73]. The use of DNase1 for the cleavage of extracellular DNA strands has been authorized for other clinical applications. The significance of endogenous DNase1 and DNase1-like 3 in NET regulation was initially demonstrated in a knockout mouse model. In these models, intravascular accumulation of NETs triggered clot formation and obstructed blood vessels in the lungs, liver, and kidneys, resulting in organ damage; this mechanism was subsequently confirmed in patients with severe inflammatory diseases exhibiting reduced DNase activity [74]. Additionally, the depletion of NETs through DNase1 treatment resulted a reduction in fibrosis and enhanced thrombolysis in pulmonary hypertension. Furthermore, administration of DNase1 improved cardiac systolic function. A recently identified downstream inhibitor of NETs is the histone inhibitory peptide HIPe, which exerts its action by binding to the N-terminal tail of histone H4 and prevents its interaction with smooth muscle cells (SMCs), thereby inducing membrane cleavage [75]. The relationship between these mechanisms and hypertension is currently under investigation.

5 | Outlook and Conclusion

NETs exert a significant impact on the occurrence and progression of hypertension. They also induce and facilitate thrombosis and inflammation. A comprehensive understanding of in vivo NETosis, encompassing both structural components and their specific functional modifications above and below, is imperative for clarifying the role of NETs in hypertensive populations. This understanding is also crucial for identifying, validating, and for the use of optimal molecular candidates for therapeutic targeting. Future therapeutic strategies may involve the increase of neutrophil extracellular chromatin degradation or the reduction of the levels of inflammatory factors through related mechanisms. These investigations suggest that NETs are involved in the genesis and pathophysiology of hypertension, potentially serving as biomarkers and drug targets for its diagnosis and treatment.

Author Contributions

Fei Yu wrote the manuscript, Jianshu Chen conceived the presented idea. Jingtao Wang consulted the relevant literature. Qiang Wu and Zhengke Ma made the tables and figures. Xiaowei Zhang provided valuable feedback and approved the manuscript.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The authors have nothing to report.

Ethics Statement

The authors have nothing to report.

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