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REVIEW

Relationship Between Neutrophil Extracellular Traps and Venous Thromboembolism: Pathophysiological and Therapeutic Role

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Abstract

Venous thromboembolism (VTE), which includes pulmonary embolism (PE) and deep vein thrombosis (DVT), is a serious vascular disease that ranks third in cardiovascular-related deaths. Inflammation along with neutrophil extracellular traps (NETs) play a key role in the pathophysiology of VTE. This review focuses on articles that that evaluate the role of NETs in the development of VTE and their potential as therapeutic targets. Research has demonstrated that when NETs become overactivated, they take part in thrombotic activities, which is the opposite of their defensive functions under healthy conditions. When endothelial cells are activated, neutrophils are recruited very early, releasing NETs and initiating a thrombotic process. NETs promote thrombosis by directly activating factor XII (FXII), ultimately triggering platelet recruitment, and initiating the intrinsic coagulant pathway. Subsequently, monocytes and factors such as tissue factor join the process, further increasing NET formation, the inflammatory reactions and progression of venous thrombus. NETs play a crucial part in the intricate interaction between inflammation and thrombosis, where each triggers the other. High levels of NETs also correlate with the severity of VTE. These properties of NETs make them potential therapeutic targets for VTE prevention and treatment. This article aims to describe NETs, their occurrence, and how they relate to VTE. Taking into account what is now known, the function of NETs as targets for treatment in VTE using various approaches, the benefits and drawbacks of these approaches, and suggestions for the future are examined.

Key words: neutrophil extracellular traps; NETosis; venous thromboembolism; immunotherapy; neutrophil

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Introduction

Venous thromboembolism (VTE) is a serious vascular disease that significantly impacts individuals' well-being and quality of life (Ding et al, 2023). Despite being one of the top three causes of cardiovascular death, much of the mortality and morbidity associated with VTE is preventable (ISTH Steering Committee for World Thrombosis Day, 2014). Inflammation is believed to play a crucial role in the initiation and progression of VTE (Cocoi et al, 2017). VTE typically encompasses deep vein thrombosis (DVT) and pulmonary embolism (PE) (Ding et al, 2023). DVT is a blood clot that usually occurs in the deep veins of the legs, but less commonly

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in the arms and in the mesenteric and cerebral veins (Waheed et al, 2024). PE is a common clinical condition that occurs when a thrombus originating from elsewhere disrupts blood flow in the pulmonary artery or its branches (Vyas et al, 2024). DVT is usually asymptomatic and resolves once mobility is restored. However, it can become chronic, and some can even cause pulmonary embolism (Roderick et al, 2005). Hemodynamically unstable PE results in severe hypotension (systolic blood pressure 40 mm Hg or more from baseline), while hemodynamically stable PE ranges from clinically asymptomatic to mildly symptomatic and responds to fluid therapy (Vyas et al, 2024).

Three main contributing factors to thrombosis formation have been identified, known as Virchow's Triad: venous stasis, vascular endothelial injury, and hypercoagulability (Kushner et al, 2024). In the development of VTE, damaged venous endothelium plays a key role in venous thrombus formation, with inflammation and thrombosis exhibiting a close interdependence (Jackson et al, 2019). When endothelial cells are activated, a complex relationship between inflammation and thrombosis is mediated by endothelial cells, leukocytes, erythrocytes, and platelets, where inflammation triggers thrombosis and vice versa (Najem et al, 2020).

After the activation of endothelial cells, neutrophils are recruited very early during the thrombotic process and release neutrophil extracellular traps (NETs). NETs promote thrombosis by directly activating factor XII (FXII), ultimately triggering platelet recruitment, and initiating the intrinsic coagulant pathway (Ding et al, 2023; von Brühl et al, 2012). Monocytes also participate in the early stages of thrombosis. Monocytes stimulate the extrinsic coagulation pathway via tissue factor (TF) delivery and ultimately support the formation of NETs (called NETosis) and blood clotting (Budnik and Brill, 2018; Ding et al, 2023). Proinflammatory cytokines, especially interleukin (IL)-6, affect the coagulant system and the fibrinolytic system, increase adhesion molecules such as P-selectin, and lead to neutrophil migration. This process eventually results in NET formation and TF release, thus further reinforcing the inflammatory reactions in the formation and progression of venous thrombus (Ding et al, 2023; Vazquez-Garza et al, 2017).

Neutrophils and monocytes also play an important role in the resolution stage of venous thrombosis by regulating collagen and fibrin degradation through the secretion of matrix metalloproteinases (MMP), mainly MMP-2 and 9, and plasminogen activators (Najem et al, 2020). Monocytes also contribute to neovascularization, which is part of thrombus remodeling and vessel recanalization, by secreting vascular endothelial growth factor (VEGF) (Cocoi et al, 2017; Najem et al, 2020).

This study aims to briefly highlight the role of NETs as treatment targets in VTE in the light of current knowledge. The study was also highlighted possible molecular mechanisms of the relationship between NETs and thrombus formation. as Additionally, the preventive role of targeting NETs in the development of VTE was evaluated.

Structure and Formation of Neutrophil Extracellular Traps

NETs are reticular structures consisting of uncondensed deoxyribonucleic acid (DNA) strands coated with granule proteins. They contain numerous proteins such as citrullinated histones, neutrophil esterase (NE), myeloperoxidase (MPO), high mobility group protein B1 (HMGB1), peptidoglycan binding protein, lactoferrin, serine proteases, matrix metalloprotease-9 (MMP-9), and proteinase 3 (PR3) (Hidalgo et al, 2022; Yu et al, 2022).

Although NETosis play a defensive role in various diseases, they have also been confirmed to be linked to many disease pathologies. NETosis due to endothelial damage amplifies the inflammatory response and thrombosis, accelerating disease progression by creating a vicious cycle between NETs, endothelial damage, and inflammation (Hidalgo et al, 2022; Zapponi et al, 2022).

The formation of NETs is activated by a variety of stimuli, including some proinflammatory mediators. Following stimulation, nicotinamide adenine dinucleotide phosphate (NADPH) oxidase or mitochondrial reactive oxygen species (ROS) activate myeloperoxidase (MPO) and neutrophil elastase (NE) in neutrophils, leading to histone degradation and chromatin disruption in the nucleus (Yu et al, 2022). ROS also activates peptidyl arginine deiminase 4 (PAD4), catalyzing the deamination and citrullination of histone arginine residues and chromatin decompensation. The fundamental process in NET formation is chromatin decondensation, which in turn depends on the presence of PAD4, NE and MPO (Fig. 1) (Li et al, 2023).

Role of Neutrophil Extracellular Traps in Thrombus Formation

Despite their beneficial roles, excessive secretion of NETs can also have harmful effects on tissues through compounds such as cell-free DNA (cf-DNA), MPO and NE (Boeltz et al, 2019). Under normal physiological conditions, thrombosis plays a role in repairing vascular damage and achieving haemostasis. However, when overactivated, it can cause various adverse events such as vascular obstruction and vascular thromboembolism (Mackman et al, 2020). A complex molecular mechanism is involved in the formation of thrombus, including the interplay between NETosis and coagulation pathways (Noubouossie et al, 2019; Wang et al, 2018).

Following vascular injury, platelets become activated as a result of exposure to subendothelial collagen, subsequently leading to the release of thromboxane A2 (TXA2) and the recruitment of more platelets along with red blood cells for the haemostatic plug (Caillon et al, 2022). With increased expression of cell adhesion molecules such as P-selectin and von Willebrand Factor (vWF), inflammatory cells infiltrate the haemostatic plug (Ley et al, 2007). In this process, leukocytes are recruited via vWF A1 domain–beta-2 integrin interaction and P-selectin/P-selectin glycoprotein ligand-1 (PSGL-1) interaction, while platelets are recruited via vWF

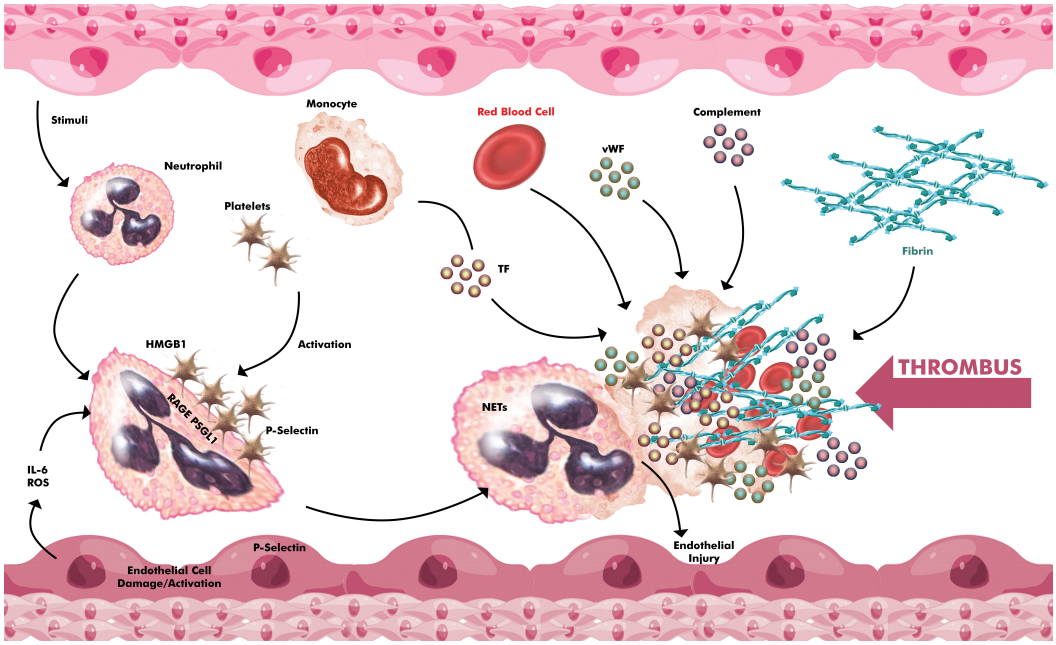

Fig. 1. Representative illustration of the role of NETs in the formation of venous thrombosis. Molecules such as IL-6 and ROS are released from damaged endothelial cells in response to various stimuli, activating neutrophils. Activated platelets also contribute to neutrophil activation, primarily through interactions involving HMGB1/RAGE and P-selectin/PSGL-1. Neutrophil activation leads to endothelial damage through the creation of NETs and the secretion of enzymes such as elastase, cathepsin G, and protease 3. NETs serve as a framework for structures like tissue factors, clotting factors, red blood cells, and platelets, ultimately leading to venous thrombosis. Drawing with Adobe Creative Suite Package [(Illustrator, version 28.7.1 and Photoshop, version 25.12) (Adobe Systems Incorporated, San Jose, CA, USA)]. Adapted from "Neutrophil extracellular traps mediate deep vein thrombosis: from mechanism to therapy (https://www.frontiersin.org/journals/immunology/articles/10.3389/fimmu.2023.1198952/full#h12)" by Yao et al (2023) that is licensed under CC BY 4.0 (https://creativecommons.org/licenses/by/4.0/). NETs, neutrophil extracellular traps; IL-6, interleukin-6; ROS, reactive oxygen species; HMGB1, high mobility group protein B1; RAGE, receptor of advanced glycation terminal; PSGL-1, P-selectin glycoprotein ligand-1; TF, tissue factor; vWF, von Willebrand Factor.

A1 domain–glycoprotein Ib interaction (Kimball et al, 2016). Complement activation and tissue factors (mainly released from monocytes) also participate in the process (Ley et al, 2007). The involvement of TF initiates the extrinsic coagulation pathway and catalyses the formation of thrombin, converting fibrinogen to fibrin (Noubouossie et al, 2019).

In addition, NETs released from neutrophils promote thrombosis by attracting platelets and activating intrinsic and extrinsic coagulation pathways (Noubouossie et al, 2019). NETs also act as a scaffold for the accumulation of platelets, erythrocytes, fibrinogen, vWF, TF and coagulation factor XII, which may contribute to thrombosis. Consequently, they create a procoagulant state and increase endothelial damage (Hidalgo et al, 2022; Wang et al, 2018; Yu et al, 2022). The network structure of NETs also enhances the stability of the fibrin skeleton within the thrombus (Li et al, 2023). Histones in NETs may promote thrombosis by activating platelets by utilizing toll-like receptor (TLR) 2 and TLR4 and increasing TF ex-

pression (Noubouossie et al, 2019; Osada et al, 2017). Similarly, NE and cathepsin G may contribute to inducing the coagulation pathway by activating TF and factor XII (Li et al, 2023).

All these events, which aggravate inflammation and thrombosis, lead to the formation of an organized thrombus characterized by a fibrotic appearance and damage to the venous wall and venous valves over time (Yao et al, 2023). NETs may play a role in both the early and late stages of thrombosis, although perhaps with different roles (Li et al, 2023).

Neutrophil Extracellular Traps as Therapeutic Targets in Venous Thromboembolism

An imbalance in the clearance of NETs can lead to excessive tissue damage and pathological conditions, including VTE (Xiang et al, 2023). NETs not only mediate inflammation but also activate platelets, coagulation, and thrombosis (Li et al, 2023). In a study by Zapponi et al (2022), patients displayed increased levels of neutrophil adhesive and chemotactic molecules, MPO-DNA complexes (residual markers of NETs), sICAM-1 (secreted intercellular adhesion molecule-1), and sVCAM-1 (secreted vascular cell adhesion molecule-1) even 2 years after an acute VTE event. Therefore, inhibiting NET formation or promoting NET degradation, which plays a crucial role in the cycle of endothelial damage-inflammation-thrombosis, can be considered an important strategy in VTE treatment and prophylaxis (Table 1). In this context, PAD4, the key enzyme in NET formation, is an important target. Additionally, research is focused on NET-specific targets such as cf-DNA and NE, as well as non-NET-specific targets such as ROS and certain chemokines.

Activated platelets are known to induce NETosis and NETs contribute to thrombosis by creating a procoagulant state (Vazquez-Garza et al, 2017). Strategies aimed at blocking this cycle by preventing essential adhesive interactions may reduce NET-mediated thrombosis. For instance, inhibiting P-selectin by blocking PSGL-1 can reduce NET release (Martinod et al, 2015). NET-associated cf-DNA is recognized by TLR9 on both monocytes and polymorphonuclear leukocytes (PMNs) (Cherpokova et al, 2019). Patients with mutations in this receptor and in TLR9-/- experimental models have shown an increased risk of VTE (Ahmad et al, 2017; Dewyer et al, 2015). Furthermore, since NETs act as a scaffold for platelets and erythrocytes to adhere, disrupting the NET structure using DNase I can prevent platelet aggregation and fibrin deposition (Hidalgo et al, 2022). Blocking NET release is the most suitable therapeutic approach to reduce the risk of VTE or prevent secondary thrombotic events. In patients with VTE where NETs are already present in their thrombi, lysing or removing NETs should be the primary approach (Yao et al, 2023).

Activated platelets expressing HMGB1 can independently trigger the recruitment of PMNs to the area of the developing thrombus, NETosis, and monocyte infiltration (Nicklas et al, 2020). It was observed that anti-HMGB1 antibody administration suppressed NET formation in experimental models via receptor of advanced glycation terminal (RAGE), TLR2, and TLR4 (Kim and Lee, 2020). PDA4

Table 1. Some selected therapeutics targeting the formation of neutrophil extracellular trap and their important major targets.

Target	Therapeutic compound/strategy	Major action	Reference
Preventing NET formation			
Protein-arginine deiminase 4	GSK484	Specific inhibition of PAD4	(Lewis et al, 2015)
	GSK199	Specific inhibition of PAD4	(Lewis et al, 2015)
	Cl-amidine	Pan-PAD inhibition	(Biron et al, 2017)
	F-amidine	Pan-PAD inhibition	(Biron et al, 2017; Li et al, 2015)
	PAD4 gene knockout	Decreased PAD4 expression	(Martinod et al, 2015)
	Neonatal NET inhibitory factor	Inhibition of NET formation	(Yost et al, 2016)
Neutrophil esterase	Sivelastat	NE inhibition	(Li et al, 2023)
Citrullinated histone H3	Anti-CitH3	CitH3 inhibition	(Chirivi et al, 2021)
Promoting NET degradation			
cf-DNA	DNAase1	DNA degradation	(Peña-Martínez et al, 2019)
Histone	Thrombomodulin	NET degradation, Histone detoxification, limiting procoagulant response	(Osada et al, 2017)
Other strategies			
von Willebrand Factor	ADAMTS13	Reduced platelet activation and neutrophil accumulation	(Carestia et al, 2016)
Thromboxane A2	Acetylsalicylic acid	Reduced risk of recurrent VTE	(Simes et al, 2014)
Platelet P2 receptor Y_{12}	Clopidogrel	Reduced platelet accumulation	(Carestia et al, 2016; Thomas and Storey, 2015)

Table 1. Continued.

Target	Therapeutic compound/strategy	Major action	Reference
Reactive oxygen species	N-acetyl cysteine	Reduced NET formation	(Aldini et al, 2018)
	Molecular hydrogen		(Shirakawa et al, 2022)
	Ascorbic acid		(Shirakawa et al, 2022)
Neutrophil depletion	Adhesion molecule inhibitor (against CXC chemokine receptor 11/CXC chemokine receptor 12, Intercellular adhesion molecule-1, P-selectin etc.)	Reduced neutrophil count	(Etulain et al, 2015)
	Anti-Ly6G antibody	Reduced neutrophil count	(Kang et al, 2020)
Combinational approaching			
Thrombus DNA, histone vWF	DNAase1+tPA DNAase1+Heparin DNAase1+ADAMTS13	Acceleration of thrombolysis Reducing the risk of thrombotic events Prevention in thrombosis (by decreasing initial platelet and neutrophil recruitment)	(Peña-Martínez et al, 2019) (Manfredi et al, 2017) (Schettert et al, 2010)

PAD4, peptidyl arginine deiminase 4; CitH3, citrullinated histone H3; NET, neutrophil extracellular trap; VTE, venous thromboembolism; NE, neutrophil esterase; cf-DNA, cell-free DNA.

inhibitors, such as GSK484 and YW4-03, block not only NET release but also thrombosis (Yao et al, 2023). Such observations also reveal that there is a reciprocal modulation relationship between PAD4 and HMGB1.

Activated protein C (APC) is an enzyme that leads to the breakdown of histones (Xiang et al, 2023). It has been observed that the use of recombinant thrombomodulin promotes the disruption of NETs by enhancing APC activity (Osada et al, 2017). Thrombomodulin also prevents thrombosis and NETosis by cleaving HMGB-1 (Nicklas et al, 2020; Yao et al, 2023).

Blocking platelet alpha granule release with acetylsalicylic acid (Schettert et al, 2010) and vWF with ADAMTS13 (a disintegrin and metalloprotease with thrombospondin-type motifs) reduces P-selectin and vWF-mediated accumulation of platelets and PMN, ultimately reducing NETosis (Hidalgo et al, 2022; Ley et al, 2007).

Some traditional drugs such as heparin, aspirin and clopidogrel have been observed to prevent the formation of NETs (Napirei et al, 2009; Simes et al, 2014; Thomas and Storey, 2015).

Blocking neutrophil recruitment and activation is one of the indirect strategies to inhibit NET formation (Martinod et al, 2015). In experimental models, resolvin D4 (RvD4) administration significantly reduced thrombus load, neutrophil and monocyte infiltration, and also prevented NET formation (Cherpokova et al, 2019).

Citrullinated histone H3 (CitH3), which is formed by citrullination of histones in the chromatin structure by PAD4, is a central player in the release of NETs and has been postulated to be associated with the severity of VTE (Thålin et al, 2018; Ząbczyk et al, 2020). Experimental studies have shown that anti-CitH3 antibodies reduce the inflammatory response (Chirivi et al, 2021). In support of this, Cl-amidine, a potent pan-PAD inhibitor, reduced CitH3 and NET levels in various disease models (Mondal and Thompson, 2019). Similarly, successful results were obtained in terms of NET inhibition with the administration of F-amidine, which is also a pan-PAD inhibitor (Lewis et al, 2015; Li et al, 2015).

Neonatal NET inhibitory factor (nNIF) has been shown to inhibit key terminal events in NET formation, including histone H3 citrullination and PAD4 activity (Yost et al, 2016). P-selectin/PSGL-1 inhibitors have been observed to block P-selectin-mediated platelet-neutrophil interactions and reduce pathological thrombosis and inflammation by inhibiting NET formation (Etulain et al, 2015).

Studies targeting other key players in the thrombus formation pathway (e.g., such as vitamin B1 for ROS, Sivelastat for neutrophil elastase, anti-HMGB1 antibody and resolvin D4 for multi-potency against thrombo-inflammation, eculizumab for complement) have also shown significant improvements in NET inhibition, regulation of thrombo-inflammation, and thrombus resolution (Cherpokova et al, 2019; Liu et al, 2007; Riyapa et al, 2019). It has been observed that various antioxidant molecules such as N-acetylcysteine and ascorbic acid (vitamin C) (Aldini et al, 2018; Shirakawa et al, 2022) prevent NET formation by inhibiting ROS production. Observations also indicate that molecular hydrogen therapy can effectively inhibit neutrophil activation and excessive NET formation without inhibiting the basic function of neutrophils (Shirakawa et al, 2022).

In an *in vitro* study by Hawez et al (2019), transfection with antagomiR-155 reduced PAD4 mRNA, DNA histone complex levels, and NET induction in PMA-stimulated neutrophils.

Although an imbalance in their clearance can lead to extensive tissue damage and pathology, NETs play a vital role in the innate immune response as anti-infectious agents (Xiang et al, 2023). Some have noted different observations (Martinod et al, 2015), suggesting that preventing NETosis by reducing neutrophil counts or enzymatic inhibition of PAD4 increases susceptibility to life-threatening infections (Ngo and Gollomp, 2022). Additionally, NET degradation may lead to the release and subsequent dissemination of bacteria captured by NETs, along with toxic products such as cf-DNA, histones, and NE (Ngo and Gollomp, 2022). Bacterial dissemination may result in infection in different foci, while toxic products can induce tissue damage and inflammation. Pan-PAD inhibitors such as F- and Cl-amidine inhibit not only PAD4 but also several other PAD subtypes simultaneously (Lewis et al, 2015; Mondal and Thompson, 2019). The observations by Martinod et al (2015) that PAD4 deficiency does not lead to increased susceptibility to bacterial infection may be because PAD4 inhibition does not significantly impair host immune function.

In addition to promoting chromatin decondensation and NETosis, ROS are also vital for bacterial killing. Similarly, to pan-PAD4 inhibition, some drugs that inhibit ROS production for NETosis increase susceptibility to bacterial infection when they excessively inhibit ROS production (Ostafin et al, 2016).

These observations should not deter the pursuit of such approaches. Prophylactic or therapeutic approaches aimed at promoting NET degradation and inhibition of NETosis still require further investigation to better understand their efficacy and safety. Alternative strategies, such as neutralizing/reducing the toxic effects of circulating products and improving the ability of NETs to capture pathogens, may be considered based on the data. For example, the tight packing of histones and DNA in the nucleosome may partially reduce their ability to interact with the coagulation system without increasing susceptibility to infection.

The nanoparticle carrier strategy is applied in various diseases to both increase the efficacy of therapeutic agents and prevent their off-target consequences (Dinc, 2024). In their study, Lee et al (2021) reported that the application of DNase-1-coated nanoparticles effectively inhibited NET formation in blood samples from patients with COVID-19. In our preliminary opinion, intravenous administration of magnetic nanoparticle carriers loaded with ligand and inhibitor molecules targeting NETs and application of a magnetic field extracorporeally will both increase the effectiveness and reduce off-target effects.

The elevation of specific molecules such as MPO-DNA, CitH3, cf-DNA, nucleosomes and DNAse-I in the peripheral blood, which are considered indicators of NET formation, may guide the treatment option to some extent (Xu et al, 2022).

Study Limitation

This review article has several limitations. Studies investigating the link between human VTE events and NETosis are scarce. Evidence regarding the actual effect of inhibiting NETs on promoting thrombolysis and preventing thrombosis is poor. Furthermore, the results of existing studies are inconsistent. In this context, the challenges of targeting NETs as a therapeutic approach remain due to the lack of sufficient evidence demonstrating their specificity, long-term inhibitory effects, and adverse effects. Evaluations rely mostly on study data from animal studies and other clinical conditions other than VTE.

To determine the optimal treatment modality in the future, randomized and observational clinical studies are still needed that prioritize the short-, medium- and long-term efficacy and safety of different treatment strategies in different clinical settings.

Future Perspective

The role of NETs in thrombotic diseases has been confirmed not only in experimental studies but also in clinical samples (LiW-52, XuX-2022). In this context, NETs deserve to be investigated as antithrombotic treatment targets. However, current data are not yet sufficient to offer it as a recommendable treatment option for VTE. As the relationship between NETs and thrombosis-related diseases becomes more well-established, more appropriate treatment protocols will be able to be established.

Biomarkers for NETs may provide new tools for precision medicine and outcome prediction. In some clinical studies, the presence of NETs or their essential components such as cf-DNA, nucleosomes, MPO, CitH3 or NE has been shown to be markers for thrombosis-related diseases, including DVT and PE (Ząbczyk et al, 2020). For example, Mauracher et al (2018) found that high plasma levels of cf-DNA and nucleosomes predicted VTE in the short term. Although these markers have been detected in venous thrombi from surgical specimens or autopsies, tissue samples are often difficult to obtain in clinical practice (Xu et al, 2022). Therefore, key NET components are often tested using blood samples. Unfortunately, these tests provide relatively limited information (Li et al, 2023).

The dosage and timing of NET treatments in VTE will be one of the target points of the upcoming clinical studies. Because these issues are very important in terms of providing the best efficacy and minimum side effects. Combination therapies may be an important option due to their synergistic effects and lower dosage requirements, resulting in fewer side effects. Again, specific targeting is needed to avoid potential off-target effects or complications such as increased susceptibility to infection, without compromising the beneficial effect.

Conclusion

VTE is a significant clinical condition with high mortality, poor prognosis, and limited effective treatment options. As our understanding of the pathophysiologi-

cal mechanisms deepens, we can identify more effective therapeutic and diagnostic targets for venous thrombosis. Inflammation and coagulation play a crucial role in VTE formation, with NETs being key in creating a deleterious cycle between the two. Elevated NET levels are linked to the severity of VTE, making NETs potential therapeutic targets for prevention and treatment, as well as biomarkers for diagnosis, and monitoring. Among the factors in the NET formation pathway, PAD4 is central, and PAD inhibitors have shown promise in reversing poor outcomes. NET inhibition could be a valuable alternative or complement to current VTE treatment, or even a preventive measure. However, due to uncertainties regarding the efficacy and side effects of these strategies in humans, further large-scale studies spanning short-, medium-, and long-term periods are necessary to fully understand the potential benefits of NET inhibition. Future research should include different therapy strategies such as modified versions, combinations and targeting different pathways.

Key Points

- Neutrophil extracellular traps (NETs) have been observed to play a role in the pathophysiology of thrombotic events, including venous thromboembolism (VTE).
- Current data have shown that targeting the formation of NETs by blocking or digesting them is a promising treatment strategy.
- There are many challenges to determining the optimal treatment modality, including uncertainties regarding timing, dosing, and side effects of different modalities, and lack of adequate human trials.
- Blocking NET formation could potentially increase susceptibility to infection. Therefore, alternative methods such as combination therapy applications and some modifications should be investigated.

Availability of Data and Materials

All supporting data are included in the article.

Author Contributions

RD and NA designed the study. RD and NA drafted the manuscript. NA wrote the manuscript. RD and NA supervised and edited the manuscript. RD and NA contributed to critical editorial changes. Both authors read and approved the final version of the manuscript. Both authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Not applicable.

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Conflict of Interest

RD is president of Medical Innovation Institute. NA is a volunteer consultant for Med-International UK Health Agency Ltd.

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