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Neutrophil extracellular traps (NETs): Relevance to thrombosis and hemostasis. A narrative review

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ABSTRACT

Background: Neutrophil extracellular traps (NETs) are released by neutrophils and comprise web-like structures that play a vital role in fighting infections. NETs have also been involved in various thrombosis and hemostasis mechanisms. This review aims to outline the current understanding of NETs in these processes, their mechanisms of action, their clinical importance, and potential treatment strategies to counteract adverse events.

Methods: A search of the literature was conducted up to June 2024 via PubMed, Scopus, Google Scholar, and Web of Science, with a focus on studies linking neutrophil extracellular traps to thrombosis and hemostasis.

Results: NETs have been directly implicated in thrombosis by activating platelets, providing a substrate for thrombus formation, and directly activating coagulation. NETs are associated with venous thromboembolism, arterial thrombosis, and cancer-related thrombosis, among other thrombotic events. NETs can also support clot formation and hemostasis at sites of vascular damage. NETs could serve as potential biomarkers for thrombotic events, and various strategies are being explored to reduce their adverse events, such as inhibiting their formation, degrading extracellular DNA, and modifying associated proteins.

Conclusions: An improved understanding of NET-mediated thrombosis and hemostasis processes might aid in the development of effective strategies to prevent life-threatening thrombus formation and aid in the prevention of thromboembolic diseases, ultimately benefiting affected patients.

Keywords: hemostasis, neutrophil extracellular traps, NETs, platelet activation, thrombosis, thromboembolic disorders

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INTRODUCTION

Thrombosis is a pathological process characterized by the formation of blood clots (thrombi) in blood vessels [1]. Although normal hemostasis is necessary to control the risk of bleeding, abnormal thrombus formation can instead lead to adverse events such as deep vein thrombosis, pulmonary embolism, heart attack, and stroke [2]. Conventionally, thrombosis has been conveniently conceptualized in terms of platelets, coagulation factors, and endothelial cells as the major and most critical participants in this process [3]. However, emerging evidence indicates that more attention should be given to the role of neutrophils, particularly arising neutrophil extracellular traps (NETs), in thrombus formation and hemostasis [4, 5].

Neutrophil extracellular traps (NETs) were first described in 2004 by Brinkmann et al. who reported that activated neutrophils release web-like structures composed of DNA, histones, and granular proteins that trap and kill pathogens [6]. Since then, NETs have gained recognition not only for their role in host defense, but also for their involvement in various pathological conditions, including thrombosis and inflammation.

Neutrophils are the most abundant white blood cells and are recognized for their innate immune functions, such as phagocytosis and degranulation [7]. Recent discoveries have revealed that neutrophils can also release NETs in response to various stimuli, including DNA, histones, and antimicrobial proteins [8]. NETs are extracellular web-like structures formed by decondensed chromatin, granule proteins, and neutrophil elastase released by a neutrophil in a defensive response against pathogens [6, 9]. Initially, identified for pathogen capture, NETs have emerged as key players in thrombosis and hemostasis [5, 10].

NETs influence thrombosis through multiple pathways. They directly activate platelets, facilitating their adhesion and aggregation at sites of vascular injury [11]. Addition-

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ally, NETs act as scaffolds for coagulation factors, promoting fibrin formation and enhancing clot stability [12]. Moreover, NETs express tissue factors, thereby facilitating the initiation of the coagulation cascade and thus promoting thrombus formation [13,14].

Histones and neutrophil elastase within NETs further increase their procoagulant properties, linking them to various thrombotic disorders, including venous thromboembolism, arterial thrombosis, and cancer-related thrombosis [15,16]. In addition to promoting clot formation, NETs play diverse roles in physiological and pathological conditions [17].

NETosis

NETosis is the process of the formation of neutrophil extracellular traps (NETs), which are extracellular web-like structures composed of DNA, histones, and antimicrobial proteins [18]. NETosis is triggered by a number of factors, such as pathogens, inflammatory cytokines, activated platelets, and cholesterol crystals [19-22].

NETosis is a process that is accompanied by certain changes in the morphology and biochemistry of neutrophils. These alterations include nuclear decondensation, chromatin condensation, and the mixing of nuclear and cytoplasmic elements [23.24]. Finally, due to cell membrane disruption, NETs are released into the extracellular compartment [25,26].

NETs are involved in the initiation and growth of a thrombus and in its stabilization and propagation. Platelets adhere to and aggregate on them, coagulation factors are activated, and fibrin formation is stimulated [4, 27-29].

This has led to the investigation of the therapeutic potential of NETs because of their contribution to thrombosis. Approaches concerning NET production, degradation, and related proteins are being explored [30]. Additionally, NETs are being investigated for their potential use in the diagnosis of thromboembolic diseases, owing to their occurrence in thrombi and their link to thrombotic complications [4, 31,32].

Given the expanding knowledge of NETs in thrombosis, this review addresses the gaps in understanding their multifaceted roles, interactions with coagulation components, and therapeutic targeting. Previous studies have often overlooked the broader role of NETs beyond antimicrobial defense, especially in thromboembolic disorders. By providing a comprehensive synthesis, this study aims to bridge this gap by exploring NET-targeting strategies and their potential diagnostic and therapeutic applications. Improved management of thrombotic complications may emerge from ongoing research into NET involvement in these processes.

METHODS

An initial search involving various bibliographic databases was performed via PubMed, Scopus, Springer, and Web of Science to evaluate articles that focused on NETs, thrombosis, and hemostasis. The search terms used included MeSH terms for "neutrophil extracellular traps", "NETs", "thrombosis", "hemostasis", "venous thromboembolism", "arterial thrombosis", and "platelet activation", "coagulation", "fibrinolysis", "cancer-associated thrombosis", "antiphospholipid syndrome", "DNA", "histones", "elastase", and "DNase". The search included all the articles that were published in English until June 2024.

The criteria for study inclusion covered the following topics: NETs and thrombosis or hemostasis, NETosis and its involvement in thrombus formation, the clinical association of NETs with thrombotic disorders, or possible pharmacotherapeutic approaches targeting NETs. The exclusion criteria included nonpeer-reviewed papers, studies that investigated only the antimicrobial properties of NETs and data unrelated to thrombosis or hemostasis, or studies that did not contain any new data (i.e., reviews, editorials, or commentaries).

Data concerning the study type (experimental and observational, clinical trial), findings concerning the function and role of NETs in thrombosis and hemostasis, the mechanisms by which NETs might influence platelets, clotting factors, and endothelial cells, the specific impact of NETs on different thrombotic disorders, and the use of potential treatments targeting NETs in potential treatment were sought in the selected articles.

The quality of the included studies was assessed via standard criteria for research quality issues, such as study design, sample size, methodology, and orientation of the produced outcomes to the objectives of the presented review. This review identified 59 eligible studies on the relevance of neutrophil extracellular traps (NETs) to thrombosis and hemostasis.

RESULTS AND DISCUSSION

A total of 1,114 articles were initially retrieved through the database search. After screening for relevance and removing duplicates, 634 articles were deemed eligible. Following the application of predefined eligibility criteria, 164 publications were excluded, leaving 59 studies included in the review.

Quality assessment

The literature search identified 59 eligible studies on NETs and thrombosis/hemostasis. The studies were assessed for quality, considering research design, sample size, methodology, and alignment with review objec-

tives. These studies provide insights into the roles of NETs in thrombosis and hemostasis; their impact on platelets, clotting factors, and endothelial cells; and their relevance to thrombotic disorders.

The role of NETs in hemostasis

The role of NETs in hemostasis is complex and involves the interplay of their procoagulant and anticoagulant effects. Some studies have established the connection of NETs with the coagulation and innate immune systems and their functions in the initial response to injury, including hemostasis [33,34]. NETs come into contact with platelets, increasing their activation and aggregation, which is vital for the formation of platelet plugs in the initial stage of hemostasis [35]. Additionally, NET proteases such as elastase can activate coagulation factors, resulting in the formation of thrombin and fibrin, which enhances clot stability [36,37].

Each of the NETs, DNA, histones, and elastase, has its own hemostatic mechanism. DNA activates coagulation of blood through the intrinsic system, especially in conditions where there is cell damage [38]. DNA also participates in thrombin-dependent activation of factor XI and enhances the tissue factor-dependent coagulation pathway [39]. Furthermore, DNA exhibits antifibrinolytic activity by increasing the formation of the tPA-PAI-1 complex and decreasing the conversion of plasminogen to plasmin [40]. The concentration of DNA in NETs in thrombi affects thrombolysis; higher DNA concentrations support faster thrombolysis, whereas lower concentrations may support fibrinolysis [41].

Histones promote coagulation in vitro by activating coagulation factors and, in vivo, compromise the anticoagulant endothelial barrier, exposing prothrombotic proteins like tissue factor and activating platelets. The prothrombotic effects of endothelial injury predominate leading to thrombosis. [42]. Histones also activate neutrophils, leading to increased NET formation, and interact with platelet membranes to activate them [43]. Additionally, histones increase the rate of prothrombin autoactivation and decrease the rate of thrombin inactivation by antithrombin [44]. They stabilize clots by binding to fibrinogen and fibrin and can interfere with thrombin–thrombomodulin interactions, which in turn affects the generation of anticoagulant-activated protein C (APC) [10].

Elastase is the major protein constituent of NETs that influences clotting and fibrinolysis. It enhances fibrinolysis by inhibiting α2-PI, which has a direct fibrinolytic effect, and increasing the transformation of plasminogen into miniplasmin, which has high fibrinolytic activity [45]. Nevertheless, elastase also has procoagulant activity. It breaks down and inactivates coagulation inhibitors, namely, tissue factor pathway inhibitors, thrombomodulin and antithrombin, implying that the subendothelial surface is more thrombogenic [46,47]. Table 1 shows the pathogenesis of NETs in thrombosis and hemostasis.

Table 1. The pathogenesis of NETs in thrombosis and hemostasis

Component	Role in Hemostasis	References
NETs (general)	• NETs interact with platelets, increasing their activation and aggregation, essential for forming platelet plugs during the initial hemostatic response. • NET proteases, such as elastase, activate coagulation factors, enhancing thrombin and fibrin formation to stabilize clots.	[34- 38]
DNA	• Activates coagulation via the intrinsic pathway, especially during cell damage. • Participates in thrombin-dependent activation of factor XI and enhances the tissue factor-dependent coagulation pathway. • Exhibits antifibrinolytic activity by increasing the formation of the tPA-PAI-1 complex and reducing plasmin generation. • Higher DNA concentrations in NETs promote faster thrombolysis, while lower concentrations may favor fibrinolysis.	[39- 42]
Histones	• Promote coagulation *in vitro* by activating coagulation factors. • Compromise the anticoagulant endothelial barrier *in vivo*, exposing prothrombotic proteins (e.g., tissue factor) and activating platelets. • Activate neutrophils, enhancing NET formation, and interacting with platelet membranes to stimulate activation. • Increase prothrombin autoactivation and reduce thrombin inactivation by antithrombin. • Stabilize clots by binding fibrinogen and fibrin and interfering with thrombin–thrombomodulin interactions, affecting anticoagulant protein C (APC) generation. • The prothrombotic effects of endothelial injury predominate, leading to thrombosis.	[10, 42- 45]
Elastase	• Enhances fibrinolysis by inhibiting α2-PI and promoting the conversion of plasminogen to miniplasmin, which has strong fibrinolytic activity. • Exhibits procoagulant activity by degrading coagulation inhibitors (e.g., tissue factor pathway inhibitors, thrombomodulin, and antithrombin), making the subendothelial surface more thrombogenic.	[46- 48]

Abbreviations: α2-PI- α2-proteinase inhibitor, DNA- deoxyribonucleic acid, NET- neutrophil extracellular trap, PAI-1- plasminogen activator inhibitor-1, tPA- tissue plasminogen activator.

Arterial thrombosis

Arterial thrombosis, which forms the basis of myocardial infarction and stroke, has been described as the development of a thrombus within an artery that blocks blood flow to tissues [48]. NETs are associated with arterial thrombosis through various processes, including the following. First, NETs form a structural framework that traps and activates platelets and stimulates platelet aggregation and, therefore, contributes to thrombus formation [4]. Additionally, NETs release anionic phospholipids such as phosphatidylserine, which bind to coagulation factors, hence strengthening the clot [38]. Moreover, NETs incorporate neutrophil elastase and cathepsin G, which can activate coagulation factors, increase thrombin generation, and increase fibrin deposition [49,50].

Studies have been conducted on human subjects and have provided important information on the involvement of NETs in myocardial infarction. Acute myocardial infarction and NETs, which are markers of cell-free DNA, are associated with thrombotic events and adverse cardiovascular outcomes [51,52]. Additionally, NETs have been identified in coronary thrombi obtained from patients with myocardial infarction, thus supporting their role in thrombus development [53,54]. These findings underscore the role of NETs in the pathogenesis of myocardial infarction and the possibility of their use in treatment and diagnosis.

Venous thrombosis

Venous thrombosis, deep vein thrombosis (DVT), and pulmonary thromboembolism (PTE) are outcomes of the formation of a thrombus within a vein, particularly in lower limb veins or pulmonary veins [55]. NETs have been detected in thrombi isolated from patients with VTE, and their involvement in this process has been indicated [56,57]. Various studies focusing on humans have been useful in identifying the involvement of NETs in venous thrombosis.

Studies have shown that NETs contribute to venous thrombosis by activating factor XII and enhancing thrombin formation [56, 58]. Moreover, Neutrophil extracellular traps (NETs) promote endothelial procoagulant activity through multiple mechanisms. They induce tissue factor (TF) expression on endothelial cells, initiating the extrinsic coagulation pathway and thrombin generation [59]. Additionally, NET components such as histones and neutrophil elastase disrupt the endothelial anticoagulant barrier by degrading thrombomodulin and the endothelial protein C receptor, leading to unchecked fibrin formation [60]. NETs also activate the complement cascade, creating a proinflammatory and prothrombotic feedback loop. These combined effects contribute to thrombus expansion and stabilization [12].Increased levels of plasma cell-free DNA, a sign of NET formation, are also linked to the risk of VTE in humans [57].

Furthermore, NETs have been identified within fresh venous thrombi and are known to contribute to the genesis of post-thrombotic syndromes, which are frequent sequelae of deep vein thrombosis [10]. Similar NETs have also been observed in patients who suffer from cancer-associated venous thromboembolism, where their contributions to the hypercoagulable status of cancer have been recognized [61].

The clinical significance of NETs in venous thrombosis is supported by their potential as biomarkers. The identification of NETs or their components in VTE may be useful for the diagnosis and risk assessment of patients [32]. Moreover, the general circulatory cell-free DNA count or other NET-derived biomarkers can help assess the therapeutic outcome of anticoagulation therapy and, potentially, the prognosis of a relapse [62]. Table 2 shows the mechanisms of NET-mediated thrombosis.

Therapeutic potential and biomarker role of NETs

The existing knowledge of NET involvement in thrombosis and hemostasis has motivated the attempt to target NETs to treat thromboembolic diseases. Several strategies are currently being researched, with the aims of inhibiting the formation of NETs, degrading extracellular DNA, and regulating NET-associated proteins.

Table 2. Mechanisms of NET-mediated thrombosis

Mechanism	Description	References
NET-mediated platelet activation	NETs activate platelets and provide a scaffold for their adhesion and aggregation, promoting thrombus formation	[4, 11, 19-22, 27-29, 36]
Tissue factor	NETs express tissue factor, initiating the coagulation cascade.	[13,14, 47, 48]
Procoagulant properties of NET components	DNA, histones, and elastase within NETs have procoagulant properties, enhancing thrombin generation and fibrin deposition	[7, 18-22, 37-39, 42-48, 50-53]
Endothelial cell activation	NETs induce tissue factor expression on endothelial cells, disrupting the endothelial anticoagulant barrier by degrading thrombomodulin and the protein C receptor, leading to unchecked fibrin formation.	[12, 42, 59, 60]
Thrombus stabilization	The DNA scaffold and associated proteins in NETs stabilize the thrombus structure, preventing its dissolution and promoting clot propagation.	[4, 27-29]

Abbreviations: DNA- deoxyribonucleic acid, NET- neutrophil extracellular trap.

In some studies, anti-NET therapeutic approaches have shown promising results. For example, neutralization of peptidyl arginine deiminase 4 (PAD4), the enzyme that triggers NET release, by PAD4 inhibitors has been shown to reduce thrombosis in a clinical trial with patients with arterial and venous thromboembolism [63,64]. Additionally, neutrophil elastase has recently been identified as a possible antithrombotic target. Early-phase clinical trials have investigated neutrophil elastase inhibitors, such as GW311616A, for the management of DVT and PTE [65,66].

Another therapeutic approach involves the degradation of extracellular DNA, which is one of the components of NETs. Clinical studies have demonstrated that DNase-I treatment, which disaggregates the DNA scaffold of NETs, enhances thrombus resolution in patients with overall deep vein thrombosis and pulmonary embolism [67,68]. Moreover, the inhibitory role of histones by histone deacetylase inhibitors has been discovered as a treatment strategy. Early findings suggest that targeting histones might decrease the prothrombotic properties of NETs [69]. Table 3 summarizes the therapeutic strategies targeting NETs. In addition to targeting NETs with specific therapeutics aimed at counteracting their role in thrombus formation, NETs have the potential to act as biomarkers of thromboembolic events. The potential biomarker applications of NETs in thromboembolic disorders are summarized in Table 4.

CONCLUSION

This review highlights the importance of NETs in preventing blood clotting and bleeding, as well as their interactions with different aspects of coagulation. NETs are also indicators of thromboembolic disease. NETs are involved in thrombus formation, maturation, and development, suggesting that they may play a crucial role in future therapeutic intervention. Additional work is required to optimally understand what transductions are at work when NETs influence thrombosis and hemostasis; ongoing preclinical and clinical studies will help provide insights to improve existing anticoagulation and thrombolysis strategies. An improved understanding of NET biology may help improve patient outcomes and reduce the thrombotic burden on those afflicted with thromboembolic disorders.

ABBREVIATIONS

DNase-I - deoxyribonuclease I

DVT- deep vein thrombosis

NETs- neutrophil extracellular traps

PAD4- peptidyl arginine deiminase 4

PTE- pulmonary thromboembolism

VTE- venous thromboembolism

AUTHORS' CONTRIBUTION

AA contributed to the conception, design, literature review, data interpretation, and writing of this manuscript. The author also reviewed and approved the final version for submission.

CONFLICT OF INTEREST

None to declare.

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Table 3. Therapeutic strategies targeting NETs

Therapeutic Approach	Description	References
PAD4 inhibition	Inhibiting the PAD4 enzyme that triggers NET release	[63,64]
Neutrophil elastase inhibition	Targeting neutrophil elastase with inhibitors such as GW311616A	[65,66]
DNase-I treatment	Disaggregating the DNA scaffold of NETs to enhance thrombus resolution	[67,68]
Histone deacetylase inhibition	Targeting histones to decrease the prothrombotic properties of NETs	[69]

Abbreviations: DNA- deoxyribonucleic acid, NET- neutrophil extracellular trap, PAD4- peptidyl arginine deiminase 4.

Table 4. Potential biomarker applications of NETs in thromboembolic disorders

Biomarker	Description	References
NETs and their components	Detection of NETs or their components in thrombi	[31-33, 57, 58]
Free DNA and NET-derived biomarkers	Assessment of general circulatory cell-free DNA or other NET-derived biomarkers for risk evaluation and prognosis	[65]
NETs in cancer-associated thrombi	Identification of NETs in thrombi associated with cancer, contributing to hypercoagulability	[15, 16, 62]

Abbreviations: DNA- deoxyribonucleic acid, NET- neutrophil extracellular trap.

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